THE INTEGRATION OF KNOWLEDGE WITHIN SCIENCE, TECHNOLOGY AND INDUSTRY: ENZYMES A CASE STUDY

by

Zbigniew Towalski

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S U M M A R Y

Enzyme technology is widely regarded as an exciting new technology possessing great opportunities for commercial interests and is one of a small group of key technologies singled out by the Science Research Councils during the 1960's as worthy of special support. In this thesis I outline the basic characteristics of this technology analysing the nature of the Government's policy towards it. The approach I have chosen requires an in depth analysis of the innovation process for enzymes which forms the basis for a model.

This model is then used to focus on aspects of the UK science policy towards innovation in enzyme technology, assessing its impacts, and appraising the usefulness of this approach for future policy initiatives.

KEYWORDS

biotechnology, enzyme technology, science policy, modeling, innovation

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CHAPTER 10 : -

CHAPTER 1 : A DISCUSSION OF THE RESEARCH PROBLEM AND RESEARCH METHODOLOGY

1.1. Introduction

Innovation is one of the processes which bring about change into an industrial society. This process describes the factors that govern the production and commercialisation of new knowledge. In the general literature the expression 'innovation' has also a much narrower meaning. Used in a specialised way it is also used to refer to the first commercial introduction of a new product or process.[1] In this thesis to avoid confusion between these different meanings I shall make use of the phrase 'process of innovation' to apply to the processes which lead to innovation and use 'innovation' as the noun to refer to the first commercial introduction of a new product or process.

Industry in the United Kingdom (U.K.) has been repeatedly criticised for failing to implement innovations from the research carried out by U.K. scientists. Government sponsored agencies such as the National Research and Development Corporation and the National Enterprise Board, entrusted with taking up new ideas and arising from public funded research have borne much of the criticism directly and led to the formation of the British Technology Group in 1981. Much of the basis for this criticism is the result of unfavourable comparisons in the exploitation of these ideas with countries such as the U.S.A., Japan and West Germany which appear to be more successful in converting new ideas into profitable ventures. Thus the prevailing view today held by many is that the U.K. industry is bad at innovating especially in areas that involve new technology.[2]

This thesis sets out to examine an aspect of this problem and show how aspects of the U.K.'s science research policy exacerbate this problem. Understanding of the process of innovation is central to my analysis and to my thesis. Fortunately it has been the focus of much attention by economists, social and political scientists, especially since the end of World War II. I shall review some of their work in section 1.2 and again in section 9.1. In chapter 9 I shall discuss models of innovation in some detail and assist with my analysis of the U.K.'s science policy. I shall now turn and introduce aspects of U.K. science policy that have a bearing on this thesis.

The relationship between science policy and the perception of the role of science in society is interwoven, since the one affects the other. Before World War II most economists regarded the process of innovation as an external influence on the main economic factors of production land, labour and capital. The post World War II's expansion of science research had a profound influence on a re-evaluation of this relationship. World War II in many respects was the physicists War.[3] The contribution of scientists to the Manhattan Project was clear and uppermost in many governments minds. It is not surprising therefore that immediately after the war the race to make the atom bomb by other world powers became the focus of much of their research efforts. The perception of the process of innovation as the orderly progress of ideas, beginning with pure research, leading to the discovery of new knowledge, its application and development, and incorporation in an invention, and its first exploitation by society as an innovation to be followed reinforced the linear nature of that process. Such a relationship between science and technology was articulated by Vannaver Bush in

the U.S. during 1945.[4] Bush successfully argued for continued support of basic research by government on the basis that basic research performed without thought of its practical applications resulted in useful discovery. This view of the link between science and technology was again restated by P.M.S. Blackett in 1968[5] in the U.K. who urged for the continued support of undirected science research since clearly basic research cost money but its practical applications resulted in useful discoveries from which industry made money.

The idea that science creates new knowledge and industry applies it prevailed for about 20 years in most post War Industrialised countries.[6] Scientific research blossomed, judged by the numbers of research publications that began to appear.[7] Research budgets also grew. In the U.K. research funds were allocated by the Department of Education and Science and administered by the Research Councils. The allocation of research funds to research projects was by a system of peer review, which extensively meant that science was administered by scientists for scientists. The Council on Science Policy was essentially made up of members of the five research councils and policy amounted to dividing up the available funds between them. In science big science projects in physics and astronomy continued to receive the lion's share of research funds, and continued to support new and increasingly sophisticated and costly projects.[8] This trend towards more and more costly research tools focused attention on big science. Escalating costs forced branches of physics to enter into international collaborative research ventures. International collaboration in research had political implications and was encouraged within the EEC. The escalation in the cost of research in the U.K. exceeded the rate of economic growth, a situation that had to eventually come to an

end. The U.K. postwar economy grew and continued to expand into the 60's. However the relative growth of the economy compared to the economic growth of other industrial nations was low. During this period heavy industries such as steel production, mining and shipbuilding continued their decline, and domestic light engineering based industries associated with car manufacture and the production of electrical goods began to lose ground to foreign competition. The need to revitalise the economy was seen as an important political platform which the first Wilson Government embraced making a firm commitment to science and technology.[9]

The need to stimulate innovation in the science based technologies focused attention on the innovative process and in particular the contribution of science. During the late 60's pressure was put on to the Research Councils to increase research effort directed towards meeting broader 'national needs'.[10] The question of accountability was also raised, directed at making better use of available resources. The fact that no coherent plan existed between the research actively on a commodity and its commercial importance and to the system of directing research in bodies such as the Agricultural Research Council,(ARC) prompted an investigation of the way governments supported research was regulated. In 1971 the Rothchild Report and the subsequent white paper ' Framework for Government Research and Development' altered the way in which government research was to be carried out and established the customer contractor principle whereby the Ministry of Agriculture Fisheries and Food received over half of the ARC's budget and became the contractor for ARC projects.

Table 1.1 Council committees constituting subject areas

Area	SRC committees	NRC committees (including programme grants)
Biology	Biology	Biology Animal Cellular Plant Population Agriculture and Forestry
Chemistry	Chemistry Enzyme Chemistry	Chemistry
Mathematics	Mathematics	Mathematics
Physics	Physics Nuclear physics Neutron beam Reactor technology Reactors Radio	Physics Nuclear High energy Atomic Energy Control Board
Computing	Computing science	Computer facilities Computing and information science
Engineering	Aeronautics and civil Aeronautics and mechanical Chemical Control Electrical and systems Manufacturing Mechanical and production Metallurgy and materials Polymer Transport Board	Engineering Chemical Electrical Civil Industrial Mechanical
Space and astronomy	Space Astronomy	Space Space and astronomy

Source : I.D.Chapman et al Funding of university research [10(iv)]

This change in focus was symptomatic of a change in government policy from a policy for technology to a policy for science which has become an important element in government thinking.[11]

The problems facing the Research Councils were considerable. The Science Research Council (SRC) was committed to supporting long term collaborative big science projects and to implement these changes in an economic climate where rising costs were growing at a faster rate than the funds allocated to carry out that research. The SRC now called the Science and Engineering Research Council (SERC) responded to these pressures by redefining the criteria by which new projects were allocated these funds. The criteria that had been used by the 'peer review' system of timeliness and promise, selectivity and concentration, sophistication and threshold needs were broadened. Thus timeliness and promise now incorporated social and industrial considerations.[12] Faced with the need to protect the research base, to improve on existing equipment and the need to train new scientists, resources were to be deliberately concentrated on certain disciplines and on certain centres. Such a policy in turn required that the SRC panels responsible for allocating funds identify areas which would benefit 'national needs'. The traditional 'internalist' mechanisms by which scientists allocated funds to other scientists by a peer review system continued. Target areas were selected for priority funding and are listed in Table 1.1.above.

All policy makers face the problem of converting intentions into reality. Studies conducted by Farina and Gibbons[13] and Chapman, Farina and Gibbons[14] found that these areas generally received constant levels of funding over the decade they studied, with two exceptions, Polymer Science and Enzyme Chemistry and Technology. In general the

funding patterns continued to favour 'big science' projects in such areas as physics and astronomy at the expense of little science areas such as biology.

This thesis examines two other aspects of the policy, the targeting of research and the coupling of research findings to industrial needs. During the 80's targeting of research has been the subject of much investigation aspects of which will be discussed in chapter 3.

Recent studies such as those of Martin and Irvine[15] address two issues in science policy; the extent to which it is possible to exercise foresight in relation to strategic research and on the role that government should play in providing infrastructural support to industry. Important as these studies are to improving the efficiency of pure science research they ignore the question of how research findings that are the result of 'needs orientated' research are coupled to meet industrial needs and stimulate innovation and the process by which 'national needs' are selected.

1.2. Survey of innovation literature

An extensive literature exists on the process of technological innovation which embraces a number of different approaches from a number of different disciplines to a lot of the work that has been carried out which also focuses on different aspects of the innovation process, some analytical, others directed at proving or disproving a particular aspect of innovation theory, and some describing the process itself. The result of this research is the existence of many 'facts' about innovation which have had an impact on policy thinking but because of conceptual difficulties of relating abstract concepts such as knowledge or scientific

discovery to innovation, little progress has been made to produce a unified theory of the innovation process that can be used to predict outcomes. Progress in the area of innovation was slow because to begin with, economists sought to explain economic growth in terms of three production functions; land, labour and capital. Schumpeter who had begun to examine the economy from the perspective of the innovative process, worked ahead of his time since most of the economists considered innovation to be an exogenous influence on the growth of the economy.[16] His work was resurrected and reintroduced in the 1950's when economists were forced to reconsider the influence of R & D on the economy; a vast expenditure on R & D was having conspicuous effects in certain areas of the economy such as agriculture and must have been seen as having a purpose. Jewkes et al explain this neglect of innovation as being the result of three factors, ignorance of science and technology by the economists; a preoccupation with trade cycles and employment; a lack of data.[17] Initial work was concerned with directing the importance of technological change as a factor influencing economic growth at a macroscopic level of the whole economy. One approach is to start with the observed growth and work back. To economists concerned with analysis of an industrial economy in terms of three production functions this would require the subtraction of the effects of the capital labour and land functions from growth total. Solow[18] did precisely that, when he fitted production functions to available data on inputs and outputs for the USA economy between 1909 and 1949. He ignored the land function on the basis that in an industrial economy land does not restrict production. To everyone's surprise he found that only 12½% of the increase was attributable to the use of more capital and 87½% of the increase was due to the residual he called 'technical progress'. A more comprehensive and sophisticated

analysis was carried out by Denison.[19] He attempted to identify and eliminate other factors from the residual, such as education, the economies of scale etc. For the U.K. for the years between 1950 and 1962 the contribution to 'advances in knowledge' was estimated at 33.2%. Despite the sophistication, the end result is always a residual which is open to criticism on the grounds that it is bound to contain the effects of other factors.

The alternative approach is to start with some measurable aspect of the innovative process and to establish a link between it and economic growth. Various approaches have been tried.

Schumpeter's analysis focused on innovation. He carefully distinguished between invention, innovation and imitation distinctions which have become part and parcel of economic thinking. Schumpeter was concerned about the discontinuous nature of innovative activity, which he used as an explanation of business cycle theory. He considered the making of an invention and the corresponding act of invention were two entirely different things economically and socially. Thus inventive activity was an exogenous influence in Schumpeter's conceptualisation of the process.[20]

Jewkes et al.[21] examined a series of case studies on innovation and stressed the importance of the inventor as the innovator which seemingly supported Schumpeter's analysis. Studies on the relationship between invention and innovation as carried out by Enos[22] revealed that the process was by no means as straightforward as Schumpeter had assumed it to be. Short intervals of time between invention and innovation were found associated with mechanical inventions and longer periods of time were found between inventions and innovations in other areas.

There also appeared to be some interfirm differences.

Other studies focused their attention on different aspects of the process such as innovation and market structure (see the review by Kamien and Schwartz)[23] and innovation and the size of the firm (Mansfield)[24] Considerable analytical work has been carried out on the diffusion of innovations (see review by Oavis[25]). There are three basic approaches to the study of interindustry innovations. Interindustry comparisons pioneered by Griliches[26] and Mansfield[27]. Interfirm comparisons pioneered by Mansfield[28] and international comparisons such as the results of a study completed by a consortium of research institutes and presented as a final report by Nabseth and Ray.[29]

The first formal study of diffusion rates was obtained by Griliches (1957)[30] on the spread of hybrid corn across the corn growing belt of the U.S. Griliches found that the data he obtained on this diffusion fitted a logistic curve developed for the mathematical theory of epidemics and that diffusion could be related to economic forces in this case to the profit expectations shaped by market size. Mansfield (1968)[31] studied the rate of diffusion of 12 innovations in four industries and confirmed a symmetrical sigmoid curve as used by Griliches was the most appropriate way to describe spread of innovations in the medium and large firms in his analysis. Ray,[32] working at the National Institute of Economic and Social Research, also found that the rate of diffusions fitted a Sigmoid curve, but that the Gompertz curve (a positively skewed sigmoid curve) fitted this data better. Chow[33] and Stoneman[34] developed a stock adjustment model to analyse investment in computers and preferred the Gompertz curve

on the basis of its superior empirical performance. The developments have little in common with economic theory. An exception to this is the vintage growth model proposed by Salter[35] and colleagues, It assumes that an industry's stock of machines will comprise machines of different ages, and that the rate of innovation will be influenced by the age distribution of the machines, their profitability and the cost of the new equipment, and that when the costs of keeping the machine exceed the potential actions made by a new machine, then the old machine will be replaced. This work laid the foundation for further work. Schmookler[36] addressed not this issue but attempted to explain the pattern of inventive activity itself. Schmookler developed his ideas during a period of study of several American industries. He found a close correspondence between increases in demand and later increase in inventive activity of goods to satisfy that demand. Schmookler concluded that inventive activity could be incorporated into an economic framework.

Down and Mohr[37] have criticised such macroscopic studies in their inability to account for innovative behaviour in the real world because such studies obscure extensive variations that occur between elements of the innovation process.

During the 50's and 60's a number of other retrospective studies of technological innovation were carried out. In 1957 Carter and Williams[38] carried out an extensive study of innovative decisions of British firms for the Board of Trade. In a study of 152 firms the authors concluded that explicit considerations of market demand factors were used to make decisions concerning the level of investment in research and development.

Myers and Marquis[39] examined 567 innovations in five different industries to find empirical knowledge about the factors which stimulate or advance the application in the civilian economy, of scientific and technological findings. They concluded that recognition of a need was a more frequent factor in innovation than recognition of technological potential or recognition of scientific opportunity.

These and results of other studies[40] suggested that innovations did have a market component and were in fact the result of a market pull.

The first major retrospective study of this phenomenon was carried out by the United States Department of Defence (DoD). The purpose of the study was to measure the payoff to Defence of its investments and see if some patterns of management led more frequently than others to usable results. Project HINDSIGHT[41] examined the development of 20 weapons systems including Polaris, Minuteman missiles, C.141 aircraft and the Mark 46 torpedo over a 20 year period. The basic approach involved a cost benefit analysis, comparing the costs of performing a given military function with the current and preceding weapons systems at an equivalent level of effectiveness. Savings attributed to research expenditure were found to have been paid back many times over. Of the 710 problem events identified in this study only 9% were linked to basic research, and 91% were associated with technological events. Of the 9% of the basic research events however 97% were orientated towards 'needs' and only 3% arose from undirected scientific research i.e. 0.3% of the 710 sample. The impact of this study on science policy and funding was dramatic. New terms began to be used such as targeted research or mission initiated research. It led to an upsurge of contract sponsored research in many areas of science.

These results horrified the scientific community who questioned these findings on issues such as equating the 710 events with individual innovations, the preselection of the projects for analysis and the arbitrary 20 year cut off period used to trace back these events. The scientific community argued that this time period was probably too short to establish the scientific connections that related to technical breakthrough in areas such as electronics. The National Science Foundation (NSF) invited proposals for a study in which non-mission orientated research contributed to innovations of economic or social importance. Such a study was carried out by the Illinois Institute of Technology. The report published in 1968 called Technology in Retrospect and Critical Events in Science better known by its acronym TRACES[42] focused on the development of scientific knowledge in five innovations. It identified 341 research events of which 70% were non mission orientated, 20% were mission orientated and 10% were linked to development. All these events in turn could be linked to basic research. The TRACES study in turn came under criticism because the sample of five innovations was not considered to be a representative sample.

A fuller study was commissioned by the NSF to carry out a more extensive study of TRACES. This study, carried out by the Battelle Research Institute on the Interaction of Science and Technology in the Innovative Process[43], re-examined three of the five TRACES cases and considered 5 others, a total of 10 innovations. As was the case with TRACES events were divided into three classes, pure research, mission orientated and development. The Battelle study also distinguished between decisive and significant events. Significant events were judged to encapsulate an important development in history whilst decisive events were those without which the innovation could not have

occurred or would have been seriously delayed. 533 (83%) of events were judged to be significant and only 89 (17%) were judged to be decisive. Of the significant events 34% were non mission orientated, 38% were mission orientated and 26% were attributable to development. Of the decisive events 15% arose from non mission orientated research, 45% had mission oriented origins and 39% were development orientated. For all research and development 34% of events were associated with non mission orientated research, 38% with mission orientated research and 26% with development work.

The Battelle study also asked the question if innovations could be managed, concludingthat non mission orientated research could not be controlled or programmed. It also provided a list of key factors which affected innovation which included the recognition of technical opportunity; recognition of need; the presence of a technical entrepreneur; certain institutional factors such as internal R & D management, availability of funding etc; general external factors of a social, political or economic nature.

Despite these findings the controversy continued since all three approaches involved the selection of the case studies and were open to the criticism of bias. A study of the Top Ten Clinical Advances in Cardiovasculor Pulmonary Medicine and Surgery carried out by Comroe and Dripps[44] overcame this source of criticism in their analysis. The ten clinical advances were selected by a poll of 40 physicians. Comroe and Dripps proceeded to identify 663 key articles essential for the 10 major advances and of these 41.6 reported work whose goal at the time was unrelated to the clinical application. Analysed yet another way. 61.5% of the key articles described basic research, 20% reported

descriptive clinical investigations without any experimental work, 16.5% were concerned with the development of new apparatus or procedures and 2% were involved in the review or synthesis of earlier work.

67.4% of the key research was carried out in colleges, universities or medical schools, and associated hospitals. Important contributions came from all of the basic non-medical science disciplines such as botany, biology, chemistry, mathematics, physical chemistry, plant physiology, physics and zoology, as well as agriculture, dentistry, engineering, photography and veterinary medicine. In most cases there was a lag phase between discovery and its clinical application. These findings were dramatically opposed to those obtained by the 1966-7 Project Hindsight study. Comroe and Dripps also commented on the fact and that the studies on how to obtain new or improved military weapons have no relationship on how to accelerate biomedical research.

These retrospective studies indicate that the process of innovation is very complex and that neither science push nor demand pull models are representatives of the 'real world' situation.

All of these studies show that the process of innovation involves the synthesis or confluence of distinct areas of study. The Battelle study in particular concluded that the innovation (the process) cannot be fully controlled, but it felt that it had discerned four ways in which management could help it along. Firstly that the technical entrepreneur is important, perhaps the most important, driving force behind innovation. Secondly that recognition of a technical opportunity and recognition of the technical need were next in order of importance. Thirdly that funding was important and fourthly that the confluence of technology, unplanned in most instances was important.

In conclusion the report stated that it presented an opportunity for management by promoting interdisciplinary R & D teams to accelerate the innovative process.

These rather inconclusive findings encouraged further work in the process of innovation. Von Hippel[45] sampled 111 scientific instrument innovations in a what is considered to be a high technology industry found that approximately 8% of the innovations judged by users to be most useful were in fact invented and developed by users and that the producers in fact only provided the product engineering and manufacturing functions. Evidence for a symbiotic relationship between users and producers in that industry.

A great deal of statistical analysis has been carried out on the pattern of success and failure in industrial innovations by such projects as Scientific Activity Predictors from Patterns with Heuristic Origins SAPPHO[46] focusing on the management of innovation and the Falk Institute Project FIP[47] concerned with identifying comparative advantages of innovation. SAPPHO identified a number of variables that were most important in distinguishing successful innovations from failures. Amongst them were included a better understanding of user needs, more attention to marketing and publicity, development work was performed efficiently and drew on and made use of outside help in science and technology. Project personnel backing successful innovations were usually more senior and held greater authority. A painstaking analytical study of the innovation process carried out by Langrish et al (1972)[48] who examined 84 innovations which received the Queen's award for technological innovation in 1966 and 1967. For 51 of the 84 innovations the major technical ideas or concepts were identified, in an

effort to study the sources of and transmission channels for knowledge used in the innovation process. The authors concluded that innovations had multiple origins, on the basis of these findings the authors rejected the linear flow of ideas from science to technology in favour of a model where a two way flow of ideas and resources takes place. Sociologists have also contributed extensively to our knowledge about innovation. Studies by Cotgrove [49] have drawn sharp destinations between the norms and values shared by scientists engaged in pure and applied research. Marquis and Allen[50] were struck by the important role played by individuals they called technological gatekeepers whom colleagues turn to for technical advice and comment.

Baker and Sweeny[51] postulate that differences in the pattern of innovation over time in the U.S. may be attributable to the changing pattern of the management of the process of innovation, frequently referring to Rosenbloom's seminal paper.[52] They suggest that the way the R & D activity is structured determines which view of innovation will be predominant within an organisation. During the 1945-55 growth period for R & D, R & D departments were organised to fit into the existing structure as a corporate laboratory. This separation reinforced the phase dominant view of the organised process of innovation and the perpetuation of the linear progression of ideas from research through development to engineering scale up and production. Rosenbloom proposed a different model of organised innovation associated with the corporate concept of project management. Technological change is represented as a social process made up of three phases of tasks, idea generation, problem solving and implementation. This model of the process will be discussed in greater detail in chapter 9.

Although this all too brief survey of the literature on innovation is far from complete it should be sufficient to establish the following points.

- It is impossible to make generalisations about innovation. Different industries show markedly different patterns of innovation and in the contribution made by pure science, applied research and development.[53]
- Combinations of factors are exceedingly important in innovation, and the combination of these factors will vary with time and circumstances.[54]
- Market factors do influence innovations, and provide a selecting environment for their diffusion.[55]
- Much of the information gathered to date about the process of innovation is of a factual kind with very few analytical links between research projects such as SAPPHO, TRACES, the diffusion studies of Griliches or Mansfield or those of Jewkes, Sawers and Stillerman.[56]

It would be unwise on academic grounds to use a theoretical framework developed in another area of innovation and apply it to my specific research situation. Although the observations and findings of these various initiatives should be borne in mind.

1.3. The research problem refined

The interpretation of 'national needs' by the SRC's and SERC's committees was made deliberately wide, to accommodate social, industrial and scientific criteria in the selection of projects and research areas. In this thesis I shall restrict my analysis to two aspects

of that policy :

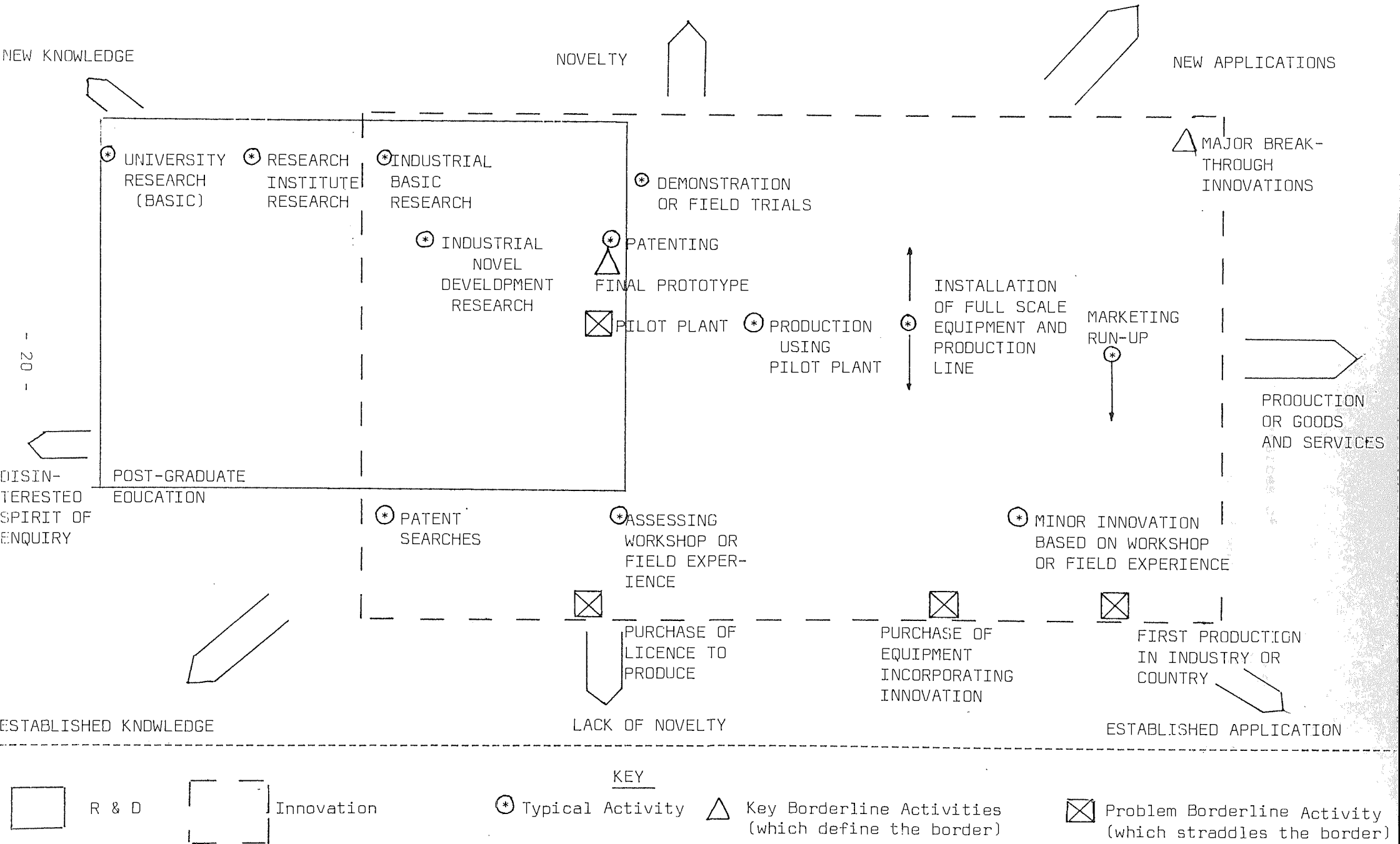
1. The selection of 'industrial needs' areas for research
2. The coupling of the products of public funded research to meet those needs.

I propose to restrict my analysis further by examining only a narrow sector of the U.K. science policy that dealing with Enzyme Chemistry and Technology.[57] I propose to evaluate that policy in terms of meeting national (in my case industrial) needs by comparing the intentions of that policy with its outcomes and to analyse the reasons for its success or failure.

My choice of this subject area has been made for the following reasons :

1. I have some expertise in this area from previous courses of study
2. Enzyme Chemistry and Technology were selected by the SRC as areas of science worthy of special encouragement in the belief that their development could lead to a new era in pure and applied chemistry.[58]
3. Enzyme chemistry and technology received increases in the proportion of resources but had the lowest level of concentration of funds of any of the committees shown.[59]
4. Enzymes and enzyme systems continued to feature as elements in the U.K's research policy for biotechnology.[60]

Figure 1.1. The Science and Technology Enterprise[63]



5. The presence of accessible sources of information on aspects of enzymes and enzyme technology.[61]

6. Access to statements on aspects of science policy relating to Enzyme Chemistry and Technology.[62]

7. The time available to carry out the research and analysis.

8. The development of a suitable algorithm for the analysis of the problem.

1.4. Research Methodology

1.4.1. Introduction

In this section I shall set out the limits of the project and discuss the method of research and analysis.

1.4.2. The project's boundaries and method of analysis

There is no logical boundary to the project to speak of, since its aim is to examine the flow of knowledge uniting scientific research, applied research technology and the commercial use of enzymes. Effectively the project crosses traditional boundaries between several distinct areas of academic study. The OECD Directorate for Science, Technology and Industry have used the extended business column approach to map this area and plotted representative activities on different stages of the process such as patenting, marketing run up, minor innovations based on workshop experience etc. (See Fig.1.1.[63]) They have identified four dominant factors that interact with one another and which represent different elements of the process i.e.

knowledge, novelty, applications and motive. They acknowledge the complexity of the process by calling this the map of the STI minefield. However firms do not innovate into a vacuum, nor do they exist as the only enterprise but as clusters of companies trading within a particular sector, exploiting different niches within the commercial environment. Changing that environment in any way will improve the lot of certain firms at the expense of others. Thus in effect it is important to consider all the commercial organisations within an industrial sector to evaluate the effects that a change in policy will have on the industrial firms and that business sector as a whole.

Despite my reduction of the problem to a small area of science and redefinition of the policy problem into an evaluation of its effectiveness it still remains too complex to analyse by opinion analysis alone, since use of opinion analysis techniques are likely to generate a very wide range of alternative views and opinions as to what were the policy failures or successes, because each opinion will depend on the perspective adopted by the person interviewed, their vested interests and their experiences. Academically each opinion would be equally valid under the terms and conditions of such an analysis and lead nowhere. The complex nature of the problem also precludes the use of any one analytical technique, (c.f. the innovation literature) and the situation of the problem within a socio-political framework, effectively precludes the use of experimentation on my part. With so many avenues of research closed the only remaining course of action is to model both the policy and the science to industry coupling process.

Thus a model of the 'real world' can be used to test a past policy initiative , to evaluate its outcomes and if it proves to be reasonably accurate, it can be used to examine more recent policies and predict their outcomes in turn.

According to modelling theory it is possible to construct a number of different classes of models. Models may be mental models identified through personal action, or verbal models which are implicit in the speech or writing. They may be physical models, such as maps or three dimensional constructions, Other models may be mathematical, or dynamic physical models such as simulations or games.

In this thesis I propose to develop two dimensional diagram models of the innovative process in an industrial system since they are clearer and easier to understand and compare, they show sequences of activities well and are able to accommodate simultaneous relationships and interactions.

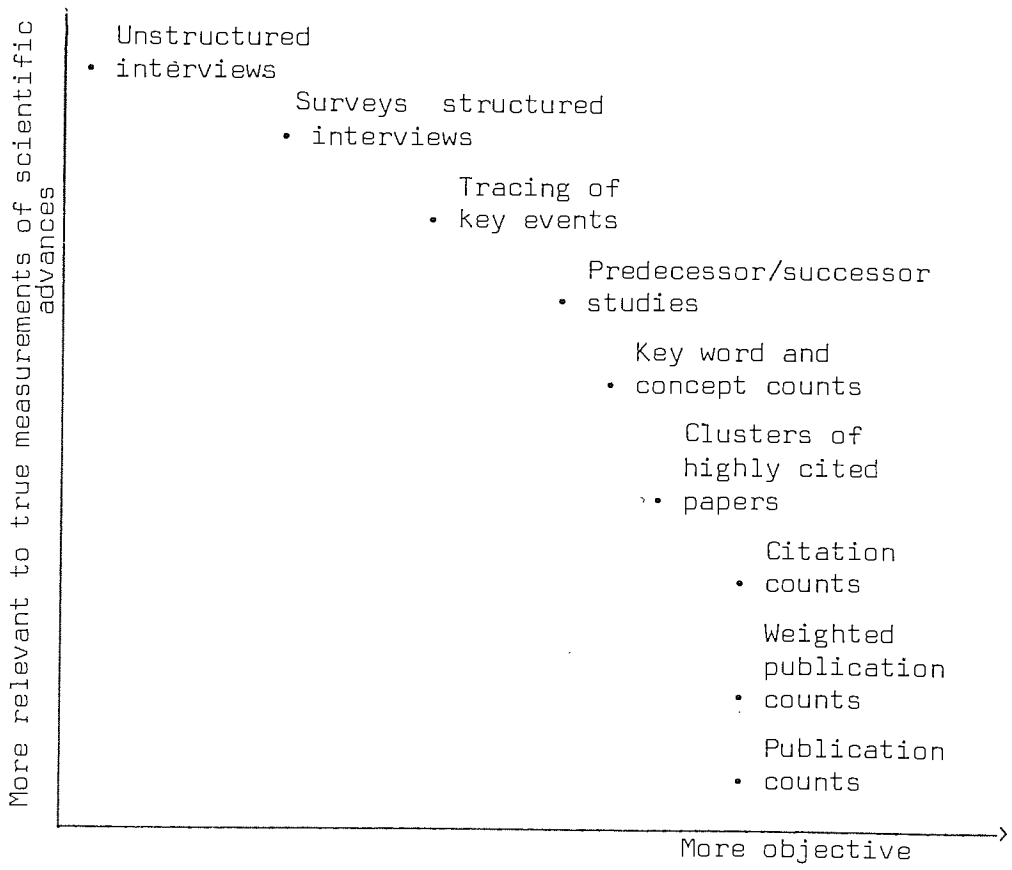
Despite their value diagram models suffer from a number of limitations. They are essentially static and cannot be used to compare a number of different situations without a lot of effort involved in translating each situation into another model. Neither can they be used easily to make precise predictions of the effects of changes acting on the different variables. To be able to do so I would need to develop a mathematical model of the process, and thus be able to work out a complex problem with various changes acting on the inter-dependent steps and conditions. This in effect becomes another research and analytical problem for which this thesis lays the foundations.

From the outset the quality of the analysis rests very much on

the quality of the model, which in turn depends on the ability of the model to represent a wide number of different situations which link enzyme science to the industrial needs. The model should represent as best as possible the different components of the science technology enterprise. I therefore propose to develop the model by recourse to several different forms of analysis.

Each technique has its advantages and disadvantages and makes a slightly different contribution about the coupling of scientific knowledge about enzymes with industrial requirements. The different analytical techniques also vary in the level of objectivity and the level of the personal bias that may influence the analysis. Narin[64] has described the tradeoffs between objectivity and relevance and his representation of the merits of the different techniques are shown in Fig.1.2.

Fig.1.2. Techniques for measuring the advance of a science or technology (F.Narin, Scientometrics 1(1) 30, 1978).



For a discussion of the merits of each approach see the Analysis of Scientific Disciplines Germane to the technology C.1.4. Final Report by H. Rothman. March 1984.[65]

I have divided the research problem into three tasks. The first task is to identify links between scientific knowledge and their industrial application in sufficient detail to be able to model the process. I propose to develop the model of enzyme innovation by recourse to these four different forms of analysis :

1. Qualitative methods involving literature surveys and interviews.
2. Quantitative methods of publication counts.
3. Retrospective analyses of enzyme innovations.
4. Case studies of four different enzyme innovations.

The second task involves the examination of U.K. SRC and SERC policy to develop two dimensional diagram models of the innovative process implicit from statements and reports of the various enzyme committees.

Thirdly to compare the two models and predict outcomes for the science policy in question and make recommendations for its improvement, and evaluate current policy statements in terms of that model.

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CHAPTER 2 : ENZYME SCIENCE, TECHNOLOGY AND APPLICATIONS

2.1. Introduction

Enzyme technology is concerned with the production, purification, immobilisation and commercial applications of enzymes. Many of the recent discussions of this technology have emphasised its "high-tech" aspects.[1] In general, different aspects of the technology have been developed by two distinct groups of companies, those concerned with the production of enzymes as catalysts and those interested in their use. Enzyme technology has been applied in a number of areas, which currently span industrial, healthcare and research situations. It is a complex business sector, in which technological, economic, social and political factors operate, and where interactions determine the shape of this technology today.

This chapter aims to acquaint the reader with the current state of development of the technology; its use; to examine how the various factors that influence enzyme production and use operate, and to identify the various companies that trade in enzymes and make this technology.

Information about enzymes can be found in most textbooks in biochemistry. The work by Dixon, The Enzymes[3], is a useful but dated source. More wide ranging but nevertheless excellent reviews are found as Topics in Enzyme and Fermentation Biotechnology[28] now in 10 volumes, and Handbook of Enzyme Biotechnology Edit. by Alan Wiseman.[27] Excellent reviews of the application of enzymes in industry are found in Industrial Enzymology, by Godfrey and Reichelt.[4] Other useful reviews are found in the Encyclopedia of Chemical Tech-

nology.[20] Commercial aspects of biotechnology are discussed in Enzymes; the interface between Technology and Economics, Edit.Danehy and Wolnak.[25] For reference to Japanese enzyme technology the book Immobilised Enzymes, Research and Development, Edit.by Chibata[24] is very useful.

During the course of this work I have also used unstructured interviews during my research, to evaluate and to corroborate findings.

2.2. What are Enzymes?

In 1877 Kuhn coined the word 'enzyme' from the Greek 'in yeast' in order to emphasise their extracellular role as the agents responsible for bringing about hydrolytic reactions. In so doing he sought to distinguish the enzymes' roles as specific catalysts from the general processes of fermentation that are carried out by the cell.[2]

Put simply, enzymes are protein catalysts capable of altering the rate of a chemical reaction, whilst remaining unchanged upon the reaction's completion. They do not need to be in a high state of purity to achieve this, and small quantities of enzyme can act on and change a large quantity of material. Enzymes operate under a fairly narrow range of defined parameters, within which they show a high affinity for their substrate. As protein molecules they have a complex three-dimensional structure, usually distinguished from other proteins by possessing a marked cleft in which lies their catalytic or 'active site'. The substrate on which the enzyme operates is induced into this site in a specific stearic fit, so precise as to be analogous to the way in which a key may fit a lock. This degree of precision partially explains why enzymes can work selectively on one substrate when presented with a choice

of many. Once an enzyme-substrate complex is formed it lasts only long enough to allow the reacting groups to interact. The complex then breaks down to release the reactants as products and liberates the enzyme; thus freed, the enzyme is available to repeat the process.[3]

Enzymes are useful as industrial catalysts because they are non-polluting, and biodegradable. They operate in conditions of mild pH usually 4-8, relatively low temperature (10°-80°C) and at normal atmospheric pressure.[4] They may be produced at relatively low cost in virtually unlimited quantities, and hold out the potential for re-use and extension of their operating range through immobilisation. So enzyme users reap the benefits of energy savings and reduced process costs. Their adoption also makes savings possible in fixed capital costs of equipment as enzyme-catalysed processes operate under milder conditions of pH, temperature and pressure than their chemical counterparts.

Enzyme utilising processes have their own special problems. Their complex protein structure, so vital for their function, is also the cause of their vulnerability. It makes enzymes susceptible to denaturation and inhibition even by slight alterations of their physical environment or mild forms of chemical change. Enzymes are also susceptible to a range of chemical poisons, and therefore, require for their use a pure substrate and clean handling conditions. Many enzymes are only active in the presence of specific metal ions, which may need to be supplied if the reaction is to proceed efficiently.

Such requirements vary from enzyme to enzyme, and need to be determined for each enzyme individually.

Other problems with enzymes relate to the difficulties of producing

them in a form suitably for assay. This is made more complex for enzymes derived from animal sources where several enzymes catalysing the same reaction can occur. They have similar molecular weights but differ in their amino acid compositions. These iso-enzymes, as they are called, vary considerably in their properties such as their pH and temperature optima, and their reaction kinetics.[5] The polymorphous state of such enzymes enables similar reactions to proceed under the different conditions as found in different organs of the body.

Many enzymes also require cofactors; thermostable, dialysable, non-protein molecules which facilitate the transfer of groups of chemicals which participate in the enzyme catalysed reaction. Co-factors act as chemical carriers, which facilitate the transfer of a particular chemical group to another enzyme which removes the chemical group, and regenerates the carrier. Without these 'carriers', or co-enzymes a large number of enzyme catalysed reactions would be unable to proceed. Since cofactors are generally costly to produce and need to be regenerated by another reaction, cofactor regeneration has been a bottleneck in the commercial application of enzyme catalysts to industrial processes. These difficulties do not stop here, for upon being 'purified' most extracellular enzymes have poor keeping qualities. Some require metal ions to enhance enzyme stability; however, not all metal ions are useful since some bring about the degradation of the catalysts.[6] It is common practice to add suitable salts to stabilise commercial enzyme preparations. Alternatively, immobilisation may sometimes be used to stabilise the enzyme; however, this is always accompanied by some loss of enzyme activity.[7]

Although these are broad generalisations as to the range of operating

conditions, there are no clearcut rules. For the use of specific enzyme catalysts each individual case requires careful analysis of the conditions and process trade-offs.

The potential for enzymes to be used as catalysts is large, over 2000 enzymes have been classified to date. Enzymes are named and classified according to the system developed by the International Union of Pure and Applied Chemistry (IUPAC) and the International Union of Biochemistry (IUB). This classification uses three principles to arrive at a unique identification code for each enzyme. First that single enzymes should end in the suffix 'ase' and enzyme systems containing more than one enzyme be clearly identified as such. Second, enzymes should be classified and named according to the reaction they catalyse. Third, that enzymes should be divided into groups on the basis of the reaction type they catalyse. Before this system was agreed, an earlier 'trivial' classification was in general use, so it was suggested that the two names exist side by side; as the IUPAC-IUB name is generally quite long, the shorter trivial name is frequently preferred for general use (e.g. maltase for -D-Glucosidase).

The four code numbers are allocated according to the class, sub-class, sub-sub-class, and serial number in the sub-sub-class respectively. There are six main classes:

E.C.1 - Oxido-reductases
E.C.2 - Transferases
E.C.3 - Hydrolases
E.C.4 - Lyases
E.C.5 - Isomerases
E.C.6 - Ligases (synthetases)

For further guidance the reader is recommended to examine the latest volume on Enzyme Nomenclature.[8]

Yet despite the fact that some 2000 enzymes have been identified, only about 150 have found a commercial application. In addition to these there are about 400 others that have been identified recently and are now available for use within the rapidly expanded field of genetic engineering, i.e. restriction endonucleases, ligases and editing enzymes. Calculations based on theoretically possible amino-acid configurations for proteins with molecular weights in the enzyme range suggests that up to 10^{1300} different combinations of amino-acids are possible. Naturally, only some of these are going to have the spatial configurations that will give them catalytic properties. It therefore appears likely that many more enzymes still remain to be discovered. Natural materials are no longer the only source of potential new enzymes discoveries as the synthesis or semisynthesis of enzyme analogues opens up another dimension which may provide new enzyme catalysts in the future.[9]

2.3. A Brief History of Enzyme Use

Use of enzyme-mediated processes can be traced to ancient civilisations, who have recorded their ability to use plant and animal extracts or micro-organisms to bring about what they considered were desired and useful changes in goods. Although many of the skills were originally domestic, some became separated and were developed and practised as specialist crafts. Knowledge of these processes was invariably empirical. It included aspects of the processing of foods (such as leavening bread, the brewing of beers, the fermentation of wines and vinegar), as well as the treatment of hides during their processing into leather.

In all these processes centralised methods of production enabled considerable benefits to be reaped from the economies of scale, and were partly responsible for their evolution from cottage to centralised town-based crafts. Often empirically devised processes failed when attempts were made to scale them up into larger volume processes, so although production became centralised, small batch processing practices persisted because of the high incidence of large batch failures.

Because these failures in processing were an obstacle to processing they became the focus for scientific investigation and the study of fermentation as a science began to emerge. With it came the understanding that fermentation was a cellular process, involving agents of cellular origin. The active components were identified and collected together under the term enzyme, as I have already mentioned.

The demonstration of enzyme activity associated with successful isolation or partial purification was often followed by a specific application of the enzyme. Many examples of these early uses are still with us today. The first enzymes to be so used invariably belonged to the group now classified as hydrolases. Their early application can probably be attributed partly to the fact that hydrolases were the active components in those processes where plant or animal extracts or micro-organisms were already in use, and partly to the fact that many of these enzymes functioned well outside the cell, i.e. were already 'stabilized' to work in an extracellular aqueous environment. This made them relatively easy to prepare and use, and enabled purified enzymes to displace the empirically derived crude enzyme extracts. (See Table 2.1.). These developments were pioneered by individual entrepreneurs who identified a market need and developed

an enzyme to supply it, e.g. Christian Hansen in 1874, produced a standardised rennet, Otto Rohm in 1917 developed an improved leather bate and Leo Wallerstien in the 1930's developed an enzymic method for chill-proofing beer.

Table 2.1. Some processes in which enzymes have replaced other industrial methods

Process	Method Substituted
1. Brewing	Enzymes used to supplement some of the enzymes in malt.
2. Cheese Making	(i) Animal rennets replace the use of plant extract and bacteria used for clotting milk (ii) Microbial rennets substitute for animal rennets.
3. Leather Bating	Enzymes replace the action of dog and bird faecal extracts.
4. Meat Tenderizing	Enzymes supplement the action of natural cathepsins.
5. Starch Conversion	Enzymes replace acid hydrolysis.
6. Textile Desizing	Enzymes replace acids as means of removing starch from fibres which in turn replaced the soaking of fabrics in stagnent ponds (the 'rotten steep').

Enzymes from microbial sources freed the enzyme user from problems of limited or seasonal supplies by making enzymes available in bulk. In addition they expanded the product range available for use.

Early pioneers in the developments were Takamine[11] in the U.S. and Calmette and Boidin[12] in France; Takamine transferred and refined the solid 'koji' fermentation techniques from Japan to produce crude fungal amylase enzymes. Initial attempts to introduce the use

of these starch-digesting enzymes 'takadiastases' in the production of potable alcohol were unsuccessful but these enzymes later became adopted for the desizing of woven cotton cloth. Calmette and Boidin in 1891 developed a method of cultivation of the fungus Amylomyces, to produce amylases used for the production of alcohol. The Amylo process flourised on the continent for many years.[13] Boidin and Effront pioneered the use of bacteria for enzyme production, and developed a film plate reactor for this purpose. They were among the first to produce enzymes from bacteria on a commercial scale.

New uses began to emerge in which the unique properties of enzymes enabled them to work simply, precisely and effectively. By way of an example I cite the development of the modern fruit juice industry. In the 1930's German and American merchandisers began to clarify fruit juice using pectinases. From this beginning a highly technological series of industrial applications has emerged allowing treatment of apples, stone fruits, citrus fruits, grapes, etc. Developments in processing have led to an increasing specialisation of pectin-digesting enzymes and also the application of amylases and cellulases to the processes. These enzyme developments were an important component of the techno-economic process which enabled an enormous growth in the world fruit juice market.[14]

The development of enzymic clarification of beer provides an example of an existing enzyme product, a papain, being put to a new use. When beer is chilled a protein-tannin complex is formed which makes the beer cloudy, pre-treatment of the beer with papain prevents this and allows a clear cold beer to be marketed.[15]

The next major development in enzyme technology was the adoption of the deep fermentation techniques for microbial enzymes production.[16] The techniques were developed during the Second World War for large scale production of the broad spectrum antibiotic penicillin. Vat culture enabled large amounts of microbial cells to be produced, cells which in turn were used for the production of enzymes. As the development of scientific knowledge progressed in the 1950's and 60's a new understanding of the microbial genetics and of the microbes environment made enzyme production more predictable (e.g. induction, genetic deregulation and control), so enzyme production was gradually transferred from an empirical craft into a technology with a sound scientific and engineering basis.

In medicine the availability of animal digestive enzyme preparations opened the way for treating those individuals who suffered from enzyme deficiencies. An early pioneer in this development was Boudalt who produced 'pepsin' which was sold as a digestive aid. Other applications followed: enzymes were used to clean wounds, to lyse blood clots, and as anti-inflammatory agents or in anti-cancer treatments. The practice of using enzymes in diagnosis either to provide simple evidence of tissue change, or as constituents of reagents, began in the 1960's and has been growing rapidly. By combining immobilised enzymes with spectrophotometers, flurometers and microcalorimeters, automation of various chemical analyses has been achieved.[17]

The use of 'enzyme electrodes' that are highly specific for biochemicals has been advancing steadily since their first reported creation in 1966 by Updike and Hicks.[18] Using different enzymes, these electrodes can be linked up to a variety of metered displays that

measure current, voltage or resistance, and which are used for the quantitative determination of a range of substrates, e.g. glucose, urea, amino acids and alcohols. A large number of such electrodes have been produced. Other developments are also possible, e.g. ATP-utilising enzyme-catalysed reactions can be monitored by chemi-luminescent means, or some reactions can be monitored by thermometric devices which can measure the temperature output of certain enzyme-catalysed reactions.

2.4. Recent applications

Today, enzymes have five distinct areas of applications:

i. As scientific research tools. (Here enzymes are often produced in small amounts, and, if available, are distributed by specialist scientific supplies. I do not propose to discuss these further.)

ii. For cosmetic uses.

iii. For diagnostic purposes.

iv. For therapeutic use.

v. As industrial catalysts.

Breakdowns of their major uses by area and type, in 1982, in Tables 2.2. and 2.3.

Table 2.2. The present state of utilisation of specific enzymes by their area of use (19)

I.U.B. Classes	Classified	Cosmetics	Diagnostics	Therapy	Industry	Total*
1. Oxidoreductases	575	1	26	6	11	38
2. Transferases	572	0	8	2	4	12
3. Hydrolases	577	3	15	35	36	75
4. Lyases	231	0	8	6	8	20
5. Isomerases	96	0	2	0	2	4
6. Ligases	87	0	0	0	2	2
	2138	4	59	49	63	151

* The totals have been adjusted to eliminate double counting.

Table 2.3. The types of applications which enzymes are currently being put to

Region of Application	Sector	Specific Areas of Use with enzyme examples
i. Analytical Research & Genetic Engineering		Most enzymes available from suppliers at a relatively high cost, for unit activity.
ii. Cosmetic		a - dental hygiene (e.g. dextranase) b - skin preparations (proteases)
iii. Diagnostics		a - blood glucose (glucose oxidase) b - urea (urease) c - blood/urine alcohol d - cholesterol (cholesterol oxidase) e - blood triglycerides (lipase) f - blood CO_2 carbonic anhydrase g - urine steroids p-Glucoronidase h - EMIT & ELISA systems EMIT - Enzyme Multiplied Immunoassay technique ELISA -Enzyme-Linked Immunosorbent Assay i - enzyme electrodes
iv. Therapy		a - anti-thrombosis agents (e.g. streptodornase) b - digestive aids (pepsin) c - anti-tumor treatments d - poison ivy treatments e - wound cleaning (trypsin) f - anti-inflammatory (super oxide dismutase) g - hypotension control (Kininogenase) i - anti-bacterial
v. Industrial	1. Food & Food	a - brewing and wine-making b - baking c - dairy products d - fruit juice production (pectinase) e - extraction of other plant products (pectinase) f - production of protein hydrolysates (pepsin) g - modification of toxic or unwelcome food components (melibiase) h - starch modifications (amylases) i - antioxidants or glucose removal (glucose oxidase) j - flavourings k - leather (proteases) l - sugar and confectionery (invertase) m - production of modified fats (lipeses)
	2. Chemicals	a - detergent formulation (subtlisin) b - paper making (amylases) c - fuel alcohol (amylases) d - lacquer production (phenolperoxidase) e - amino acid synthesis (proteases) f - inhouse modification of pharmaceuticals (ergosteroloxidase) g - immobilised enzymes used to obtain inhibitors
	3. Textiles	a - desizing cotton (proteases) b - degumming silk
	4. Waste Treatments	a - reclaiming wastes b - improving waste treatment management

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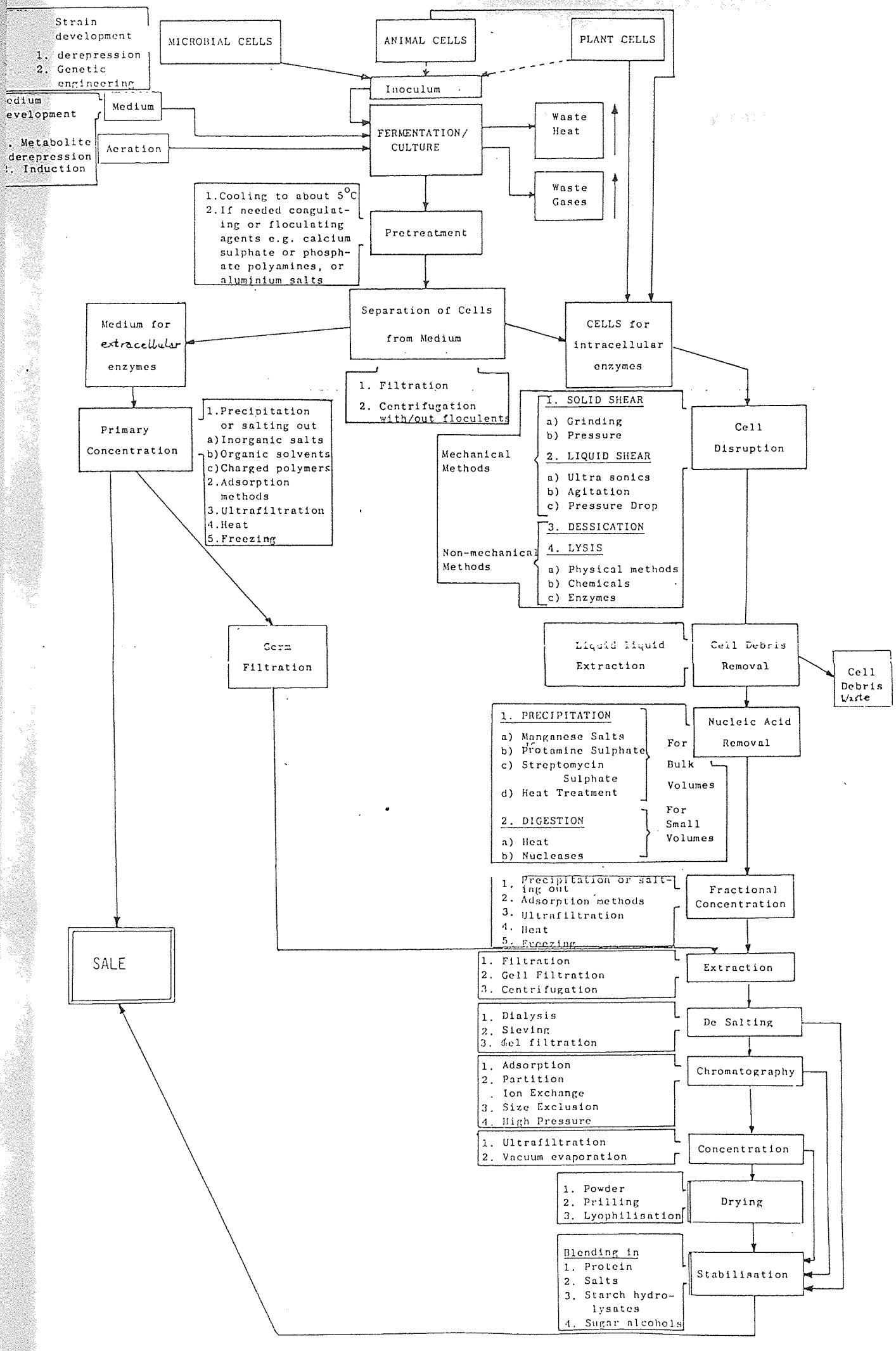
2.5. The Production of Enzymes

As it is an altogether too complex a task to chemically synthesise enzymes commercially *in vitro*, all the enzymes that are currently in use are obtained from living sources, between them they account for all of the enzymes that are traded in the world. The sources are animal organs, plant tissues, or microbes. The suppliers of enzymes have been increasingly dependent on microbial sources for their supplies. Loosely classified, enzymes fall into two categories, extracellular enzymes that are secreted by the cells into the environment, and the intracellular enzymes which catalyse the many complex reactions within the cell itself. Extracellular enzymes are easier to obtain and produce. I have already mentioned that they are stabilised to work in the extracellular environment. With animal or plant sources these enzymes are obtained by disrupting the cells that produce them, releasing the enzyme and their precursors. Enzyme precursors may need to be activated. If the enzymes are produced by microbes then they are secreted into the culture medium. The microbial cells are then separated from the medium and the medium is processed for any extracellular enzymes it contains.

These extracts have relatively low levels of enzyme activity, as they are very dilute, and they may contain a lot of impurities. It is therefore desirable to purify the enzyme. This raises the level of catalytic activity and reduces the level of contaminants for the user but increases the cost of the enzyme preparation. Purification begins with a primary concentration stage. This involves the use of empirically derived techniques to separate the enzyme either by precipitation of the enzyme or the precipitation and subsequent removal of the undesired materials, leaving the enzyme in solution. Some of these techniques

FIG.2.1. A SUMMARY IN FLOW DIAGRAM FORM OF THE STAGES IN THE PRODUCTION OF PURIFIED ENZYMES



are listed in Fig.2.1. Separation in either case is usually carried out by filtration or centrifugation. If salts have been used to precipitate the enzyme then the precipitated enzymes are redissolved and the enzyme solution is desalted. This extract is then made available for sale. For some purposes further purification may be desirable. On a commercial scale this stage often involves the use of chromatography. Further concentration increases the activity per unit volume. Drying this concentrate results in a dusty powder. The keeping qualities of the enzymes are improved by the process of stabilisation which involves the blending of proteins, salts, starch hydrolysates or sugar alcohols, with either the liquid or powder preparations.

The production of intracellular enzymes is similar to that of extracellular enzymes once the enzymes have been isolated. However, the extraction process for intracellular enzymes differs initially because of the need to disrupt the cells and 'free' the enzymes they contain. The ease with which cells may be broken varies, animal tissues are relatively easy to burst and small bacterial cells are the most difficult. There are a variety of methods available for disruption of cells. See Fig.2.1. If the enzymes remain attached to cell fragments, surfactants may be used to get them into solution. Since nucleic acids interfere with separation procedures there is also a need to separate them out from the cell debris. Nucleic acids may account for as much as 30% of the cellular matter of some microbes. There are two ways their separation can be achieved; either by precipitation or digestion. It is the need for these two additional processing stages - cell disruption and nucleic acid separation - which makes intracellular enzymes more costly to produce. Fig. 2.2. shows the various stages in

the production of both extra and intracellular enzymes. I shall now examine the factors that limit the production of enzymes on a commercial scale.

2.6. Factors influencing the production of enzymes

Although there may be other factors that influence enzyme production I shall restrict my discussion to five factors that in my opinion have had a major impact on commercial enzyme production; biological, economic, technological, commercial and social factors.

2.6.1. Biological factors

Enzymes are obtained from three sources: animals, plants, and microbes. Climate, soil structure, regional, national and international agricultural policies and social traditions interact and determine the type of the animals reared and crops that are produced. There in turn define which type of enzymes are available for processing since they are derived from available animal and plant sources.

Enzymes derived from animals are usually by-products of carcases slaughtered primarily for meat. Their supply still tends to be seasonal, and their availability may depend on the animal supply, the types available, and the slaughtering policy, itself determined in part by the demand for, and price of, meat. This source of enzyme supply is finite, since a growing demand for an enzyme by-product is unlikely to be met by slaughtering more animals. A rise in demand for cheese led to an increase in the demand for animal rennets to a point that demand outstripped supply. In the search for alternatives, microbial rennet sources were developed.

Enzymes from plant sources are available in potentially larger quantities. However, these enzymes have drawbacks, in that they are usually heavily contaminated because of the methods used to collect them. Also attempts to purify them are often thwarted by an accompanying loss in the enzyme's stability. The extraction of plant enzymes is therefore restricted to simple processing stages and this limits their commercial usefulness

These limitations do not apply to enzymes that can be obtained from microbes. Enzymes derived from microbial sources depend on sterilised batch culture for their production and are thus less susceptible to both the constraints of supply and contamination. As there is a wider range of microbes than can supply enzymes, microbial enzymes can be matched to individual processes more closely. This more than compensates for complex processing techniques used to produce enzymes from microbial sources. The cloning of animal protein genes in microbes has been difficult since they have different editing systems for the production of proteins. Recently genetic engineering has opened the way for the production of animal or plant enzymes by the use of genetically modified yeasts. The majority of the microbial enzymes with industrial uses are obtained directly from 36 species of filamentous fungi, 5 species of yeasts and 12 species of bacteria, and the economies of the various processes being what they are it is likely that the numbers of the enzymes will continue to grow.[20]

2.6.2. Economic factors

It is in the interests of both producers and users that the overall cost of the production of enzymes be kept as low as

possible. For enzymes obtained from animal or plant sources, some of the costs of production are borne by the farmer. The enzyme producer buys in these crude sources and incurs only development and running costs in the extraction and purification of the enzyme product. Economies of scale can be obtained by increasing the volume of production with animal, plant and microbial sources. However, microbial sources offer the enzyme producer another potential advantage - that of controlling the basic supply source of the enzyme.

Microbial enzyme production costs are sensitive to a range of variables such as; the choice of organism, the specific conditions necessary for enzyme production, the costs of the fermentation feedstocks, and the energy costs of the production process. The microbial enzyme producer seeks savings by attempting to minimise such costs. Although the choice or organisms for sources of enzymes seem infinite, in practice various factors narrow down the choice, e.g. if the enzyme is intended for food processing only certain microbial types can be employed because of consumer safety requirements. Amongst other factors that will influence organism choice will be the physical conditions in which an enzyme is intended to operate with respect to temperature and pH. Choice of organism will also affect the time the fermentation is likely to take to make the enzymes, and hence influence operational costs. Very roughly, bacteria have mean doubling times of 45 minutes, yeasts 90 minutes, moulds 180 minutes and protozoa 360 minutes, and so the faster growing bacteria and moulds are the preferred sources of microbial enzymes, all other things being equal.

2.6.3. Technological factors

There are two methods of microbe cultivation in general use: the semi-solid tray culture system and the submerged liquid culture system. The former is more labour-intensive but has lower capital costs. It is especially successful for cultivating filamentous fungi. Semi-solid fermentations suffer from problems of contamination, as the growth medium in this form is difficult to sterilise. However, the infections are usually localised and become swamped by the cultivated microbe. Aeration is good, so a very wide variety of enzymes can be made. Submerged liquid cultures in order to achieve high enzyme yields on the other hand need to be completely aseptic, and prevent the introduction of toxin-producing microbes; hence they are expensive to run and install. The need to maintain a single organism in the culture set a high standard both for production practice and for plant hygiene. The process may need to be protected by sterile barriers and in order to reduce human error it is preferable that much of the process is run automatically. Culture techniques for the production of enzymes, require a range of different optimal production conditions which need to be determined for each enzyme. A move away from narrowly defined conditions can influence the enzyme yield dramatically. Improvements in enzyme yields are achieved by medium manipulation and the genetic improvement of the culture strains.

Storage smoothes out the inequalities between the enzyme supply and the market demand. Enzymes become denatured and become less active with time, so to allow for prolonged storage they need

to be stabilised. The use of preservatives as enzyme stabilisers for example for food processing preparations, is complicated by the fact that such preservatives must satisfy the 'food additive' requirements. Use of brine is permissible and very effective, but other agents such as benzoates or sorbates may also be used. Usually immobilising enzymes will improve their 'keeping qualities' and this will be dealt with more fully in the section on the factors that influence the enzymes' operational use. Little is known of the underlying principles that affect enhanced enzyme stability and much of the techniques currently in use have been developed empirically.

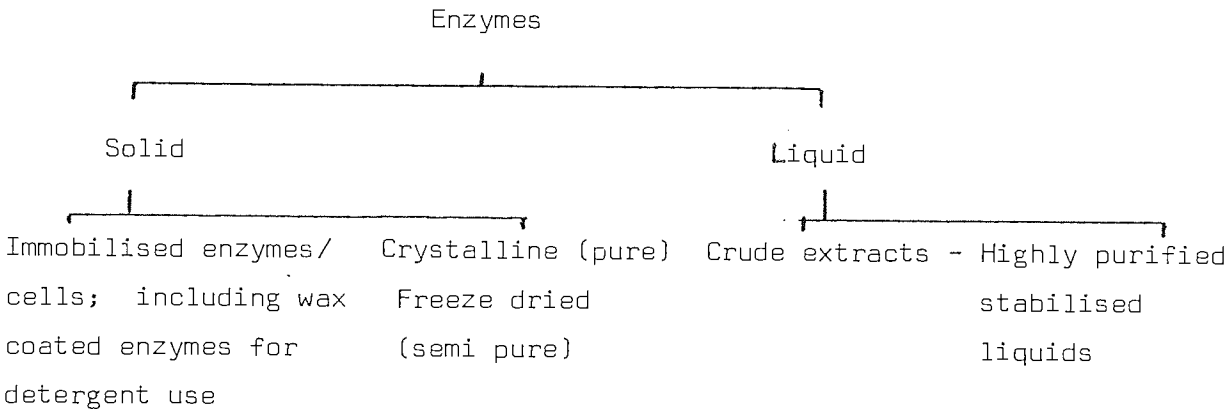


Fig.2.2 Types of enzyme preparations available today

2.6.4. Commercial factors

Enzyme applications vary according to the enzyme purity they require, thus one finds a range of purities of enzyme preparations on the market. Some preparations are crude mixtures of many enzymes

and are sold as such, e.g. diastase or pancreatin. Where enzymes are purified producers generally have to offer two or more grades of the enzyme product which have differing levels of activity. The blending of enzyme preparations to meet customer needs is also practised.

Generally enzyme producers provide an extensive technical backup to enzyme users, who need to be convinced that the additional costs of using an enzyme catalyst system are more than offset by a net saving of using the enzyme in the process.

It is at this level that difficulties are likely to be encountered as enzymes are bought and sold in units based on enzyme activity. These units of activity are defined in terms of some process or test that they serve. Many units are often assayed on a poorly defined substrate, and under arbitrary conditions. Over time a large number of different measures of activity have been developed for both similar and different enzymes.

This lack of uniform standards at the commercial level creates the need for close technical collaboration between the enzyme suppliers and enzyme users. The enzyme manufacturer supplies much of this technical information and recommends an enzyme dosage to the product user as part of the service. This is effectively a measure of the enzyme's productivity for the process in terms of mass of product per unit mass of enzyme, under the defined operating conditions.[21] This information is needed by an enzyme user before they can engineer and develop any enzyme system.

2.6.5. Social factors

New enzymes for food use need to be approved by the government bodies charged with the evaluation of such new additives. Enzymes for use in food preparation have to be thoroughly tested to ensure that they are safe for use so the final costs of producing enzymes for sale may be even higher. Extensive toxicological tests need to be carried out to satisfy these official bodies that the enzymes are safe. Such tests are not only expensive but are time-consuming and may last for 2-5 years. Consequently, it is cheaper to develop enzymes for food use from organisms that are generally regarded as safe than to develop them from new and unproven sources.

I have already stated that enzymes are proteins. This property of enzymes also presents another difficulty for if foreign proteins periodically enter the body of humans they may trigger an allergy reaction in certain individuals. The chances of contact are increased if enzymes are produced as dried powders. So that in the routine control of enzyme products it is necessary not only to cover the enzyme's performance parameters, and levels of contaminants in dried granulated preparations, but levels of the protein dust need to be monitored also. In addition to protecting the labour force, it may be necessary to protect the general public if they are likely to come into contact with enzyme dusts. The industry accepts that the use of dry enzyme dusts is best avoided. The level of risk of sensitisation of enzymes are considerably reduced if the enzymes are handled in a liquid or in a coated or encapsulated form.

Generally solutions of purified enzymes are preferred for general processing, whilst crude or semi-purified extracts suffice for the treatment of effluents. Partially purified preparations will satisfy most analytical purposes since the enzyme has a high affinity for the substrate, whilst de-dusted, encapsulated spray-dried powders or prills are available for consumer products, such as biological detergents. Medical uses generally require that the enzyme be purified as crystalline highly purified freeze-dried solids, but generally in this area of application more care is taken over their use in any case.

2.7. Factors that influence enzyme use

The way enzymes are used depends upon the specific enzyme reaction and the general process needs. Choice of an enzyme depends on the way an enzyme's characteristics match those operating needs. There are many different uses of enzyme catalysts and there are different ways of using enzymes as catalysts. These range from simply adding the enzyme in a soluble form to more complex systems, where the enzyme is insolubilised by immobilising it onto a carrier. Soluble enzymes may be :

(i) mixed with the substrate in vivo, e.g. injected into beef animals prior to killing;

(ii) mixed with the substrate and not recovered e.g. in the fondant preparations for soft centre chocolates; or

(iii) more often added to a holding vessel, tank or reactor, e.g. in the production of cheese curd from milk, or as the pre-soak formulation of

washing powder preparations in laundries. In such vessels mixing improves the overall reaction kinetics.

Reactor configurations are varied and scale-up is accompanied by an increase in mechanical complexity.

In all the three methods mentioned above the enzyme is lost after the process reaction is complete. Sometimes, therefore, it is necessary to introduce a further production stage to denature the enzyme once it has completed its task.

The immobilisation of enzymes overcomes this problem of enzyme loss and is an important development in extending the application of enzymatic processes for industrial use by reducing enzyme costs.[22] Immobilised systems:

(i) enable the cost of the enzymic catalyst to be brought down by making it re-usable;

(ii) open up the way to continuous processing with its corresponding benefits;

(iii) enhance the possibility of maintaining high catalyst concentrations to achieve fast reaction rates.

The necessity for cofactors affects the ease with which enzymes can be utilised in industrial processes. Amongst those enzymes most used industrially - hydrolases, isomerases and oxidases - only the isomerases do not require co-factors. Hydrolases require water to bring about their reactions and fortunately so many reactions are carried out in water so they are simple to use. Oxidase-using

processes require oxygen, peroxide or cofactors such as NAD. The commercial development of cofactor-requiring enzyme systems has been delayed by the initial cost of preparing cofactors and subsequent difficulties concerning the regeneration of some of them. Several research groups have successfully regenerated cofactors. Degussa A G has recently introduced a system converting keto acids into amino acids by a dehydrogenase enzyme and NADH. The NADH is regenerated by enzyme action.[23] Compromise solutions offered by purification and co-immobilisation of cofactor and enzyme together result in processes that have low efficiencies and low product yields. Research and development of enzyme systems that are capable of using electrons directly given a source of an electric current promises to extend the range of enzyme oxido-reductases still further.

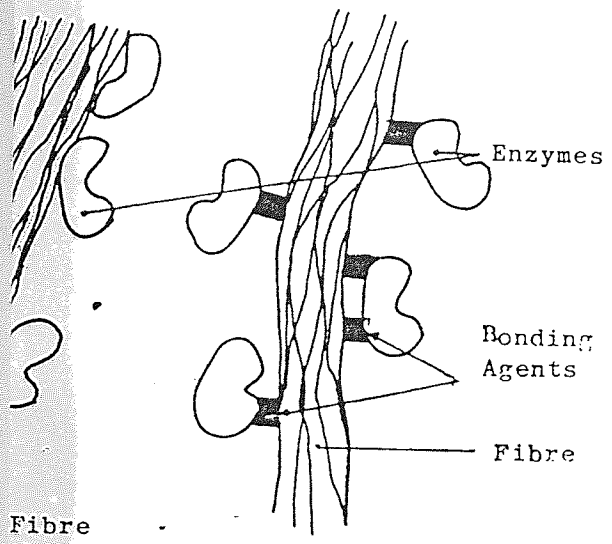
Another way out of this dilemma is to use whole cells rather than purified enzymes and cofactors. This overcomes some problems since cells contain both enzymes and cofactors together, and within them cofactors are being regenerated continuously. Whole cells prove attractive commercially as they obviate the need for enzyme extraction and purification and the need to develop systems for cofactor regeneration. There is little evidence to suggest that cellular systems are more stabilised[24], but they are well integrated within a system (the cell), their group working life is prolonged, and so continuous production becomes appropriate.

Where successive enzyme reactions (multi-step reactions) are required immobilised whole cells offer a considerable advantage over immobilised enzyme systems, particularly if within the cell the enzymes are spatially related to one another, e.g. are found together on membranes in the cell.

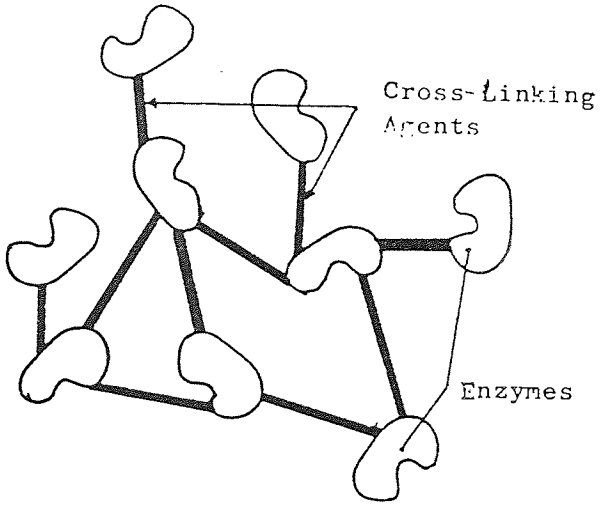
Fig 2·3 Diagrams to show the various methods available for the immobilisation of enzymes

CARRIER BINDING

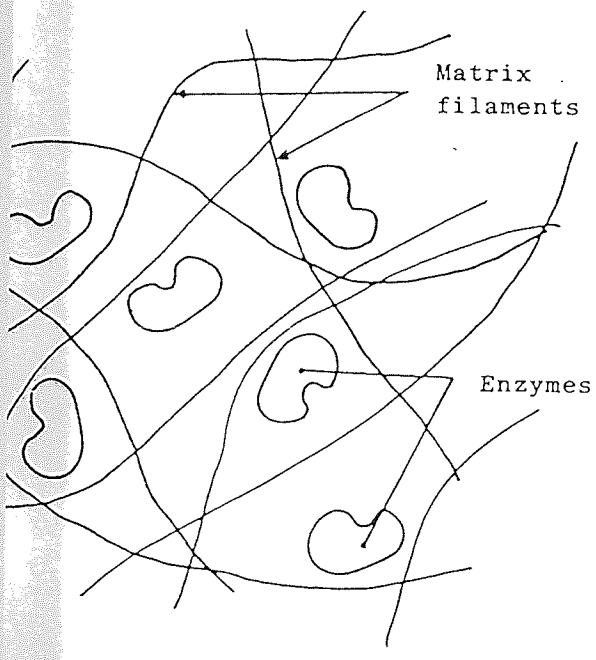
:al Adsorption b) Chemical Bonding
:arrier onto carrier



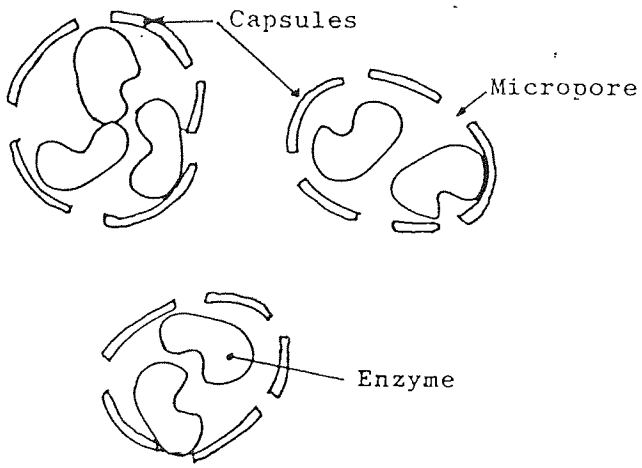
CROSS-LINKING



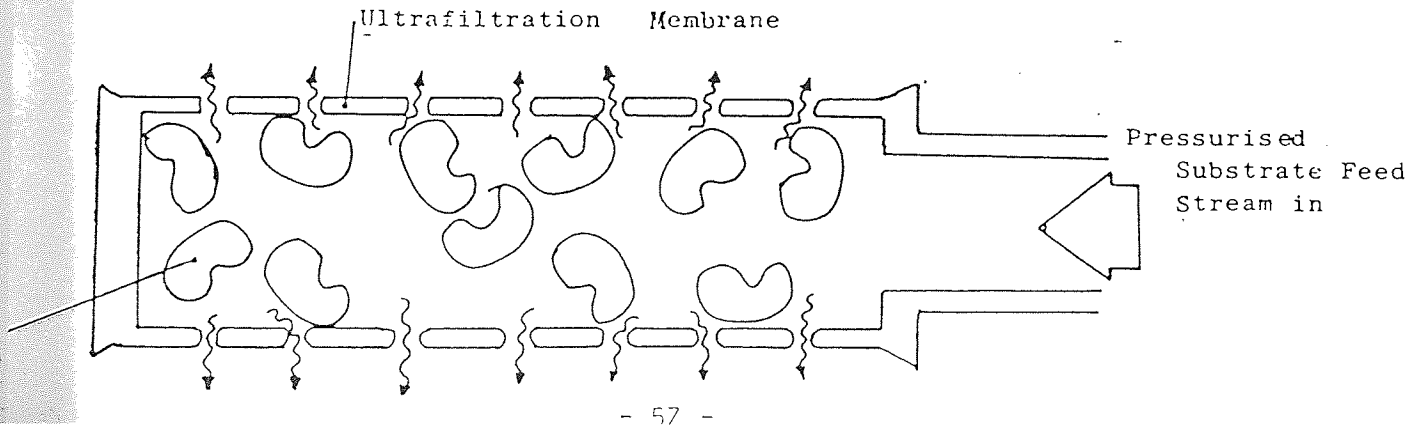
ENTRAPMENT



MICRO ENCAPSULATION



ULTRA FILTRATION



Immobilisation of cells offers considerable savings in the initial processing stages, thus allowing small product runs to become a commercial possibility. For many industrial processes, immobilisation of cells provides a more cost-effective answer than the use of immobilised enzymes.

The main disadvantages of using immobilised cells is that they often carry out unwanted side reactions. The need to prevent such unwanted enzymatic side reactions also destroys much of the advantage of using cells. It is best to use inert immobilisation supports for cells since immobilisation using harsh chemicals or high temperatures has a bad effect on their catalytic activities, either by the creation of rate-limiting diffusional barriers around the cell, or by increasing the cell's preferential affinity for the reaction products; catalytic activity may even be lost completely. Hybrid systems using immobilised enzymes and whole cells have been used in the production of gluconic acid. In this case organisms are cultivated on a glucose substrate and gradually become inactivated by the hydrogen peroxide that is produced. However, the inactivated cells contain enzymes that continue to convert large amounts of substrate into gluconic acid and hydrogen peroxide. This method of gluconic acid production is more economic than any that makes use of an immobilised enzyme system.

Enzymes can be immobilised in a number of ways, by the use of a wide range of carriers or immobilisation systems, ultrafiltration membranes, within a matrix or gel, by physical absorption or ionic bonding, or covalent bonding onto a carrier or by cross-linking with or without bonding onto a carrier. (See Fig. 2.3)

In general there are three types of industrial systems in which enzymes are used; batch processing with soluble enzymes, batch

TABLE 2.1: SOME FACTORS THAT INFLUENCE A CHOICE OF AN ENZYME REACTOR

ENZYME CHARACTERISTICS	SYSTEM . TYPE		
	Batch Processing/ Soluble Enzyme	Batch Processing / Immobilised Enzyme	Column Reactor / Immobilised Enzyme
1. High enzyme costs	Expensive	Reduces enzyme costs per unit operation	Reduces enzyme costs per unit operation
2. Low enzyme costs	Suitable	Not worth considering	Not worth considering
3. Ability to re-use the enzyme	None	Possible	Possible
4. Enzyme stability	Low	Moderate - high losses through attri tion	High
5. Enzyme kinetics most suited	Low reaction rates	Low reaction rates	High reaction rates
PROCESS CHARACTERISTICS			
1. Relative ease in controlling the enzyme reaction conditions e.g. temperature and pH	Easy	Easy	Difficult ; and high heat transfer may be a major problem.
2. Suitability for processing viscous or particulate substrates	High	Moderate	Low
3. Product yields	Generally low but higher for products that undergo substrate inhibition.	Generally low but higher for products that undergo substrate inhibition	High but useless for enzymes that undergo substrate inhibition
4. Product Purity	Low	High	High
5. Capital Costs	Low	Moderate	High
6. Labour Costs	High	Moderate	Low
7. Potential for Automation	High	High	Moderate
8. Relative Ease of Implementing Automation	Difficult	Difficult	Easy

processing with immobilised enzyme and column reactors with immobilised enzymes. The decision on which systems to use often rests on two sets of operational characteristics, those that are determined by the enzyme and those that are determined by the process. The enzyme characteristics that will influence choice include the cost of the enzyme, the ability to reuse the enzyme in the process, the enzyme's stability, and speed of the catalysed reaction. Process characteristics that will influence choice of reactor include: the ease with which the reaction conditions can be controlled; the suitability of the substrate for that type of processing; the product yields; the purity of both the substrate and the product; the absolute and relative costs of labour and capital; and the ease of automating this process. Table 2.4. summarises these characteristics for the three types of system. From an engineering point of view immobilisation of enzymes allows a variety of continuous process configurations to be used. These include continuous Stirred Tank Reactors and Fluidised Bed Reactors which, for the sake of brevity we have excluded from our discussions. Differences between the immobilised enzyme catalyst also affect the decision.

It should be stressed that none of these systems possesses any absolute advantage over the others. Choices between these systems are made after the careful evaluation of the process and the enzyme characteristics, etc. New developments will alter the decisions, e.g. fluidised bed enzyme systems have been studied in recent years but are the least studied so it is likely that they would require considerably more development if they were to be adopted commercially. There are no universally efficient formula for the design of an optimum enzyme reactor system and each system generally needs to be tailor-made. Considerable use still has to be made of empirical and experimental data during the design and

scale up of such a process. It should be stressed that a high level of care needs to be taken in choosing an enzyme catalyst to ensure that it meets the technical and commercial requirements of the process.

2.8. Enzyme Producing Companies

A number of companies trade within the enzyme market, each company offering some special product and or service to a distinct product sector.

First and foremost is the group of 10 or so enzyme producers who sell enzyme-containing plant and animal materials, or who culture their own microbes as enzyme sources. Some of these producers, particularly those who grow microbes, process these materials to extract the desired enzyme and offer this product for sale.

Second is the group of enzyme extractors and purifiers who purchase crude or commercial grade materials. The picture is further clouded by the practice amongst established enzyme producers of buying in crude enzyme preparations from one another as a means of developing their own product range, as a way of extending the service they can offer to new or existing customers.

Third are the enzyme factors or dealers who work as a marketing outlet for a parent or associated company that has its production facility based abroad. Others may have a licence or franchise to sell enzymes from a major producer who does not have a direct outlet to the market. This practice is popular because enzymes are highly reactive catalysts that are compact, low volume products which are cheap to transport. As a corollary the siting of the production facility need not be in the midst of its market.

Often any one company may be engaged in more than one of these operations, for very different enzymes, however the larger, more established companies are less likely to be involved with factoring.

I have already shown that the market for enzymes is very diverse (see Table 2.3.). Entry into it has been achieved successfully in the past from a number of different starting points.

Some companies were already making use of enzymes empirically before the science of enzymes had evolved, some traded as producers of crude complex products using enzymes, e.g. the maltsters. They supplied the brewing industry with malted grain and the enzymes contained within it. Most maltsters remained traditional concerns and only a few made the transition into the wider field of enzyme production, e.g. Associated British Maltsters (ABM).

Another point of entry into enzyme production was as a result of the growth and rationalisation of the meat packing industry particularly in the U.S., where the production of crude enzyme extracts became a valued by-product of the process and developed as such. One company has become an enzyme producer by making specialist pharmaceutical grade products from such sources, and includes in its range the gastric enzyme pepsin.

Many companies have come into existence by pioneering the development of new commercial enzyme sources or by making use of existing enzymes or cater for the needs of a new existing market. In the former category are found Takamine Laboratories (U.S.) and Grindestedverket, (Netherlands), in the latter, Wallerstein (U.S.) and Biocon (Eire).

Another strategy of companies entering this industry has been to start up as enzyme retailers and/or blenders, establish a market through

successful marketing and then if need be enter into enzyme production proper.

Many companies producing enzymes today have become producers by company acquisition. Takamine has been taken over by Miles (U.S.) which in turn was acquired by Bayer (West Germany). A.B.M. (U.K.) acquired the British Diamalt (U.K.) Co. and Norman and Evans and Rais Ltd. (U.K.), and was itself taken over by Dalgety (Australia) where they now operate as a distinct division within this larger organisation. Wallerstein was taken over by Truvenol.

There is a case for companies that use enzymes in large quantities, to opt for supplying them in-house, e.g. Anheuser Busch's (U.S.) in-house development of malting enzymes, for a captive flour milling market.

The development of fermentation technology within the pharmaceutical field prompted pharmaceutical-based companies to enter new markets, since many were faced with over-capacity in antibiotics. Novo Industri N.V. (Denmark) and Gist-Brocades A.S.(Netherlands) and Glaxo had extensive fermentation knowhow, which they applied successfully to the production of microbial enzymes. Today Novo and Gist Brocades are world leaders in the commercial production of bulk enzymes, expansion coinciding with the development of washing powder enzymes, glucose isomerases and starch processing enzymes.

The potential for developing immobilised enzyme systems and immobilised enzyme carriers has prompted the entry of chemical companies into this field. A specialist glass manufacturer, Corning Glass Works Co.(U.S.) has been particularly successful in developing porous glass carriers and immobilised enzyme systems for industrial use.

No single enzyme producer manufactures the complete range of enzymes nor does any one manufacturer hold a monopoly on any enzyme that enjoys a high volume of sales. However, two companies supply over 60% of the world's industrial enzyme production. Even so neither of these produces enzymes as their only stock in trade. Invariably enzymes form part of an extensive and diversified product range. These idiosyncracies of the suppliers and the market are best understood in terms of their past history along with current trends, developments and growth of the companies that now trade within this sector area. In the past enzymes have not been the type of products that have generated vast returns; and with few exceptions required that the producer, in order to survive and grow, either diversify the product range or enter another field of business. This latter strategy seems to have been adopted by such companies as Chr.Hansen's Laboratorium (Denmark) and Rohm and Hass GmbH (West Germany). Christian Hansen's Laboratorium produces a number of preparations for the dairy and food industry, e.g. vegetable food colours such as annatto yellow, wax and plastic emulsions for coating cheese, and cultures of bacteria for specific cheese flavour production, Rohm and Hass split their business interests by trading in seemingly unrelated areas, so that the production of enzymes became an autonomous division of what is now a well-diversified chemicals and plastics producing company.

Since historically enzyme producers have been companies that have served a certain industrial sector, they have developed an intimate knowledge and understanding of that sector's needs and requirements. We have already indicated that trade in enzymes is also affected by the quality of the technical back-up. As a consequence it is difficult for new producers lacking established links to penetrate this market on the basis of price alone.

For an extensive list of enzyme producers the reader is referred to Data Index 2 of Industrial Enzymology (see Ref.411). But what of the future? - firms using biotechnology have been increasingly attractive to take over, and recently a number have been acquired in this way. The companies that have been involved in these dealings have been large multinational corporations taking over smaller specialist concerns. It is likely that this trend will continue and further take-overs will take place in industrial, medical, therapeutic and scientific fields, of both enzyme producers and enzyme users.

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CHAPTER 3 : ENZYME SCIENCE, ENZYME TECHNOLOGY AND INDUSTRY ANALYSED USING QUANTITATIVE INDICATORS

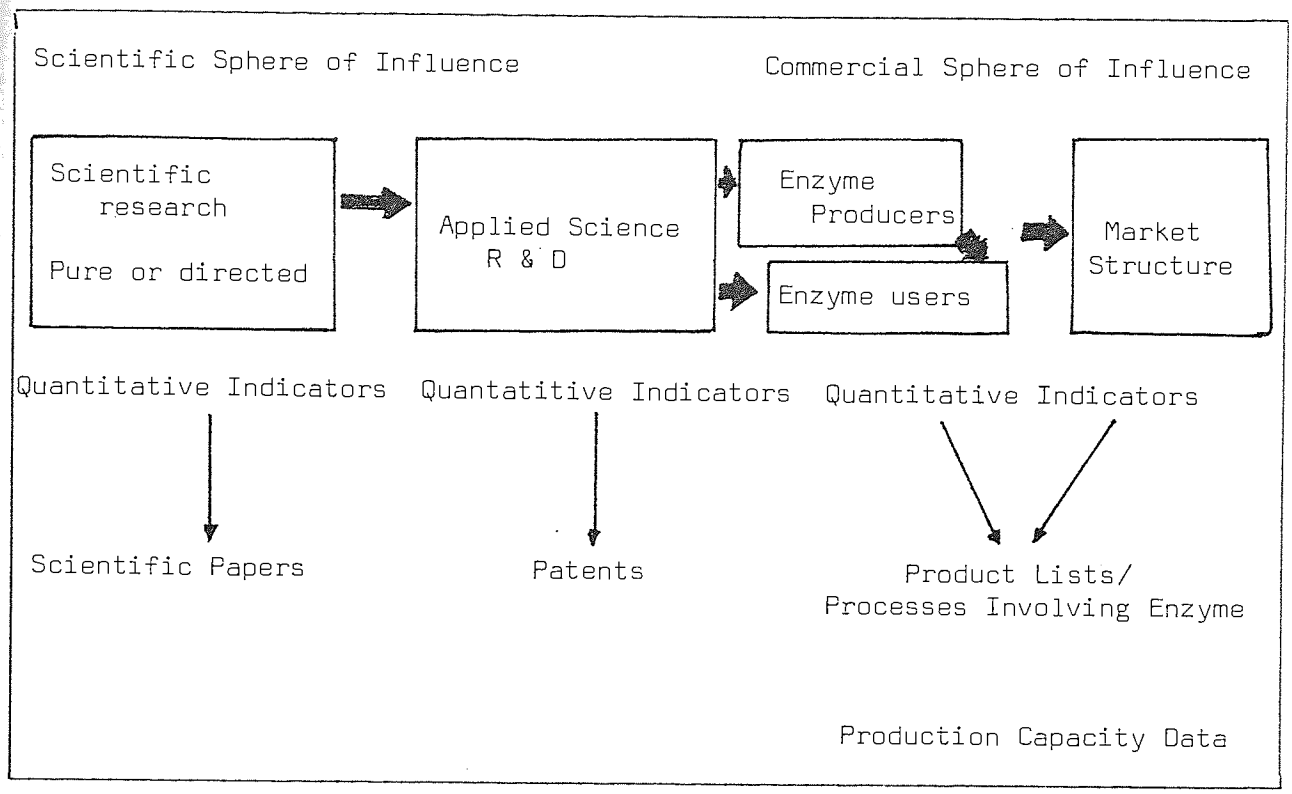
3.1. Introduction

The aim of this chapter is to identify general trends and development within the science technology enterprise by quantitative methods of analysis of published information. In chapter 1, I discussed how most studies of innovation concentrate on the input side of innovations and a few outputs such as patents, knowhow and income and how the information from these studies was balkanised. Consequently I elected to analyse a number of different aspects of the Enzyme Science Technology Enterprise by drawing on several sources of information. However time constraints restricted this section to an analysis of just four sources of data :

1. Scientific publications
2. Patents
3. Company information
4. Market information.

As shown in Figure 3.0[1] and in Figure 1.1. they provide information on different aspects of the innovative process which links scientific knowledge to industrial needs.

Fig. 3.0. The Simplest Translational Process Model of Science into Technology



3.2. Analysis of scientific publications

3.2.1. Introduction

The techniques for measuring the outputs and advances in research and development, see the reviews by Rothman, Rothman et al and Irvine and Martin.[2] I shall discuss results obtained by two of the three main techniques of publication analysis paper counting and co-word analysis, since I have access to sources of that type of data. For a discussion of cocitation techniques used see the reviews mentioned above.

3.2.2. The Productivity of Scientific Research

I have already stated in chapter 1 section 1 that scientific literature has been growing exponentially. Within this, the

FIGURE 3.1. COMPARISON OF THE ENZYMES KNOWN TO SCIENCE AND THOSE IN COMMERCIAL USE

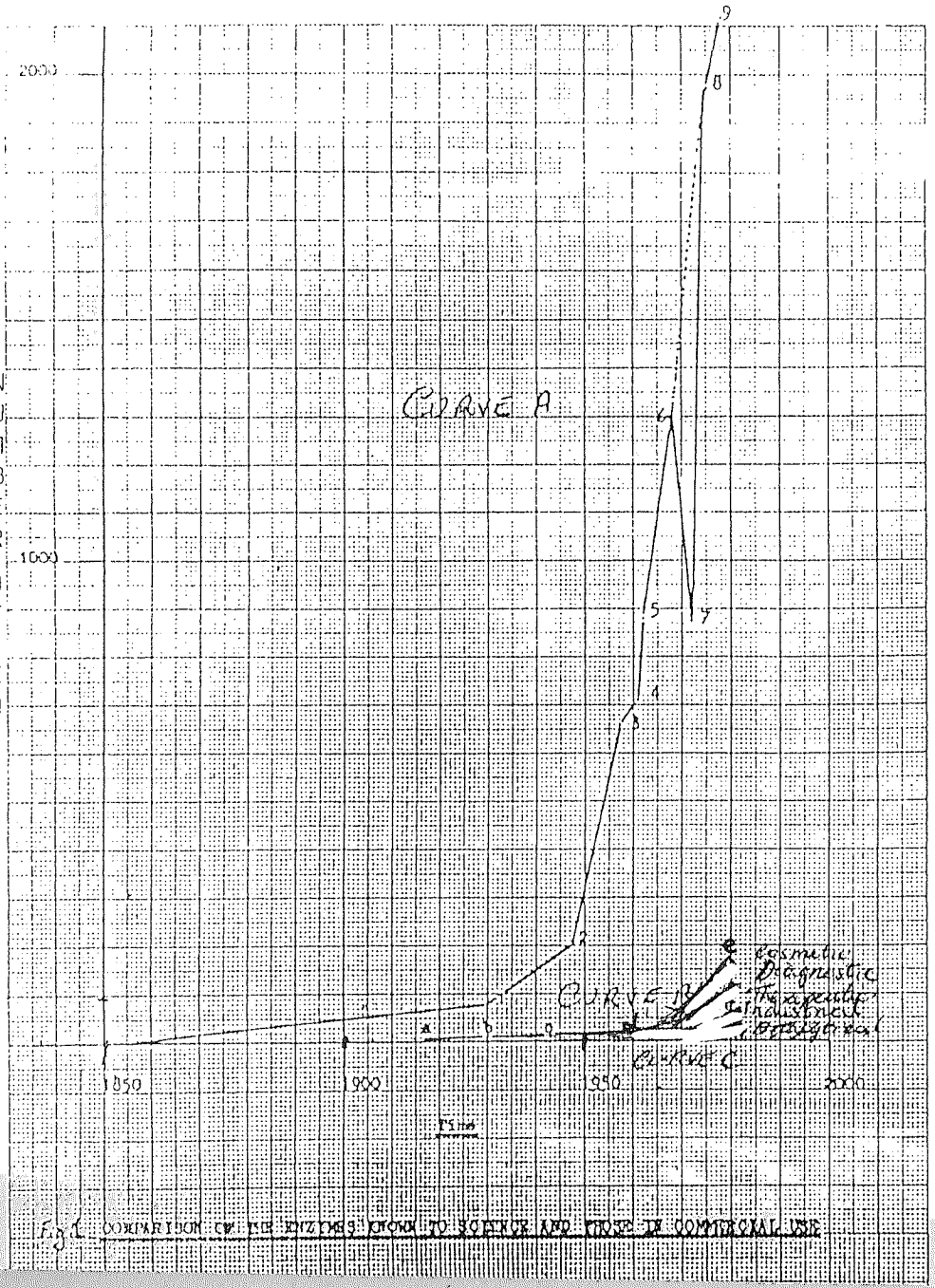


Fig 1. COMPARISON OF THE ENZYMES KNOWN TO SCIENCE AND THOSE IN COMMERCIAL USE

SOURCES OF DATA FOR THE COMPARISON OF THE ENZYMES KNOWN TO SCIENCE AND THOSE IN COMMERCIAL PRODUCTION AND USE.

Numbers of Enzymes known to science.

1.J.B.S. Haldane , The Enzymes , 1930 (80).

2.J.B.Sumner and G.F.Sommers ,Chemistry and Methods of Enzymes, 1947 (200).

3.M.Dixon and E.C.Webb, Enzymes ,1957 (660).

4.Report of the Enzyme Commission, 1961 (712).

5.M.Dixon and E.C.Webb, Enzymes ,1962 (880).

6.T.E.Barman, Enzyme Handbook I, 1969 (1300).

7.Report on Enzyme Nomenclature , 1972 (875).

8.Report on Enzyme Nomenclature plus Supplement I , 1975 (1974).

9.Report on Enzyme Nomenclature ,1978 (2122).

Numbers of Enzymes in Commercial Production.

a. J.Effront and S.C.Prescott, Biochemical Catalysts in Life and Industry, 1917

b.H.F.Smyth and W.L.Obold ,Industrial Microbiology, 1930

c.H.Tauber , Enzyme Technology , 1946

d.D.Perlman ,Fermentation Industries...Quo Vadis?,Chem.Tech 7(7) 434-43, 1977.

Z. Towalski

e.Survey of Industrial Enzymes Carried out at Aston University, 1980.

literature concerned with biochemistry has been growing most quickly, but Garfield has observed that the explosive growth in biochemistry that occurred in the 1970's has slowed somewhat during the past decade.[3] Hence it would be useful to obtain a measure of the rate of growth of enzyme science, a component of the biochemistry literature subset. This is difficult to do directly. However indirect measures can be found such as the rate of discovery of new enzymes.

To carry out a count of every single enzyme and to trace it back is extremely tedious, since this information is scattered over a good many scientific and medical journals. I have therefore opted to use two methods to obtain information about the rate of discovery of enzymes,

1. To count numbers of enzymes listed in academic publications reviewing the field.
2. To analyse the 3859 references to enzymes used by the Nomenclature Committee of the I.U.B./I.U.P.A.C. work in their Review on Enzyme Nomenclature of 1978.

The results of method 1 are presented as Curve A, Fig.3.1. The results using method 2 are plotted as Fig.3.2.

It is interesting that both methods indicate a drop in the number of enzyme discoveries around 1970-1975, and that the number of enzyme discoveries appears to continue to fall. On Fig.3.1. I have also plotted the increase in the number of enzymes that have found some commercial application using sources which reviewed the commercial applications of enzymes. (c.f. Table 2.2. in chapter 2).

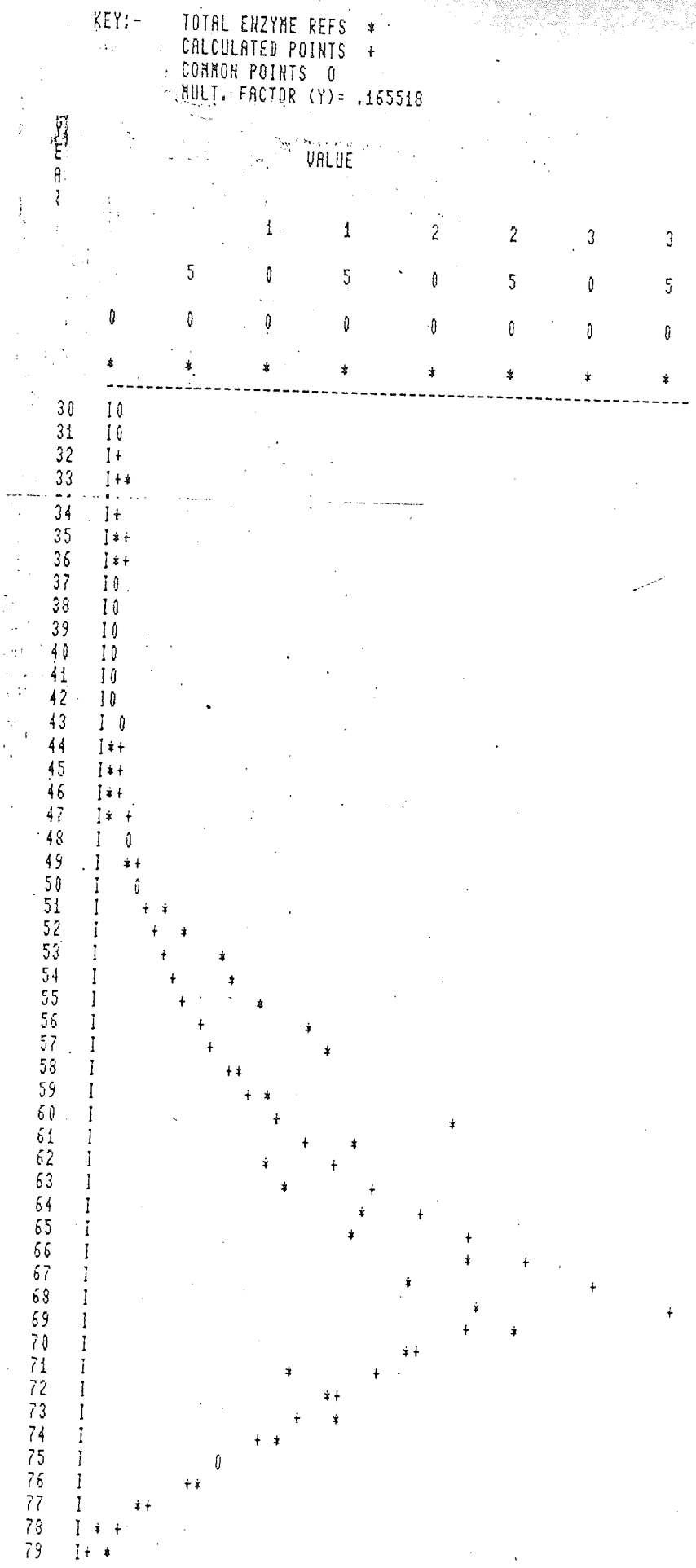


Figure 3.2. Histogram of total enzyme references from Enzyme Nomenclature 1978.

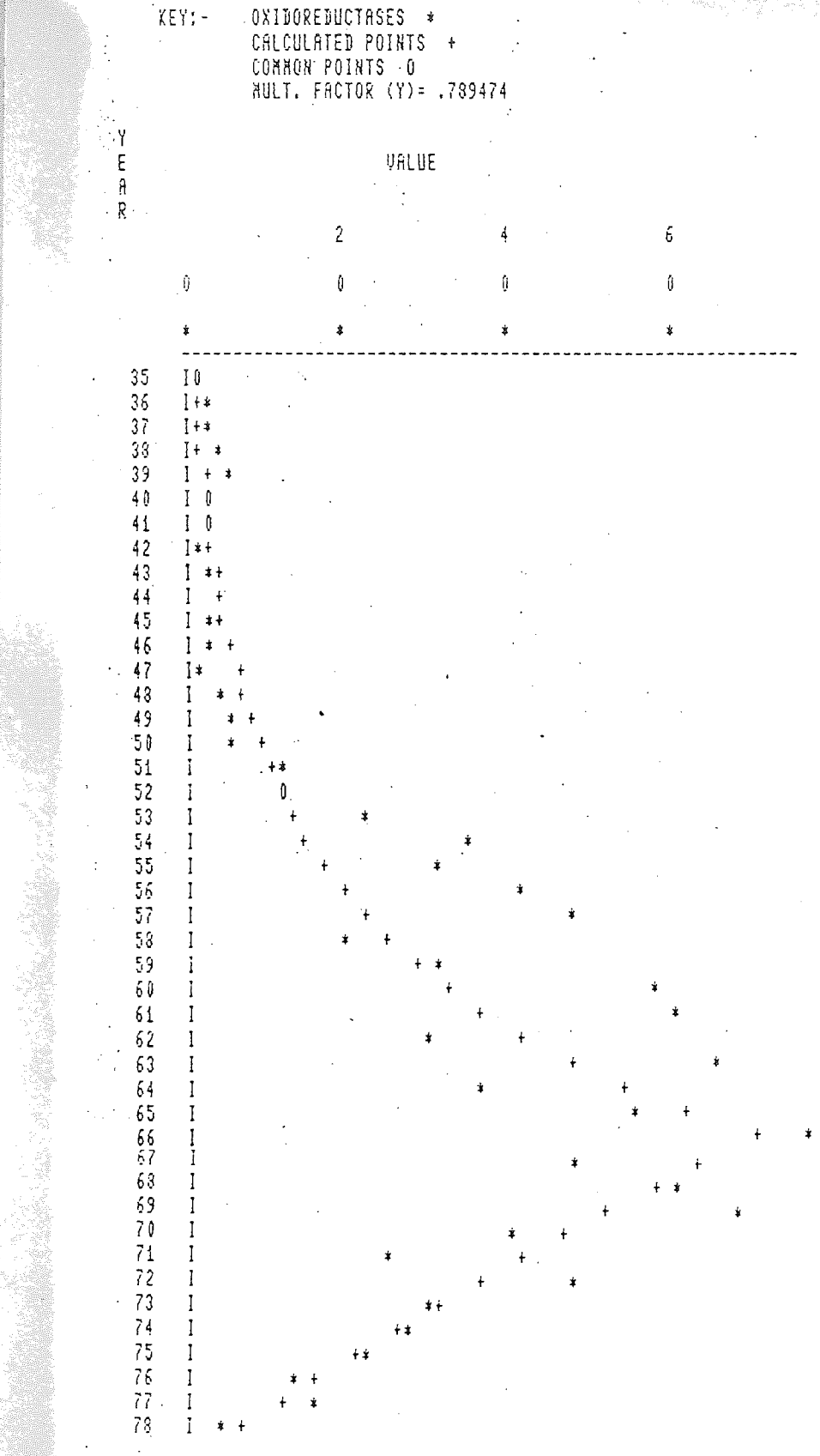


Figure 3.3. Histogram of Oxidoreductase enzyme references from Enzyme Nomenclature 1978.

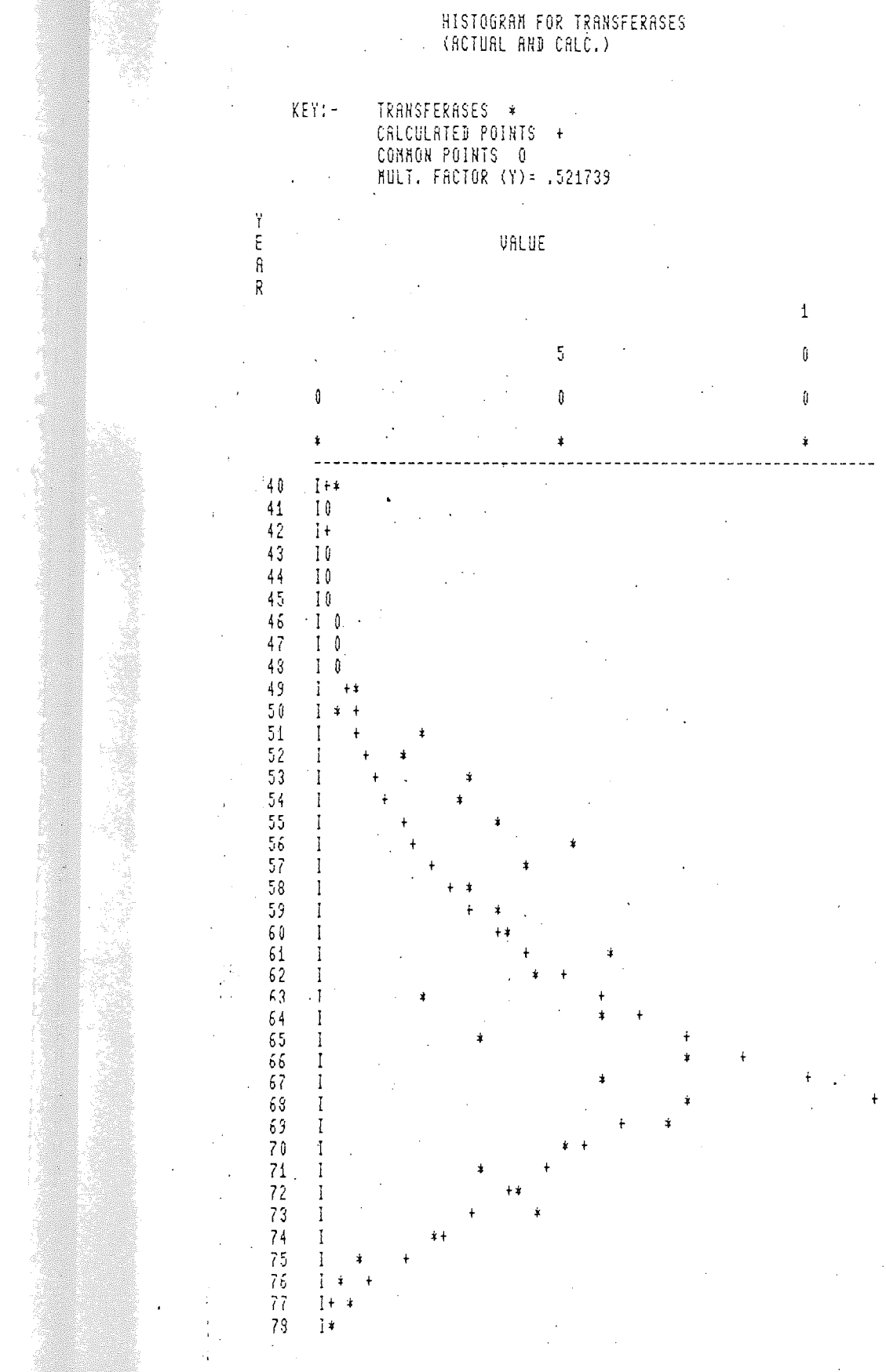


Fig.3.4. Histogram of transferase enzyme references from Enzyme Nomenclature 1978

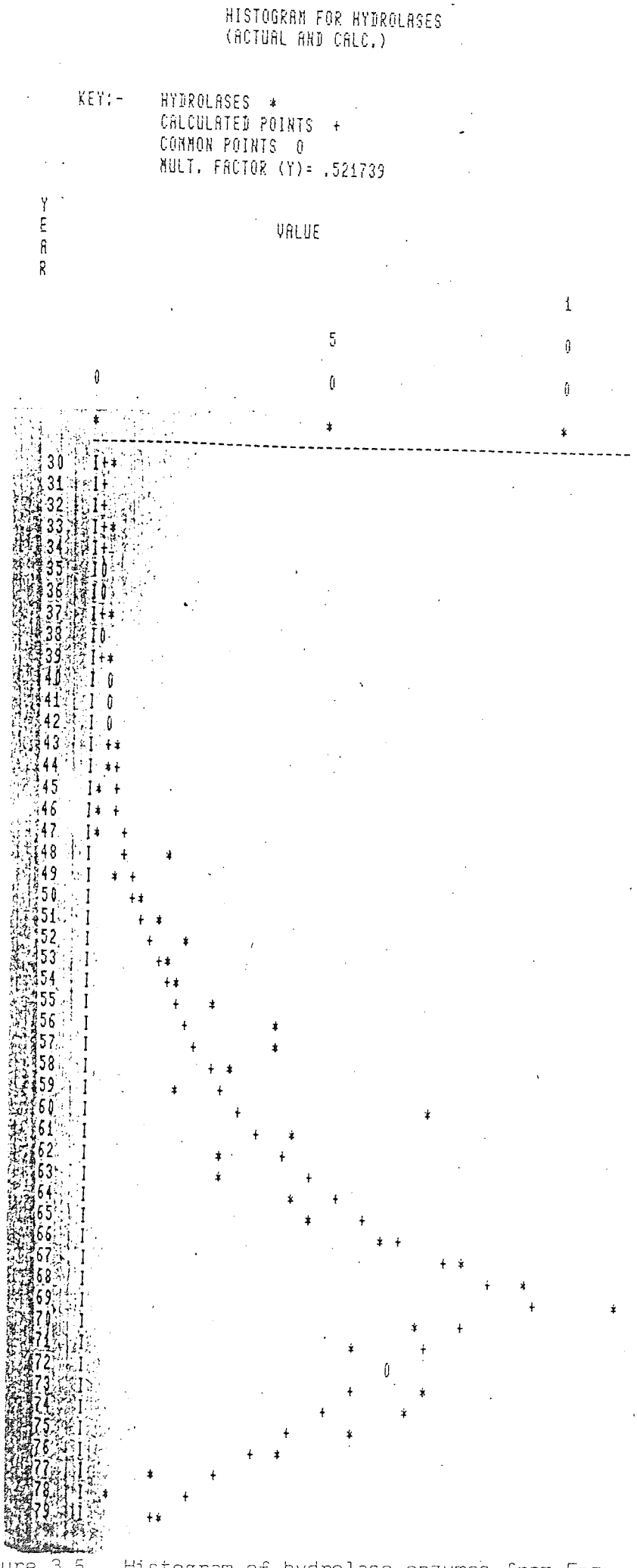


Figure 3.5. Histogram of hydrolase enzymes from Enzyme Nomenclature 1978.

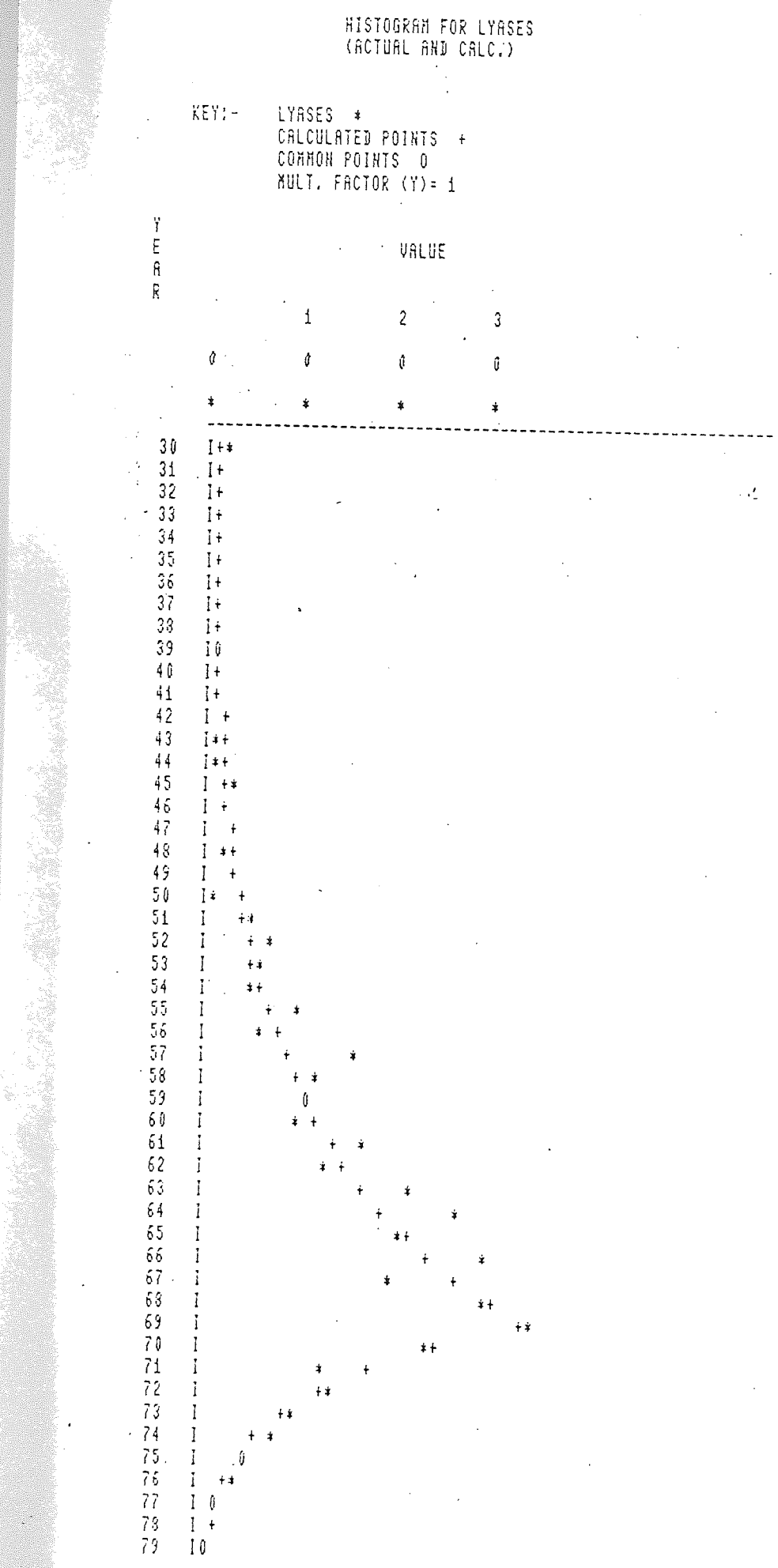


Figure 3.6. Histogram of Lyase Enzymereferences from Enzyme Nomenclature 1978

HISTOGRAM FOR ISOMERASES
(ACTUAL AND CALC.)

KEY:- ISOMERASES *
CALCULATED POINTS +
COMMON POINTS 0
MULT. FACTOR (Y)= 1

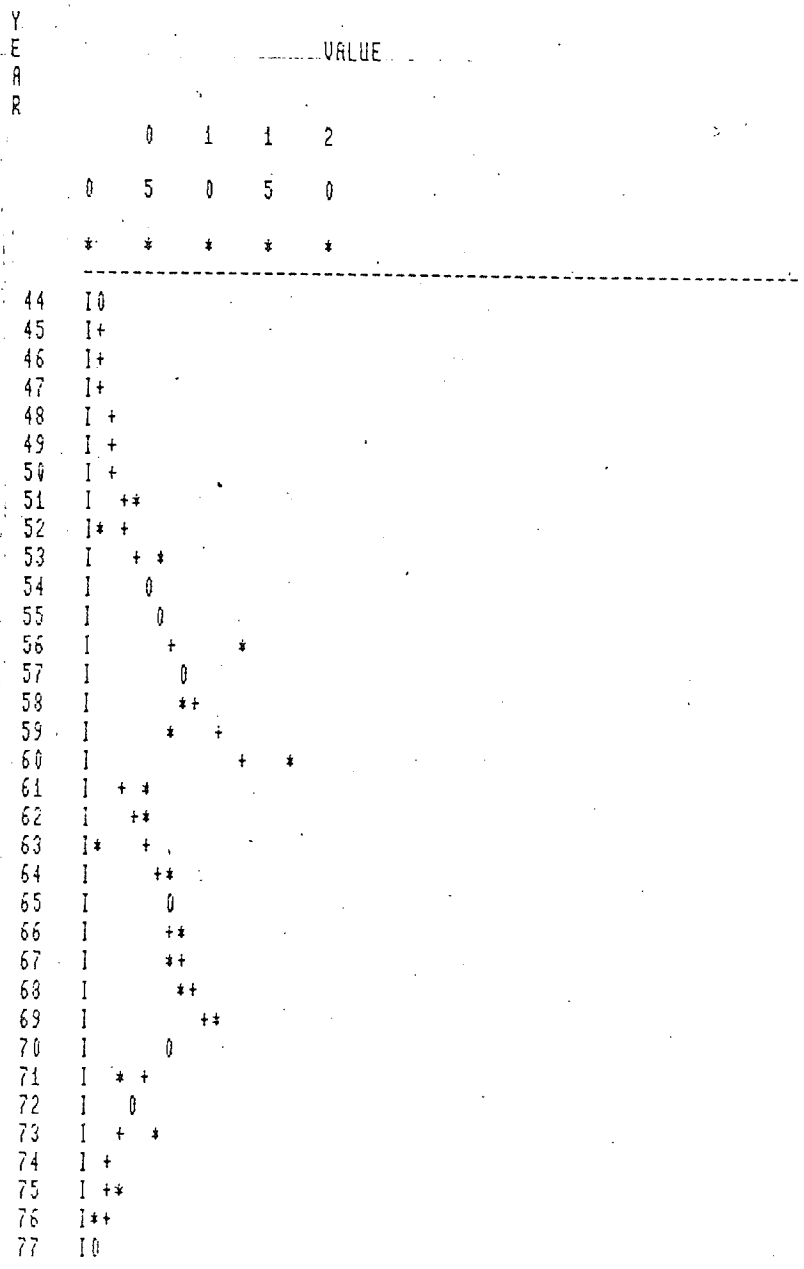


Figure 3.7. Histogram of Isomerase Enzyme references from Enzyme Nomenclature 1978.

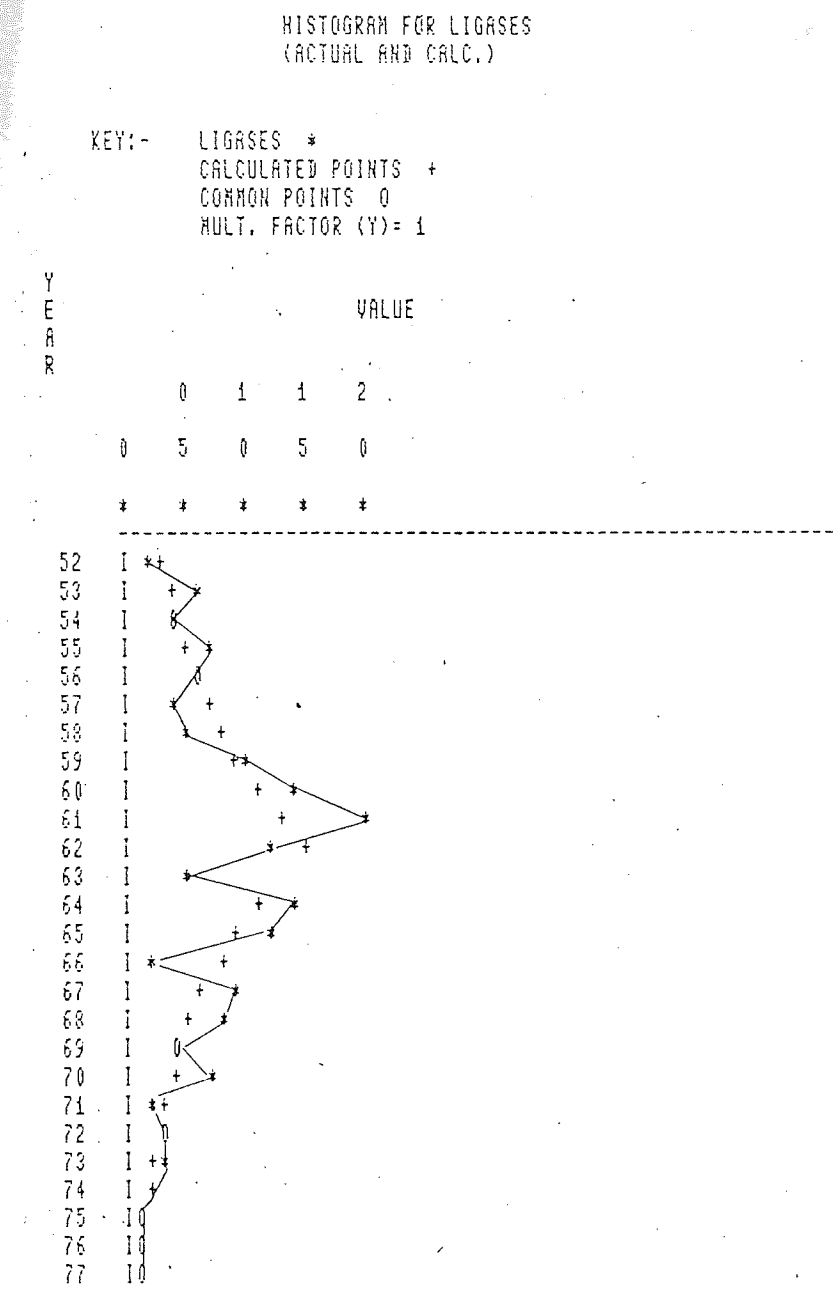


Figure 3.8. Histogram of Ligase Enzyme references from Enzyme Nomenclature 19

It shows that less than one tenth of all enzymes currently known have found a commercial application and that of these less than half have any uses as industrial catalysts. These are clear signs that the rate of discovery of enzymes is beginning to fall, a pattern that to all intents and purposes appears to be following the logistic curve for scientific discovery described by Crane.[4] I have already described the logistic curve in my discussion of the diffusion of innovations (see Griliches and Mansfield (section 2 of chapter 1).

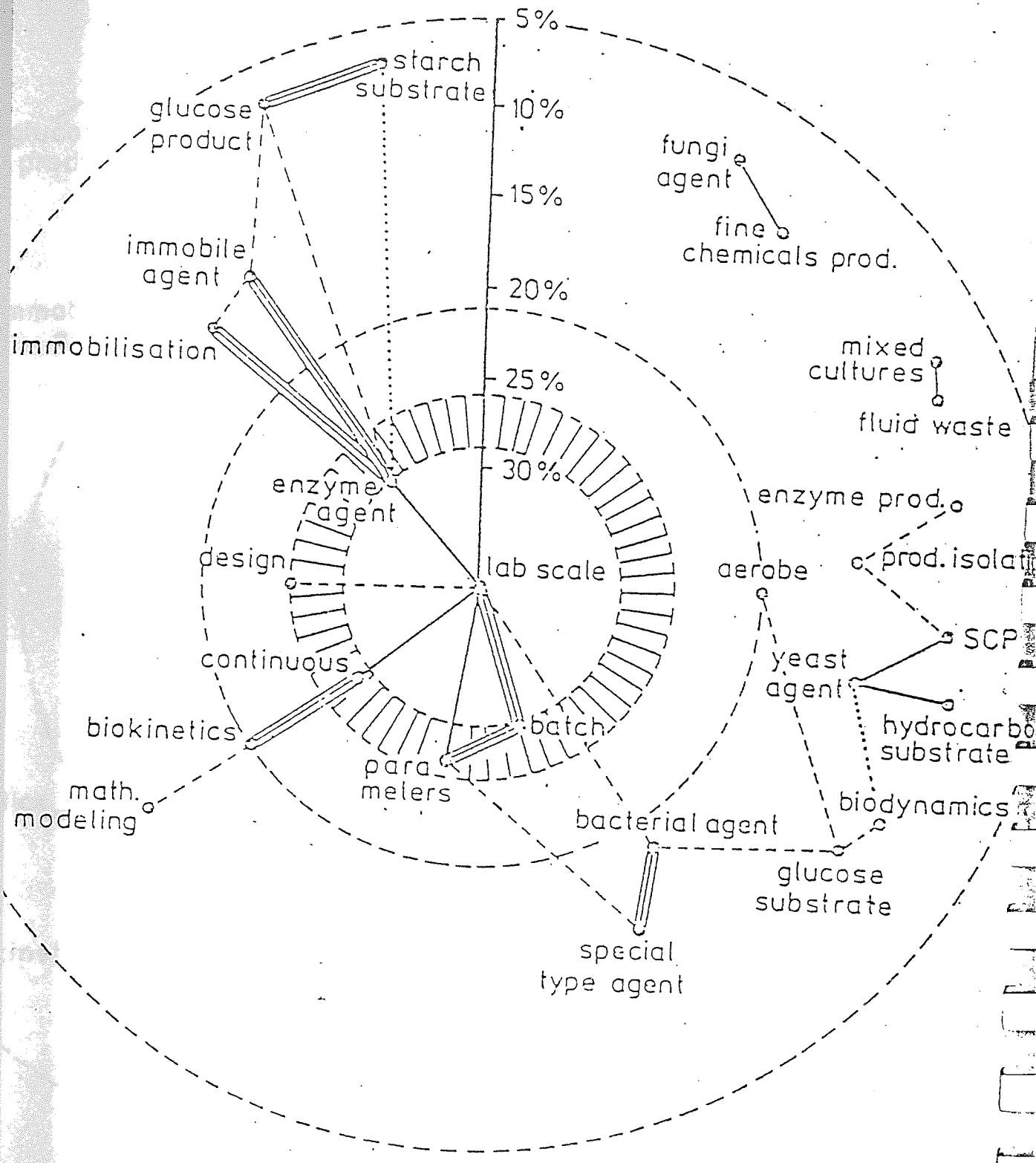
I have also analysed the relative growth rates in enzymes attributable to the six enzyme classes of the I.U.B./I.U.P.A.C. data base. These are shown as Fig.3.3-3.8. The different curves indicate marked differences between the enzyme classes. The reference to the enzymes reflect their relative numbers, see Table 2.2. (in chapter 2) but also appear to reflect the degree of research activity. It is interesting to note that work on the oxidoreductases, transferases, hydrolases and lyases appears to reach a peak around 1969, whereas work on isomerase has two peaks in 1960 and 1969 and the work on ligases peaks much earlier during 1961 and does not continue to advance. See Figs.3.3-3.8. The incidence of the peaks of discovery of enzymes and enzyme action just predate the U.S. and U.K. government sponsored research initiatives. It is possible that these rapid advances in the isolation and identification of enzyme may be attributed to the number of advances in the science of biochemistry and immunology which made the isolation and purification of small amounts of proteins such as enzymes more or less routine procedures. However, I have not had the opportunity to investigate this aspect further.

These analyses however tell us very little about the cognitive structures of science. Techniques for measuring the cognitive structure of science are reasonably well advanced. Publications and citation counts have been developed by Small and others at the Institute for Scientific Information.[5] The data base for analysis is based on the science citation index. However although the information is readily available for analysis through co-citation mapping, these maps are costly to produce. In the event of not having access to such data bases I shall refer to a co-word analyses of a Biotechnology core journal carried out by Rip and van der Es and written up by Rip and Courtial.

For a discussion of co-word analysis techniques developed at the Paris School of Mines, see Callon, Courtial, Turner and Bauin,[6] Courtial Sigogneau and Callon,[7] Callon Law and Rip,[8] and Bauin, Callon, Courtial and Turner.[9]

Co-word analysis overcomes many of the problems associated with co-citation analysis, such as the lack of a comprehensive theory of citing practices or the fact that other factors than acknowledgment of the cognitive aspect of the cited work can influence co-citation. The techniques of making co-word models have been pioneered by a group of workers at the Ecole des Mines in Paris with the support of the C.N.R.S. The approach is to build up an index of key co-word files and to measure the degree of their co-occurrence. In this way relationships between research areas can be mapped as can the passage of ideas and techniques from one area to another.

Figure 3.9. Circular map Jaccard 1970-74



Circular map, Jaccard, 1970 - 74

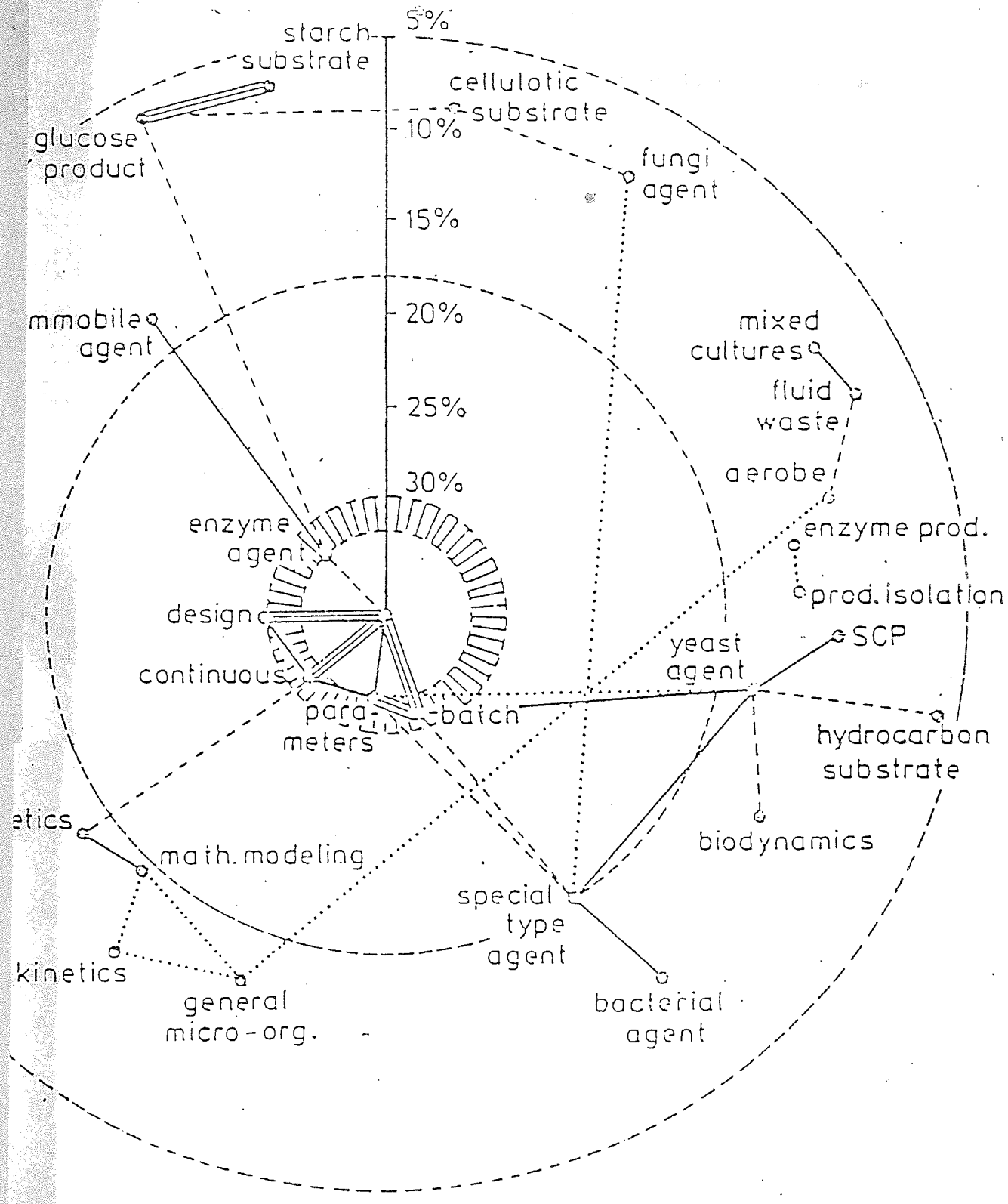
Jij ≥ .3

Jij ≥ .25

Jij ≥ .2

Jij ≥ .19

Figure 3.10 Circular map Jaccard 1975-1979



map, Jaccard, 1975-79

Jij ≥.3

Jij ≥.25

Jij ≥.2

Jij ≥.19

ard links with keywords below - 82 -

equency have been deleted

Rip and van der Es aimed to reanalyse the data described in the contents articles of a case journal of biotechnology (Biotechnology and Bioengineering) over a ten year period 1970-79.

Circular maps of keywords produced by calculating Jaccard links give an impression of linkages between keywords. The rank ordering of the keywords is taken into account by having a radial scale.

The results of the co-occurrence of keywords for 1970-4 and 1975-9 analyses are presented in Figs.3.9-3.10.

It is interesting to note that during 1970-74 enzyme activities formed an independent cluster loosely related to labscale and to a production cluster represented by glucose products and starch substrates. In the 75-79 set, these activities were more loosely associated with the labscale cluster, and immobile agent. An independent cluster can also be seen associated with enzyme production and isolation! It is interesting to speculate on its nature since during the late 70's more commercial criteria appear to enter the literature. These two aspects of enzyme science will be examined in greater detail in the case studies in chapter 5

3.3. The Analysis of Patents

3.3.1. Recent use of Patent Information as Indicators of Technological Output.

Recently a number of authors have used patents as indicators to ascertain the "status of a technology". [10,11,12] In order to evaluate these studies I have carried out an in depth analysis

of the U.S. patents that deal with Glucose Isomerase and used this source of data to establish the reliability of the patent data in these reports.

The analysis of Glucose Isomerase patents also yielded insights into company strategies, their technical strengths, the interrelationships between science and industry which I shall discuss in chapter 5.

The object of these studies is to examine the view of the status of enzyme technology presented by the various authors, and also in case studies E, F and G, to reanalyse the data bases to obtain additional information where possible.

The cases examined were as follows:-

Case A - 'Production of Biological Catalysts Stabilisation and Exploitation', prepared by D. Thomas.

Case B - Data presented by G. Tasset to an Expert Group Meeting 2.5.79.

Case C - 'Appendix 4. Patenting Activity in Biotechnology from Biotechnology Trends'. - NBST(1), presented by P. Daley.

Case D - 'Patterns of Change in Biotechnology' by P.K. Marstrand.

Case E - 'Application of Enzymes' by van de Kasteele.

Case F - 'Immobilisation of Enzymes' by M. Sigmond.

(1) National Board for Science and Technology - Eire

3.3.2. Case A

In a recent report by D. Thomas [13] on 'The Production of Biological Catalysts, Stabilisation and Exploitation', Thomas provided a breakdown of some 1300 patents and papers in broad classes - those of the U.S., Japan, Members States and Other Countries. The information came from his own data source and selection was based on key words in order to introduce some degree of detachment, but he stated that numerous references had been omitted. It gave an indication of research and patent activity in the field that was being carried out by the 4 classes mentioned. There was little point in attempting to break down the papers by groups or to analyse the patents further as the classification was controversial.*

The overall impression created was that the patents covered many countries and despite certain errors gave an 'impartial' picture of the situation in the enzyme field. "The important point is the relative weights of the U.S.A., Japan, Member States and Other Countries" (ibid p.91).

Table 3.1.

Analysis of D. Thomas information from Appendices I-IV

	Appendix I Member States	Appendix II Japan	Appendix III U.S.	Appendix IV Others
Papers	238	81	371	231
Patents	88	74	82	24

* Communication to M. Cantley by Z. Towalski

Reviewing the present state (1976, p.59) Thomas states that both for the number of patents (in his Appendix II) and present industrial applications Japan is the most important country in the field. Numerous groups in Japanese industry and universities are very active in enzyme technology. He places the U.S. as second in the field [p.66] and forecasts an industrial explosion of Enzyme Technology - based on the results obtained from the project carried out by RANN of the N.S.F. (Judging from comments such as those produced by RANN Utilisation Experience case study No.6. 'Industrial syrups' produced by G.T.Isao, NSF. C921 30p 1975, this in fact has not materialised). It is interesting how these observations enter the literature (see Endeavour, Vol.5,(3), 1981, p.96).

Observations on Thomas' use of Patents

The U.S.Patent data only

1. Thomas did not make allowance for the existence of concordance, e.g. U.S.Patent 3,519,535 and German Patent 1,944,418 are part of a much larger patent family but are listed as two separate entries within the report.
2. Entries 872,872,874 appear in another section 904-911.
3. Organisations are misclassified, e.g. NRDC, a U.K. organisation, appears in the U.S. section.
4. References 915 and 917 have same patent numbers.

A comparison of our listing of U.S. glucose isomerase patents to 1976 and the patents in the Thomas sample also to 1976, presented overleaf illustrates the limitations of Thomas' sample.

Table 3.2. A comparison of U.S. Glucose Isomerase patents in the Thomas sample

Companies/Agencies	U.S.Patents to 1976	U.S. Patents in Thomas Sample to 1976
Agency of Industrial Science & Technology (Japan)	5	-
Anheuser Busch	4	-
Baxter Laboratories	2	-
Corning Glass Works	14	3
CPC International	13	-
EI Du Pont de Nexmouros	1	-
Denkai	1	-
Miles	5	-
Monsanto	1	-
National Food Research Institute (Japan)	5	-
Novo	2	-
Reynolds Tobacco Co.	8	3
Rhone Poulenc	1	-
AE Staley	2	-
Standard Brands	10	3
Individuals	1	-
Total	75	9

Furthermore, of all the glucose isomerase patents listed by Thomas, only the Standard Brands patent, held re. Clinton Corn Processing Co., formed the basis of a commercial process, The C.P.C. patents were the only other group that had been used commercially to some extent, were not mentioned at all.* I am forced therefore to conclude that the Thomas patent sample is a poor representation of industrial innovation in this area. Nevertheless the paper was valuable in drawing attention to the use of patent information as an indicator of the sort of enzyme inventions that were being developed.

* Corning Glass licensed some of the technology to C.P.C. in both amylases and isomerases.

3.3.3. Case B

Another attempt using patents as indicators is that of Dr.G. Tasset[14] Manager of the Patents Department, Smith Kline-RIT, Rixensart, Belgium, and presented at an Expert Group Meeting, Brussels, 2nd May 1979.

He used the following search strategy of the Derwent Patent Data Base to obtain numbers of immobilised enzymes patents.

'ENZYME' (from standard Derwent thesaurus)
OR
'ENZYME, general' (from Derwent manual code)
OR
'ENZYME, general' (from Derwent punch code, Farmdoc section)
AND NOT
'BIOSYNTHESIS OF ENZYMES' (from Derwent manual code)
AND
'IMMOBILISE OR FIX' (from standard Derwent thesaurus)

A and '75/AY (accession year)
A and '76/AY
A and '77/AY
A and '78/AY
A and '79/AY (first week)

Table 3.3. Annual distribution of the immobilisation patents 1977-79

The data produced was :

YEAR	NUMBER OF NEW PATENTS
1975	37
1976	48
1977	79
1978	106
1979 (first week)	9
	279

He presented the information on the national distribution of the latest and hence most relevant patents.

Table 3.4. The National Distribution of the 194 Patents (1977-1979)

Countries	Number of Patents	%
Japan	124	64
USA	39	20
UK	1	<1
Italy	3	1.5
France	7	3.6
Bundes Republik Deutschland	8	4.1
USSR	9	4.6
Denmark	1	<1
Not Identified	2	1

Comment

This once again gave an impression that Japan was 'ahead' followed by the U.S., France, Germany and the USSR. However, the number of immobilised enzyme systems in large-scale industrial use is still very low, it being cheaper to fix the enzyme within the cell and then to immobilise the cell and use immobilised cells as the reactive part of a production process. Further, the Derwent patents data base was not searched for equivalent patent concordance. Consequently, the inclusion of double counting 'errors' is a possibility. Without re-running the programme, it would be impossible to be any more specific than that. The number of patents obtained by the interrogation of the Derwent data base produced a less extensive list than that analysed by DG XIII*, but such a difference could also be explained by the difference in the two time periods,

* If the 1970-74 trend is an extrapolation of the 75-79 figures then there will be less than <100 i.e. <30:1974, <25:1973, <20:1972, <15:1971, <10:1970.

but does warrant further analysis. It also draws attention to other potential difficulties associated with the use of computer based information,

i) This poses two problems, the first concerns the accessibility of the data to data retrieval standardised by interrogations and,

ii) the effectiveness of the data collections, its processing and classification prior to its entry into the data base itself.

I do not propose to deal with these matters further.

3.3.4. Case C

Based on 'Biotechnology Trends', Chemicals, Pharmaceuticals, Health Care and Food Processing. Prepared for the National Board of Science and Technology, Ireland presented by Peter Daley.(15)

Appendix 4 - Patenting Activity in Biotechnology.
Enzyme and Microbial Technology 1970-1976.

I have re-analysed these patents and Table 3.5 offers a summary. The analysis shows that overall, the EEC is at least in a comparable position with the situation in the U.S. and Japan and that within the EEC, the U.K. and Germany being particularly strong - if somewhat less inventive than the U.S. and Japan. The patents information nevertheless was a little dated even though the report was circulated in April 1981.

Table 3.5. Summary of Data Presented in Appendix 4 'Patenting Activity in Biotechnology Enzyme Microbial Technology 1970-1976'

	Countries	Companies	Patents
	US	19	32
	Japan	24	37
EEC	Belgium	-	-
EEC	Denmark	2	2
EEC	France	4	6
EEC	Germany	8	24
EEC	Ireland	-	-
EEC	Italy	3	5
EEC	Netherlands	2	2
EEC	UK	17	26
	TOTAL	36	65
OTHERS	Sweden	2	4
OTHERS	Switzerland	2	2

Comment

The data presented by P. Daley was far from comprehensive since companies were listed under the patent granting country and not by the parent companies country of registration. Some companies' patents were listed in the "Other Countries Section", as well as their own.

The low number of U.S. glucose isomerase patents as compared with our reference sample obtained by other methods, showed and suggested that this data should not be taken as a quantitative indicator, nor was it a representative sample of patenting of the

enzyme and microbial field in general. However, it served to give additional information to the Biotechnology Report itself and drew attention to the sort of patents and the sort of companies that were becoming involved in enzyme technology.

3.3.5. Case D

P. Marstrand carried out an analysis of world-wide patents registered in the period 1977 - June 1980 as part of a UNEP project (FP/0402-10-03[2171]), presented also as a SPRU Occasional Paper.* Patent entries from 27 countries, the European Patent Office and PCT International were scanned using the U.K. Chemical Information Service (UKCIS) at Nottingham University, England. This sought relevant entries from 2 sources, Lockheed in the United States and 'ReCon' Frascati, Italy. The initial interrogation revealed a large number of entries, so the research was limited to Chem Abs, Jan.1977 - June 1980, Vols.88-92. (This data is also the subject of a C.1.4. report.)

Each entry showed the names of the researchers, their organisational affiliations, the country of origin, the country of registration, date of application or priority, date of registration, the title and key terms describing what was claimed. The information was coded and stored on cards. A computer was used to sort the cards into various countries, and separate various product or purpose categories. The most relevant for us appear in Table 3.6 and appear under two headings:

a Enzyme or Co-enzyme;

b Sweetener.

* See also Preliminary Report, "The Use of Computerised Information Data Bases in the Analysis of Innovation", P.Marstrand (part of C.1.4. project)

The patents included under (a) are those where an enzyme or co-enzyme is named as a product. Some include the incorporation of enzymes within a sector. There were 288 cases. The following is a list of organisations which had 6 or more patents assigned to them:-

1.	Agency of Industrial Science & Technology	14	
2.	Kyowa Hakko Kogyo	13	
3.	Ajnomoto	10	were -from Japan
4.	Mitsubishi Chemical Industries	9	
5.	Amano Pharmaceuticals	6	

(b), Sweeteners. Almost all the patents were concerned with fructose enriched glucose syrup but other sweeteners derived from different substrates were also included. Unfortunately, the analysis states that there were 92 reported cases which seems to be confused with the number in the Biomass category in Table 3.6. The companies listed were the following:

1.	Kureka Chemical Industries	12	
2.	Mitsubishi	7	
3.	Ajinomoto	5	- All of Japan
4.	Kyowa Hakko Kogyo	5	
5.	Nippon Beet Sugar Co.	5	

The paper also produced a table of data of out-patenting. Our Table 3.6 is the table from p.17 of the Marstrand SPRU Paper. It appears that most industrialised countries except for Japan show a high propensity for firms to patent abroad. It is interesting to note that Japan has higher national patents counts than is evident from the Japanese patents taken out in other countries.

.6 - National Interest in Products/Purposes, period 1977 - June 1980

Country	Number of patents in the following categories		Validation for same period
	Enzyme/Coenzyme	Sweeteners	US Patents in GI** in Period
Australia	-	-	-
Austria	-	-	-
Belgium	-	-	-
Brazil	2	1	-
Britain	4	-	1
Canada	-	-	-
Czechoslovakia	6	2	-
Denmark *	at least 11 *	{ at least 1 }*	2
Finland	-	-	-
France	4	1	1
GDR	8	1	-
FGR	5	3	-
Hungary	3	2	-
India	5	1	-
Israel	-	-	-
Italy	1	1	-
Japan	183	32	7
Netherlands	3	-	-
Poland	11	-	-
Romania	1	-	-
S. Africa	1	-	-
Spain	2	1	-
Sweden	1	-	-
Switzerland	-	2	-
US	21	8	16
USSR	32	2	-
Total	293	57	27

* Missing from P.Marstrands Table 1 but included for the sake of completeness.

** GI = glucose isomerase (xylose isomerase)

This observation could reflect the strong parochial nature of Japanese science industry and technology, an observation would be borne out by citation studies, which also show that Japan's major scientific contact with the world is through the U.S.

Validation. The comparison of Glucose Isomerase patents within patents identified in this analysis.

See Table 3.6. overleaf.

The validation exercise of comparing the U.S. patents for glucose isomerase with the patents in this analysis is difficult because I was unable to separate the glucose isomerase patent component from the data presented in this analysis and I was thus unable to compare like with like. Table 3.6 shows that there are 16 U.S. glucose isomerase patents listed in an equivalent time period, this is 8 more than appear in the "Sweetener" category, however, some of the "missing" patents may have been included in the 'Enzyme/Coenzyme' category which holds 21 patents, without re-running the data the check on completeness of this data base must remain inconclusive.

However, the attempt to reconcile the counts of enzymes and companies by countries (obtained from the list of companies that have patented specific enzymes, found in the SPRU report), with the comparison of activity between countries (on p.4 and the information supplied in Table 1, p.5, 15, of the UNEP/SPRU reports)* did reveal some inconsistencies. For example, the Danish company 'NOVO' patented in 11 enzyme areas, but the report stated on p.4 that neither Denmark nor Norway registered any patents for the relevant technologies in the period. This could be explained by

* Available from Science Policy Research Unit, University of Sussex, Mantell Building, Falmer, Brighton, Sussex BN1 9RF, United Kingdom.

the fact that companies such as Novo patented abroad in preference to patenting at home, (e.g. as part of the aggressive pursuit of a patent policy interested in securing larger markets) or that Novo was misclassified. What could not be reconciled was that (i) despite Novo's 11 patents it was not identified or discussed in the section identifying companies with 6 or more products on p.28; (ii) no Danish entries appear in the analysis of out-patenting in Table 9 p.17 of the report presented as Table 3.7 in this analysis overleaf).

On a more general note the information obtained from entries in Chemical Abstracts Jan.1977 - June 1980, was presented without a check on patent concordance, and thus risked the inclusion of multiple entries of patents that belonged to the same family, although the likelihood of this error is reduced somewhat by the short time span over which the sample was taken $3\frac{1}{2}$ years.

Furthermore, although the data presented in Tables 3.6. and 3.7 purports to "Indicate the interest of various countries in the range of purpose/products, and technical integration respectively as indicated by propensity to patent", it does not reflect "national interest per se", despite the list of accompanying qualifications. This analysis fails to distinguish between domestic and foreign patents, and as the data contains both these patent sets, the breakdown by countries more accurately reflects the National and International Perception of Commercial Opportunities by the patentees as indicated by their propensity to patent. It follows that the information as presented in the form of Table 3.6 is too coarse for our use as an indicator of national strengths directly. Despite these comments, it provides valuable information about the

Table 3.7 - Incidence of out-patenting

Country	No. of Patents	No. Foreign	Countries concerned
Australia	1	1	
Austria	6	2	Belgium 1, FGR 1.
Belgium	2	0	
Brazil	3	0	
Britain	32	25	Belgium 3, Canada 2, France 1, FGR 12, Japan 3, N'lands 2. US 2, Eur. 1.
Canada	2	0	
Czechoslovakia	41	1	FGR 1.
Finland	1	0	
France	21	12	Austria 1, Belgium 1, Britain 1, FGR. 9.
GDR	34	9	FGR 1, Japan 1, US 1, USSR 2.
FGR	41	10	Britain 5, Japan 1, N'lands 2, US 1, Eur.1.
Hungary	14	3	FGR 2, Japan 1.
India	14	0	
Israel	1	1	Brazil
Italy	14	6	Britain 3, Romania 1, Spain 2.
Japan	842	101	Belgium 5, Britain 5, Canada 1, France 7, FGR 68, N'lands 2, S.Africa 1, US 8, USSR 1, Eur.3.
Netherlands	4	2	Britain 2, FGR 2.
Poland	42	0	
Romania	10	1	France 1.
S.Africa	1	1	US
Spain	5	0	
Sweden	4	2	France 1, FGR 1.
Switzerland	21	16	FGR 12, Japan 3, US 1, Eur.1.
US	152	67	Belgium 7, Brazil 1, Britain 1, Canada 2, France 4, FGR 36, Japan 10, N'lands 2, Romania 1, USSR 2. Eur. 1.
USSR	81	77	Britain 3, Canada 1, France 1, FGR 1, US 1

N.B. No cases recorded for Denmark, Norway, Europe or International.

Source : P. Marstrand. (op.cit.)

proprietory knowledge relating to enzymes that industry and individuals are laying claim for enzyme products. Also the data and methodological approach do offer profound insights into national industrial and R & D activities in biotechnology.

3.3.6. The Interpretation of the Data Presented in Cases E,F and G.

The following three cases are based on data collected for the Commission of the European Communities, Directorate General XIII, "Scientific and Technical Information and Information Management" Patents Division, Luxemburg.

Together they give the most comprehensive patent data base publicly available for analysis. They provide the closest match between the U.S. patents of glucose isomerase I collected independently and that claimed by the reports. (see Table 3.8. overleaf).

The patents for these three reports have been divided into 3 sections, all covering the period 1970-79 and are called:

1. Preparation of Enzymes - by M.A.Rosemeyer & B.R. Rabin[17]
2. Application of Enzymes - by R.P.van de Kasteele[18]
3. Immobilization of Enzymes - by M.Sigmond.[19]

However, some patents that by all rights should appear on one report appear in another, e.g. The Grace patent B48/3 appearing in the Group on page 64 of the van de Kasteele analysis is similar to, and should be included with the Grace patents of the Immobilization of Enzymes study carried out by Sigmond.

Such minor discrepancies, don't detract from the value of this data base. Its unique character is, that it lists patents within patent families. This, as mentioned earlier, is essential in presenting errors in the analysis due to multiple counting of data caused by multiple patent filings.

By allocating the assigned patents within a patent family to the holding company, it is possible to obtain a measure of the inventive strength of that organisation. I have also argued that the patents controlled by a parent company can earn revenues for that parent company's nation, thus in effect these patents can in turn be deemed as "belonging" to that nation. In this way I also overcome the problem of what to do with patents obtained by foreign subsidiaries of multinational organisations for research carried out in the subsidiary for the benefit of the whole organisation.

Caveats of this Analysis

1. It may produce a misleading impression of a nation's "inhouse" R & D expertise. A number of companies have tapped into the highly trained research personnel in Japan by setting up their own research establishment e.g. Pfizer. Alternatively, they have entered into research agreements with Japanese Research Institutes, bought Japanese patents or obtained licenses or all three e.g. C.P.C. International.

2. At least five newly commercialised enzymes that have made a major impact on the world's markets have early Japanese involvement and activity common to them. These are as follows:

(i) Microbial Rennets

(ii) Glucose Isomerase

(iii) b-Galactosidase

(iv) Cellulase

(v) Urokinase

This "analysis" will mask this important point but it will reveal an aspect of the commercial situation not available from any other source.

3. By not having strongly developed R & D facilities, a nation does not necessarily forfeit on innovations, providing that it has a well established commercial basis. However it forfeits any advantages that are to be gained through having a strongly developed "cadre" of scientific personnel which becomes more important at a time of crisis such as a war. In time of peace the least cost will be a negative balance of payments between the nations in payment for research funded abroad or for proprietory knowledge developed abroad. Money put into the rival nation's economy to develop and maintain that nation's research facilities is lost to the other to the further detriment of that nation's scientific expertise, further undermining the weaker nation's ability to provide the necessary infrastructure and the right quality of labour to enable its commercial organisations to compete successfully internationally. This may be going on despite apparent overall commercial success of the domestic enterprises at present, or apparent R & D strengths as determined by patent counts.

4. Such an analysis is only valid for a peace-time scenario. History has shown that companies that had manufacturing bases

abroad, lost them during times of war; such changes can also take place from time to time with changes of government.

5. We have not been able to carry out a check as to the accuracy of all patent families reported, but this does not seem to be a major problem since a spot check of a sample of the patent families listed proved them to be correct.

Comments concerning the Validation (Table 3.8)

1. Some of the patent families noted in the glucose isomerase validation exercise, as not having U.S. counterparts may indeed have them, and if so would make the tie up between the two sets of data even closer.

2. It has not escaped my attention that certain organisations or individuals who have taken out glucose isomerase patents have not done so in the U.S. and, therefore, don't appear in the sample that I use to validate the other patent case studies. This is part of the problem of validating data using only one set of patents. However, if we used all the patents families for glucose isomerase available to us for validation then they would only decrease the magnitude of the discrepancy between my data and the data presented in these case studies.

3. On a final note, apart from the two French Companies Rhone Poulenc and Roquette Freres, the Japanese company Nagase (which is not listed at all), then the U.S. analysis reflects the present commercial scene, as all the companies involved in marketing or developing a glucose isomerase enzyme for the manufacture of HFCS are included. If this is the case for glucose isomerase, then it is conceivable that this set of enzyme patents provides a represen-

Table 3.8- The comparison of DGXIII patents for Glucose Isomerase with the US Patents collected independently.

	Patents from 3 D.G.XIII Studies 1970-1979			Patents Aston Study 1970-79
Organisations holding Glucose Isomerase patents.	Total patent families.	Patents/patent families other than US.	U.S.Patent/ patent families.	U.S. Patents only.
Agency of Industrial Science & Technology	3	1	2	4
Amerace Corporation	1	1	0	1
American Cyanamid	2	2	0	2
Anheuser-Busch	4	1	3	5
Baxter Laboratories	2	0	2	2
Corning Glass Works	7	7	0	13
C.P.C.	17	10	7	16
Denki Kagaku Koyyo K.K.*	2	1	1	1
Gist-Brocades	1	1	0	0
L.Givaudan & Cie S.A.	2	2	0	0
W.R.Grace	5	5	0	5
I.C.I.	3	3	0	1
Kali-Chemie	1	1	0	0
Kansai Paints Co.	2	2	0	0
Miles	4	0	4	5
Millipore Corpn.	1	0	1	1
Mitsubishi Chemical Ind.*	4	3	1	5
Monsanto	0	0	0	1
National Food Research Institute of Japan	3	0	3	3
Nippon Oil	1	1	0	0
Novo	8	5	3	3
Rhone Progil	1	1	0	0
Pennick & Ford	1	0	1	1
Rank Hovis McDougal	1	1	0	0
R.J.Reynolds Tobacco Co.	4	1	3	10
Rhone Poulenc S.A.	1	1	0	1
Roquette Freres	1	1	0	0
Snam Progretti	2	2	0	0
A.E.Staley	3	0	3	3
Standard Brands	16	5	11	15
Sumitomo Rubber	1	1	0	0
Tanabe Seiyaku	1	1	0	1
U.O.P.	0	0	0	1
TOTALS	105	60	45	100

tative sample of patenting activity for other enzymes.

3.3.7. Case E - Summary and Analysis of M.A. Rosemeyer and B.R. Rabin Report.(op.cit.)

The Report

The Commission of European Community D.G.XIII for Scientific and Technical Information Management, Patents Division, Luxemburg produced a series of studies on enzymes. This case study uses the report called "Preparation of Enzymes" by M.A. Rosemeyer and B.R. Rabin and edited by M. Terpstra. Their study was based on 300 original American, Japanese and West European patents and patent applications covering the period 1970-79. The report described patents taken out by 122 companies and institutions; identified and listed the patent families, abstracted the patents and organised the patent information into sections. It did not include information on priority data or on the dates the patents were issued.

The large number of hydrolase patents obtained, required their separate analysis, although the authors report that some hydrolase patents were included in this data. This applied in particular to those enzymes that were listed in the "Preparation of Enzymes" and "Enzymes Inhibitor" sections. As hydrolases are the major category of industrial enzymes produced and used world-wide, this separation enables us to concentrate on examining the less well developed categories of enzymes and examine the development of the non-hydrolase enzyme technology. (Less well developed with the exception of Glucose Oxidase and Glucose Isomerase, both of which have already undergone considerable commercialisation).

This report has a major advantage for our purposes over the other reports, in that it eliminates the errors due to double counting by listing patent families. It lists the patents as data and allows me to carry out a further analysis that is more in line with my objectives of ascertaining trends and patterns.

In the report the patents have been divided into 12 sections :

1. Preparation of Enzymes
2. Oxidoreductases
3. Glucose Oxidase
4. Uricase
5. Transferases
6. Hydrolases
7. Lyases
8. Isomerases
9. Glucose/Xylose Isomerase
10. Cell Lytic Enzymes
11. Coenzymes
12. Enzyme Inhibitors

In some cases, enzymes are referred to in sections to which they do not belong. This may occur because the enzyme is included in a patent with other enzymes, or through mistakes made in classifying the patents and aggravated perhaps by the use of misleading names for some enzymes. To correct these discrepancies, and also to provide a cross-reference of enzymes, an Index of Enzymes was provided as an Appendix.

Summary of B.R. Rabin's and M.A. Rosemeyer's Conclusions

The authors state in their analysis that certain industrial or medical aims are apparent from the patents. These are:

1. A major effort with conversion of glucose to fructose;
2. Use of cholesterol oxidase in order to measure cholesterol levels in blood sera.
3. Use of enzyme inhibitors e.g. anti-inflammatory agents in the treatment of gastric ulcers.

They state however, that the variety of enzymatic processes precludes a simple statement of objectives.

Table 3.9 shows that the U.S., Japan and EEC have comparable numbers of patents and numbers of companies taking out patents. Within the EEC Germany appears to be particularly well placed followed by the U.K., France, Denmark and Italy, in that order.

The Analysis of these patents

Table 3.9 - Summary of Numbers of Patentees and Number of Patents by Countries

	Countries	Patentees	Patents
	US	37	106
	Japan	31 *a	90
EEC	Belgium	1	1
EEC	Denmark	1	7
EEC	France	7 *b	12
EEC	Germany	13 *c	57
EEC	Greece	0	0
EEC	Ireland	1	1
EEC	Italy	2	5
EEC	Netherlands	2 *d	3
EEC	UK	7	11
	EEC Total	34	97
OTHERS	Sweden	2	2
OTHERS	Switzerland	1 *e	3
OTHERS	Israel	1	1
OTHERS	USSR	2	3

* a. includes 3 separate entries for Hayashibara Co. as single entry.

* b. includes 2 separate entries for Rhone Poulenc as a single entry.

* c. includes Miles and Bayer as a single entry (Miles Labs. is a subsid. of Bayer).

* d. Akzo and Organon listed as 1 company.(Organon is a subsid. of Akzo).

* e. including L.Givaudan & Cie and Hoffman La Roche as single entry. (L.Givaudan & Cie SA is a subsid. of Hoffman La Roche)

ble 3.10- Analysis of Patents and patent families by Country, No. of companies and patents, and product groups.

BROAD CATEGORIES		DEVELOP-MENT	FOOD							PHARMA-CEUTICAL		CHEMICAL			GENERAL APPLI-CATION	TOTAL PATE
Finer Categories / Countries		Purification	Flavour Enhancers	Food Process Enzymes	Lipase Enzymes	Food Preservation by Lytic Enzymes	HFCS Enzymes	Other Sweetners	Amino Acids	Veterinary Medical Diagnostic	Enzyme Inhibitors	Cosmetics Antibacterials	Fine Chemical Co-enzymes	Detergents	Waste Treatment	
US	Patentees	9	1	3	-	-	6	-	1	13	-	-	5	4	1	
	Patents	10	1	11	-	-	42	-	1	30	-	-	5	5	1	106
Japan	Patentees	5	1	2	1	4	4	2	4	13	4	-	8	1	-	
	Patents	7	3	2	4	5	7	2	8	20	12	-	18	2	-	90
EEC Countries																
Belgium	Patentees	-	-	-	-	-	-	-	-	-	-	-	-	1	-	
	Patents	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1
Denmark	Patentees	1	-	-	-	-	1	-	-	1	-	-	-	-	-	
	Patents	1	-	-	-	-	5	-	-	1	-	-	-	-	-	7
France	Patentees	1	-	1	-	-	2	-	-	4	-	-	3	-	-	
	Patents	1	-	1	-	-	2	-	-	4	-	-	4	-	-	12
Germany	Patentees	8	-	1	-	-	1	-	-	9	-	1	2	1	-	
	Patents	12	-	1	-	-	4	-	-	19	-	1	16	4	-	57
Greece	Patentees	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	Patents	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ireland	Patentees	-	-	-	-	-	-	-	-	-	-	-	1	-	-	
	Patents	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1
Italy	Patentees	-	-	1	-	-	1	-	-	-	-	-	1	-	-	
	Patents	-	-	1	-	-	2	-	-	-	-	-	2	-	-	5
Nether-lands	Patentees	-	-	1	-	-	-	-	-	2	-	-	-	-	-	
	Patents	-	-	1	-	-	-	-	-	2	-	-	-	-	-	3
UK	Patentees	1	-	-	-	-	1	-	-	5	-	-	2	-	-	
	Patents	1	-	-	-	-	2	-	-	6	-	-	2	-	-	11
EEC TOTAL	Patentees	11	-	4	-	-	6	-	-	21	-	1	9	2	-	
	Patents	15	-	4	-	-	15	-	-	32	-	1	25	5	-	97
Other Countries																
Israel	Patentees		-	-	-	-	-	-	-	-	-	-	1	-	-	
	Patents	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1
Sweden	Patentees	1	-	-	-	-	-	-	-	1	-	-	-	-	-	
	Patents	1	-	-	-	-	-	-	-	1	-	-	-	-	-	2
Switzer-land	Patentees	-	-	-	-	-	1	-	-	-	-	-	1	-	-	
	Patents	-	-	-	-	-	2	-	-	-	-	-	1	-	-	3
USSR	Patentees	-	-	-	-	-	-	-	-	1	-	-	1	-	-	
	Patents	-	-	-	-	-	-	-	-	1	-	-	2	-	-	3

Comments on Analysis Table 3.10

The U.S. has a high number of patents dealing with High Fructose Corn Syrups (42), followed by veterinary and medical diagnostics. (27). The application of enzymes to foods has several categories missing, as does the category concerning the use of enzyme inhibitors.

Japan has the most comprehensive development of enzyme patents covering all areas except those concerning waste treatment and cosmetic applications. Particular strengths lie in the areas of amino acid synthesis, flavour enhancers, medical and fine chemical applications.

The E.E.C. countries are less well placed than either the U.S. or Japan individually. However, they have a number of patents on HFCS manufacture, veterinary and medical diagnostic products, and in the area of fine chemical manufacture. Quite larger gaps appear particularly in the food and flavour fields, and pharmaceutical areas also.

The Other Countries don't appear to have a great deal going on.

What on the face of things appeared to be a comparable situation is now beginning to look less so. The Japanese patenting activity seems to be most comprehensive and industrially orientated. International differences in patenting by product groups show up particularly well.

1. Japan has industrial expertise in the area of amino acid synthesis, flavour enhancers, medical and fine chemical applications.
2. The U.S. has made a major effort in the production of HFCS and in the Veterinary and Medical fields.

The E.E.C. as a whole appears to show some extensive patent activity involving enzymes in areas including medical and veterinary products, HFCS's and fine chemicals.

Graphs representing the number of enzyme inventions other than hydrolases * granted to patentees in the period 1970-1979. The U.S. & Japan.

Fig.3.11.

Patents assigned to U.S. Nationals or U.S.Organisations

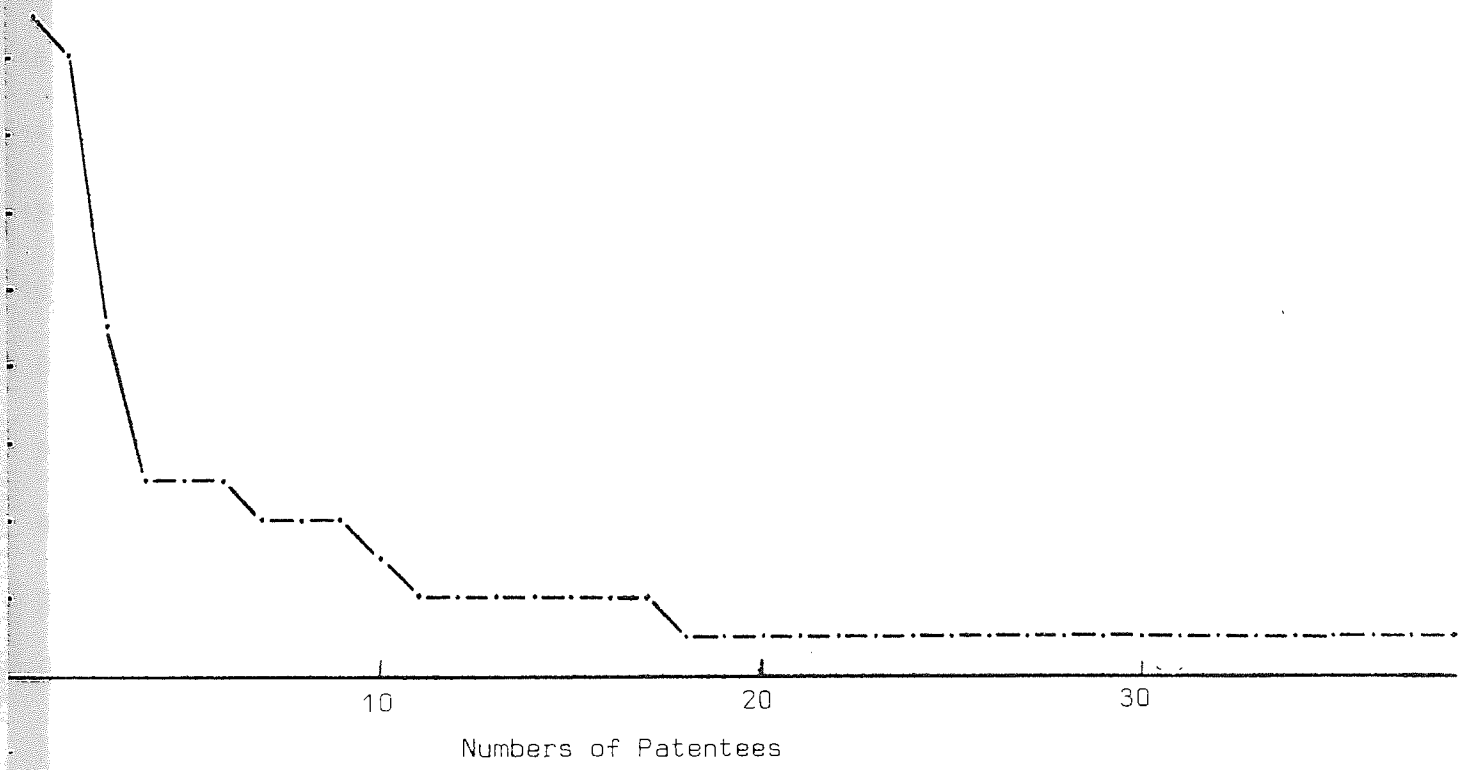
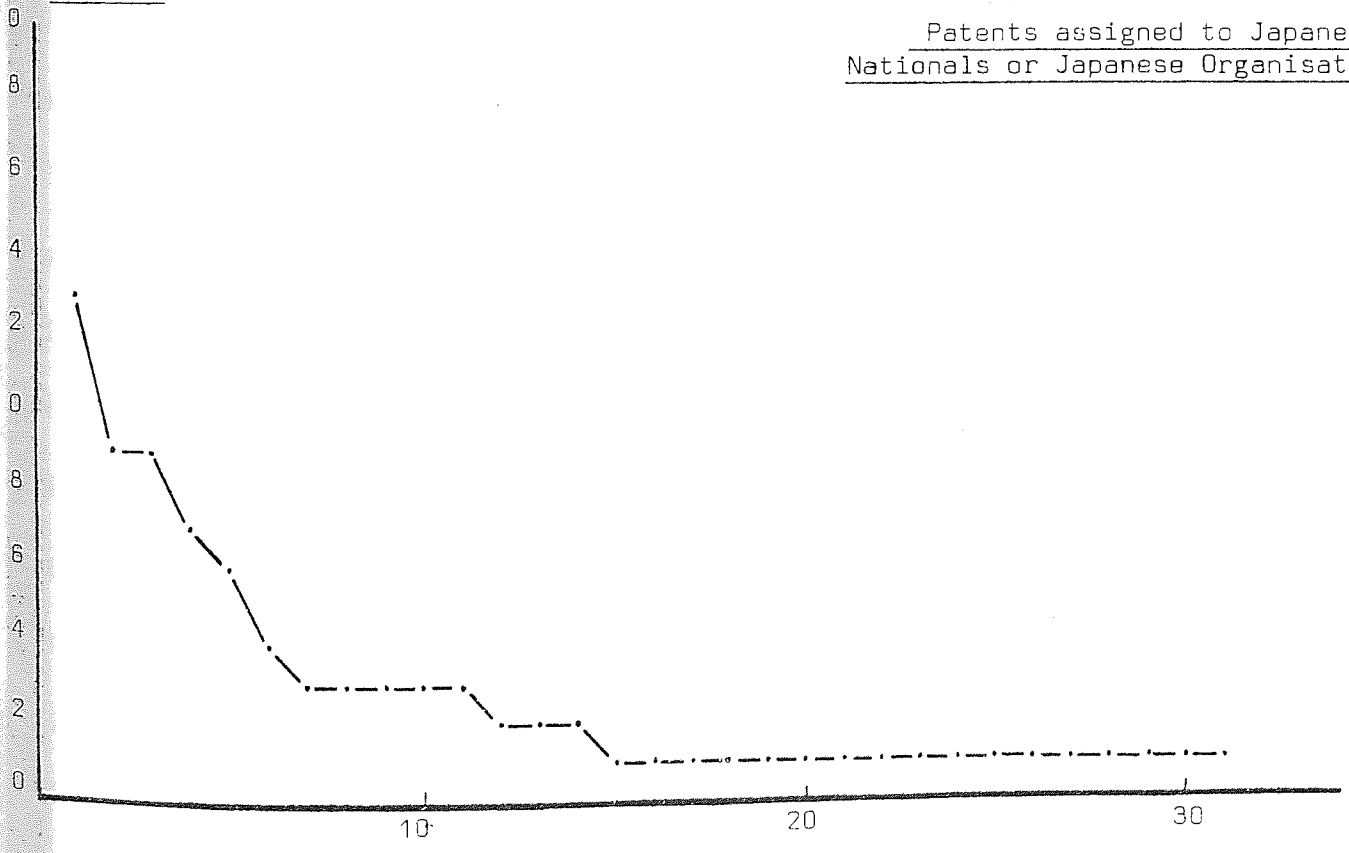


Fig.3.12

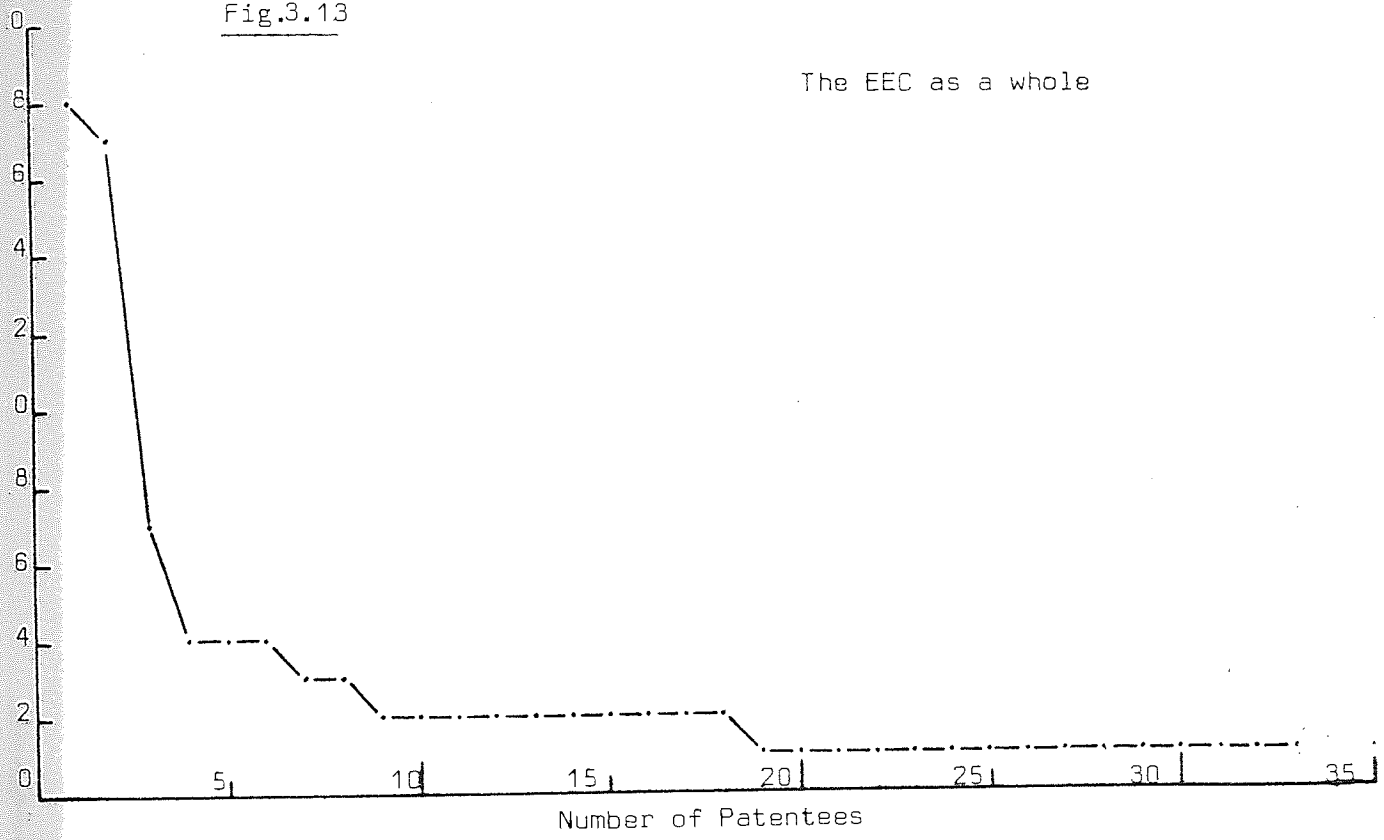
Patents assigned to Japanese Nationals or Japanese Organisations



Graphs representing the numbers of enzyme inventions other than those involving hydrolases granted * to patentees in the period 1970-79. The EEC and constituant countries

Fig.3.13

The EEC as a whole



Patenting for individual nation

Fig.3.14

FGR

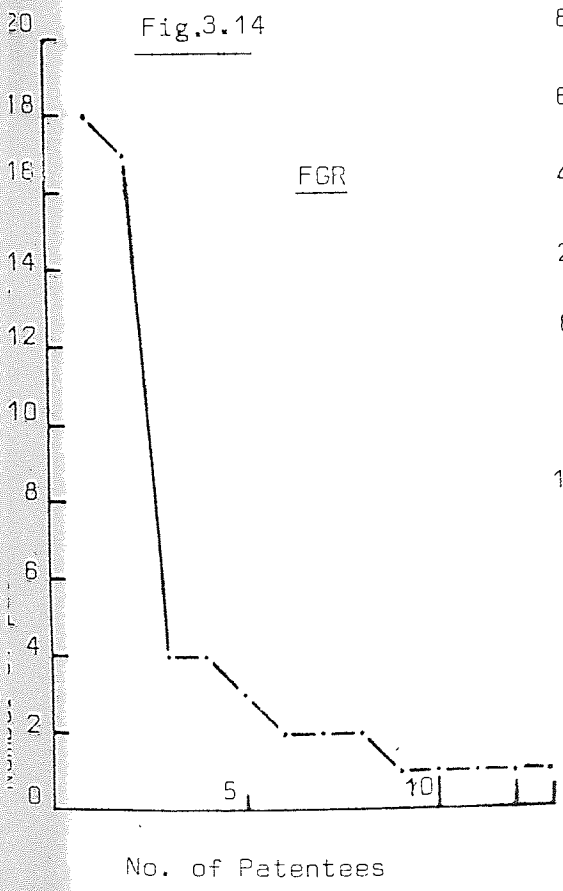


Fig.3.15

France

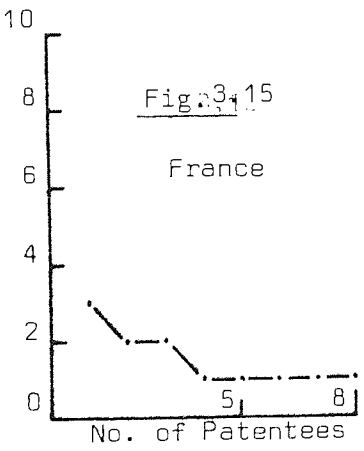


Fig.3.16

U.K.

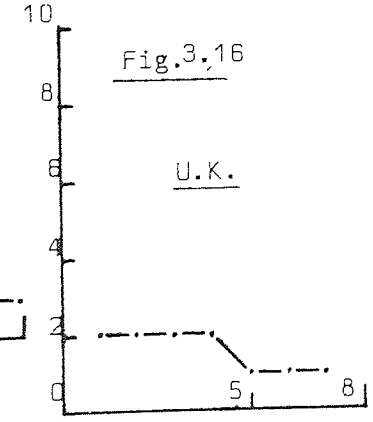


Fig.3.17

Denmark

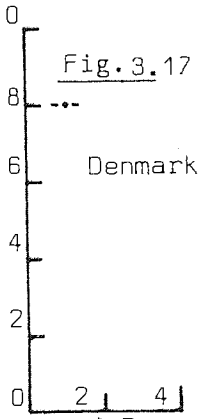


Fig.3.18

Netherlands

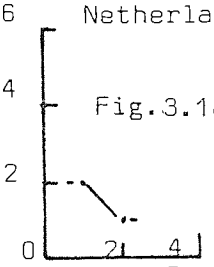


Fig.3.19

Italy

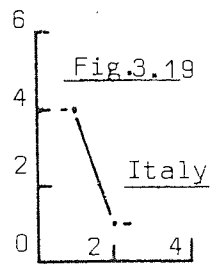
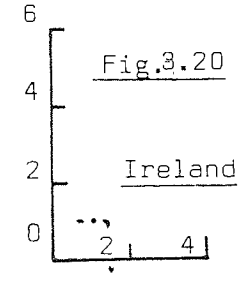


Fig.3.20

Ireland



Graphs representing the number of enzyme inventions other than those involving hydrolases * granted to patentees in period 1970-79. Other Nations.

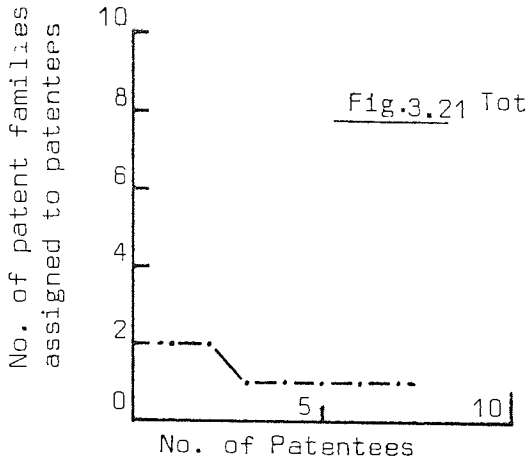


Fig.3.21 Total Other Nations

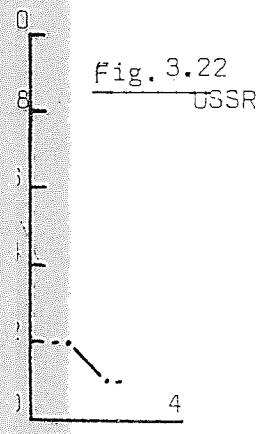


Fig. 3.22 USSR

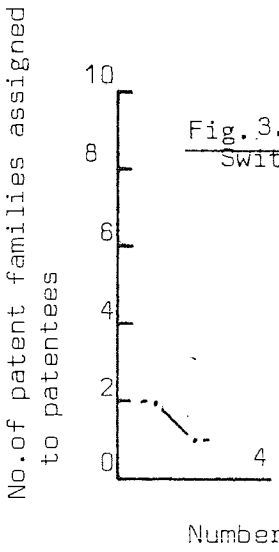


Fig.3.23 Switzerland

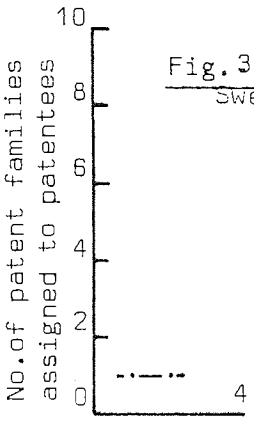


Fig.3.24 Sweden

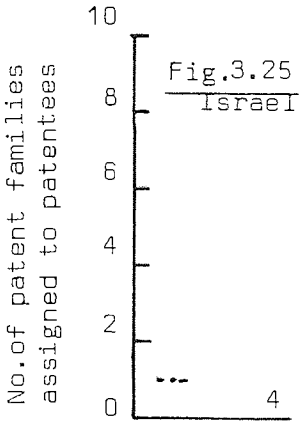


Fig.3.25 Israel

Analysis of the Propensity to patent by companies, by countries
(See Figs.3.11 - 3.25)

The plot of number of enzyme patents obtained by companies in rank order, shows that there are a few companies that have undertaken a concerted R & D programme in developing new products and/or exploiting enzyme routes as alternatives to established methods of production. However, a large number of companies have looked at enzymes and have considered their application. This is represented by the largest part of the plots of companies with a few patents. This is something that would be expected bearing in mind that the applications of enzyme technology has a potentially wide impact on the established industrial base. At present enzymes usually form only a small part of a company's range of products, and are often treated as a side line to companies' main areas of trading. It is only a few companies that have recognised the potential of the enzyme technology, have successfully identified a market and have made a commitment to its continued development and exploitation.

The national plots speak for themselves, particularly the plots of those countries that make up the E.E.C. Germany is the most active patenting country within the community.

The "other countries" category indicate very little overall patent activity.

Analysis of National patenting activities by patenting organisations and their interests.

In order to focus on national strengths I have carried out an analysis endeavouring to identify the type of organisations that are patenting in this area, their size and areas of trading. The analysis follows (Table 3.11) :

Number of Patents	Manufacturing Status	Patentees	Approx.Employees	Approx.Sales ($000,000 of $'s)	Beverages	Brewing	Corn Refining	Flavours	Food Additives	Food Processing Human Grade	Fermented Foods	Agricultural Chemicals & Specialities	Fine Chemicals incldg.Enzymes	Heavy Chemicals Inorganic	H.C.Organic	Detergents	Cosmetics	Parenteral Prod.	Vet.Pharm. Health Care	Oil	Tobacco	Photography
17	M	CPC International	42,000	3,200	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	M	Standard Brands	23,300	2,350	-	-	x	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-
8	M	Eastman Kodak Co	123,000	7,010	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x
5	M	Baxter-Travenol Labs	32,000	1,000	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-
5	M	Pfizer	40,700	2,390	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-
4	N	U.S. D.A.	Gov.Institute		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	M	Anheuser Busch Inc.	16,000	2,250	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	M	R.J.Reynolds	80,000	6,620	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-
4	M	A.E.Staley Manu.Co.	4,250	1,210	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	M	Merck & Co.	28,700	1,980	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-
2	M	E.I.Dupont de Namoures	131,000	10,500	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-
2	NF	Intellec.Prop.Dev.Corp.	?	?	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	M	Monsanto	62,800	5,010	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-
2	N	Pabst Brewing	5,000	735	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	M	Rohm & Haas	13,000	1,250	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-
2	M	The Upjohn Co	18,900	1,320	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-
2	None	Scheissberg M	None		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1	M	American Cyanamid	43,900	2,740	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-
1	M	Beckman International	10,000	338	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1	NF	Continental Oil	?	?	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1	M	Corning Glass Works	29,700	1,250	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1	NF	Davis Labs.Inc	?	?	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-
1	M	Dow Chemical Co.	53,500	6,880	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-
1	M	Drew Chemical Co. subsid.of U.S.Filter Corpn.	7,200	540	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 3.11. The US Patentees (cont'd)

Number of Patents	Manufacturing Status	Company	Employees	Sales (,000,000 of $'s)	Beverages	Brewing	Corn Refining	Flavours	Food Additives	Food Processing Human Grade	Fermented Foods	Agricultural Chemicals & Specialities	Fine Chemicals	Heavy Chemicals Inorganic	H.C. Organic	Detergents	Cosmetics	Parenteral Prod.	Vet. Pharm. Health Care	Oil	Tobacco	Photography
1	M	Eli Lilly & Co.	25,400	1,850	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1	None	Forgione	none		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1	None	Lycette, R.M.	none		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1	NF	Nevada Enzymes Inc.	?	?	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1	NF	Pierce Chemical Co.	?	?	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-
1	M	G.D.Searle	17,100	848	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-	-
1	M	Squibb & Sons	37,000	1,510	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-	-
1	N	Schenk R.U. et al	none		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1	N	Univ. of California	Academic	Institute-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1	N	US Government	Government	Institute-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1	N	Wisconsin Aluminii	Philanthropic	Organis-ation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1	M	Xerox Corp.	104,000	5,900-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1	M	(A&W) Teneco	104,000	8,760	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-

Key : M = Multinational Manufacturing status
N = National Manufacturing status
None = Indicates patents held by an individual
NF = None found

Comments on Table 3.11. The U.S. Patentees

The major group of organisations with patents in this area are the food corporations holding 45 patents in this analysis, especially C.P.C. (Corn Products Corporation) International which holds 17 patents alone. Two of these Corporations have multi-national interests.

Eastman Kodak has many patents linked to the reprocessing of silver residues. Areas outside its main trading include a growing interest in health care products and diagnostics.

Baxter Laboratories' patents can be tied in with the production of perenteral products, and diagnostics.

The companies with 1-3 patents are chemical and pharmaceutical companies which hold patents for a variety of preparations.

The bulk and specialist enzyme commercial producing companies include Baxter Travenol (Wallerstein Co.), Pabst Brewing Co., Rohm and Haas and the small company Nevada Enzymes Inc. They don't appear to have been actively involved in any of the rapidly innovating areas, from the patents assigned to them.

with additional information on company size and main areas of trading

Number of Patent famili	Manufacturing Status	Zaibatsu Affiliation	Patentees	Approx.number of Employees	Approx.Sales (000,000 Yen)	AREAS OF TRADING: Beverages	Brewing	Corn Refining	Flavours	Food Additives	Food Processing Human Grade	Fermented Foods	Agricultural Chemicals & Specialities	Fine Chemicals/ Ind.Enzymes	Heavy Chemicals Inorganic	H.C. Organic	Detergents	Cosmetics	Parenteral Prod.	Vet. Pharm. Health Care	Oil	Tobacco	Photography
13	N		Kyowa Kogyo KK	4,842	144,949	14%	-	-	-	-	20%	-	-	35%	-	-	-	-	-	31%	-	-	-
8	NF		Zaidan Hoyin Biseibutsu	?	?	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-
9	N		Takeda	11,074	373,019	-	-	-	-	-	-	18%	-	12%	-	-	-	-	-	59%	-	-	-
7	N	Sanwa	Tanabe Seiyaku KK	5,202	97,964	-	-	-	-	8%	-	-	-	-	-	-	-	-	-	92%	-	-	-
6	N		Hayashibara[1]	25	?	-	-	x	-	-	-	x[2]	-	x	-	-	-	-	-	-	-	-	-
4	M		Ajinomoto KK	5,508	308,263	-	-	-	-	28%	28%	23%	-	-	-	-	-	-	-	26%	-	-	-
3	N	Sumi-tomo	Dainippon Seiyaku	2,138	37,257	-	-	-	-	-	-	-	-	-	-	-	-	-	-	92%	-	-	-
3	N	Mitsu bishi	Kikkoman Shoyu	4,092	103,719	9%	-	-	72%	-	-	19%	-	-	-	-	-	-	-	-	-	-	-
3	N	"	Mitsubishi Kasei Kogyo KK	9,235	509,366	-	-	-	-	-	-	-	12%	-	18%	51%	-	-	-	-	-	-	-
3	None		National Food Research Inst. of Japan	Research & Patent Licensing Organisation		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	N		Toyo Jozo KK Subsid.of Ashai Chemical	1,765	42,659	47%	-	-	9%	-	-	-	-	-	-	-	-	-	-	44%	-	-	-
2	N		Eisai KK	3,058	60,974	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100%	-	-	-
	None		Noda Research Institute	Research Organ-isation		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	N		Kanegafuchi Kagaku Kogyo KK[4]	3,071	123,076	-	-	-	-	-	17%	-	-	10%	-	59%	-	-	-	-	-	-	-
1	N		Chugai Seiyaku KK + Iizuka	2,745	42,441	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100%	-	-	-
1	None		K. Hayashibara(individual)	None	None	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1	N	"	Kirin Beer KK	8,034	59,933	10%	90%	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1	M		Kumiai Kagaku Kogyo KK	918	50,144	-	-	-	-	-	-	-	100%	-	-	-	-	-	-	-	-	-	-

Number of Patent fami	Manufacturing Status	Zaibatsu Affiliation	Patentees	Approx.number of Employees	Approx. Sales (000,000 Yen)	AREA OF TRADING: Beverages	Brewing	Corn refining	Flavours	Food Additives	Food Processing Human Grade	Fermented Foods	Agricultural Chemicals & Specialities	Fine Chemicals/ Indust.Enzymes	Heavy Chemicals Inorganic	H.C. Organic	Detergents	Cosmetics	Parenteral Prod.	Vet. Pharm. Health Care	Oil	Tobacco	Photography
1	N		Marukin Shoyu KK Subsid. of Ajinomoto	374	4,522	-	-	-	87%	18%	-	-	-	-	-	-	-	-	-	-	-	-	-
1	N	Mitsubishi	Nippon Kayaku Kogyo KK	4,436	73,101	-	-	-	-	-	-	-	19%	43%	-	10%	-	-	-	-	24%	-	-
1	N		Nihon Sekiyu KK	2,995	1,611,856	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100%	-	-
1	N		Ono Yakuhin Kogyo KK	1,238	17,107	-	-	-	-	-	-	-	-	-	-	-	-	-	-	73%	-	-
1	N	Fuyo	Oriental Kobo Kogyo KK subsid. Fuyo Nisshen	521	20,697	-	-	-	-	63%	-	37%	-	-	-	-	-	-	-	-	-	-	-
1	None		Rikagaku Kenkyusho			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1	N	DKB	Sankyo KK	5,488	91,500					7%			16%							77%			
1	N		Sanraku Ocean KK subsid. of Ajinomoto	1,252	38,596	62%				32%				16%									
1	NF		Seikagaku Kogyo Co KK	?	?	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1	N	Sanwa	Tokuyama Soda	2,442	106,503	-	-	-	-	-	-	-	-	-	27%	55%	18%	-	-	-	-	-	-
1	N	Sumitomo	Taisho Pharmaceutical Co.	2,932	57,600	-	-	-	-	-	-	-	-	-	-	-	-	-	-	99%	-	-	-
1	N	Mitsubishi	Toray Industries	17,839	418,482	-	-	-	-	-	-	-	-	-	7%	93%	-	-	-	-	-	-	-
1	None		Ko Aida	None	None	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

1 Hayashibara Seibutsu Kugaku Kenkyujo
2 Low calorie sweetners syrups
3 Diversified
4 Subsidiary of Kanebo

Key: M = Multinational Manufacturing status
N = National Manufacturing status
None= Indicates patents held by an individual
NF = None found

Comments on Table 3.12

This table shows that there are few Japanese companies with a multinational production basis. Japan's commercial efforts are more geared to domestic production, consolidating the home market and exporting 'Bio.' products to foreign markets from the secured home market base. (Exceptions such as Ajinomoto however show how rapidly they can change from national to multi-national production status). This may be the reason for P. Marstrand's observation that approximately only 1:8 of Japanese biotechnology patents are patented abroad. The companies that are quite active in patenting enzymes have broadly diversified market interests and also a high number of the companies have pharmaceutical interests followed by food interests, chemical interests and then an interest in beverages.

The Mitsubishi industrial grouping (8) is represented strongly with the patent activities in the following companies. Kikkoman Shoyu (3) Mitsubishi Kasei Kogyo KK (3) Kirin Beer KK (1) and Nippon Kayaku Kogyo KK (1); The Sanwa industrial grouping (8) represented by Tanabe Seiyaku (7) and Tokuyama Soda (1): The Fuyo industrial grouping by Oriental Kobo Kogyo (1); The Sumitomo industrial grouping represented by Dainippon Seiyaku (3) and Taisho Pharmaceutical Co.(1); Mitsui Zeibatsu represented by (1) Toray Industries (1). In terms of organisation and size the industrial grouping overshadows many of the multinational corporations that we regard as dominant forces in the West. (see Table 3.13)

Kyowa Kogyo, Takeda, Ajinomoto, Eisai, Nihon Sakiyu and Hayashibara appear to be independents. Ajinomoto became an

Table 3.13 JAPANESE INDUSTRIAL GROUPINGS VS MULTINATIONALS ORGANISATIONS

Total turnovers of the leading six Japanese industrial groups are compared with some of the leading multinationals' turnovers as follows:

	1974		1976	
	Turnover (¥billion)	Index (Mitsubishi=100)	Turnover (¥billion)	Index (Mitsubishi=100)
bishi Group	18,842	100	22,503	100
i Group	14,063	75	16,360	73
omo Group	14,159	75	15,362	68
Group	11,829	63	14,654	65
roup	13,771	73	16,206	72
Group	12,020	64	13,841	62
	12,618	67	14,589	65
Dutch-Shell	9,911	53	10,826	48
	9,465	50	14,154	63
	7,086	38	8,652	38
ver	4,100	22	4,729	21
	4,024	21	4,709	21
	3,803	20	4,891	22
	3,346	18	3,529	16
os	2,827	15	3,456	15
Steel	2,756	15	2,581	11
en	2,599	14	2,384	11
	2,549	14	2,761	12
	2,074	11	2,240	10
du Pont	2,073	11	2,508	11
é	1,681	9	2,288	10
sh Steel	1,602	9	1,501	7

Note: Turnover of each group - aggregaged turnover of all the companies listed in the Full Listing of Group Companies in Part II except banks and insurance companies.

In terms of size by turnover the leading six industrial groups are larger than most major multinationals. Mitsubishi Group is about twice as large as Royal Dutch-Shell, d Fuyo and Sanwa Groups are three times larger than Unilever, G.E. and I.B.M. in terms turnover.

During the last three years from 1974 to 1976 Fuyo Group has increased turnover arply by 23.9% followed by Mitsubishi Group with 19.4%.

Among major multinationals G.M., Ford, I.B.M. and Nestlé have increased their turnover nspicuously during the same period.

Source : Industrial Groupings In Japan - Dodwell Marketing Consultants 1976 Edition

of trading.

Number of Patent Famili	Manufacturing Status		Approximate Employees	Approximate Sales (000) of national currency	Beverages	Brewing	Corn Refining	Flavour	Food Additives	Food Processing Human Grade	Fermented Foods	Agricultural Chemicals & Specialities	Fine Chemicals (incl.Enzymes)	Heavy Chemicals Inorganic	H.C. Organic	Detergents	Cosmetics	Parenteral Prod.	Vet. Pharm. Health Care	Oil	Tobacco	Photography
BELGIUM																						
1	N/M	Kali-Chemi AG [Subsid.of Solvay]	2,882	404,000DM	-	-	-	-	-	-	-	-	x	x	x	-	-	-	-	-	-	-
DENMARK																						
6	M	Novo Industri A/S	2,859	938,796,000Kr	-	-	-	-	-	-	-	-	50%	-	-	-	-	-	50%	-	-	-
FRANCE																						
2	NF	ANVAR	Patent development and licensing organisation		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	N	L'Air Liquide	5,949	2,048,360Fr	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-
2	None	Modrovich	none	none	-	-	-	-	-	-	-	-	-	x	x	-	-	-	-	-	-	-
2	M	Rhone Poulenc Industries SA	33,200	8,343,000Fr	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-
1	NF	Choay	?	?	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1	M	Les Produits Organique Du Sante Orsan	473	135,486Fr	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-
1	N	Roquette Freres SA	1,994	1,080,644Fr	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1	None	Societe des Etudes et d'application Biochemiques	none	none	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
GERMANY																						
18	M	Bayer Aktiengesellschaft & Miles Labs.	62,408	2,130,000,000DM	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-
17	M	Boehringer Mannheim GmbH			-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-
4	N	Henkel & CIE GmbH	15,280	96,000,000DM	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-
4	M	Hoechst & (Behringwerke A.G.)	178,710; (2,000)	20,201,000,000DM (2,130,000,000DM)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-

Number of Patent Families	Manufacturing Status		Approximate Employees	Approximate Sales (000) of national currency	Beverages	Brewing	Corn Refining	Flavour	Food Additives	Food Processing Human Grade	Fermented Foods	Agricultural Chemicals & Specialities	Fine Chemicals (incl.Enzymes)	Heavy Chemicals Inorganic	H.C. Organic	Detergents	Cosmetics	Parenteral Prod.	Vet. Pharm. Health Care	Oil	Tobacco	Photography
GERMANY																						
3	NF	Forschung institute für Die Garungsindustrie, Enzymologie und Technische Microbiologie	Unknown	Unknown	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	M	W.R.Grace & Co. Subsid of Frederich Flich Industrie Verwaltung KGaA	"	"	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	NF	Gesellschaft für Biotechnologische Forschung GmbH	"	Research Organisation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	NF	Veb-Arzenemittelwerk	"	"	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1	NF	Institut Fresenius	"	"	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1	M	E.Merck Patent GmbH Subsidiary of E.Merck.	7,910	942,400,000DM	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1		Imp/Pfeifer & Langen K.G. (Koln) Exp?	Unknown	Unknown	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1		None Schmidt.	none	none	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1		None G. Wober	none	none	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
IRELAND																						
1	NF	Irish Stone Foundation	Unknown	Unknown	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ITALY																						
4	N	Snam-Progretti S.p.A	3,100	60,000,000L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X
1	NF	Societa Italiana Resine S.p.A	Unkown	Unknown	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 3.14 The E.E.C. Countries cont'd

Number of Patent Families	Manufacturing Status	Patentees	Approximate Employees	Approximate Sales (M)	Beverages	Brewing	Corn Refining	Flavour	Food Additives	Food Processing Human Grade	Fermented Foods	Agricultural Chemicals & Specialities	Fine Chemicals (incl. Enzymes)	Heavy Chemicals Inorganic	H.C. Organic	Detergents	Cosmetics	Parenteral Prod.	Vet. Pharm. Health Care	Oil/Gas	Tobacco	Photography
NETHERLANDS																						
	M	Akzo & Organon	83,200	2,727,800,000DF	-	-	-	-	-	-	-	-	-	×	×	-	-	-	×	×	-	-
	M	Shell, Nederland N.V.	?	?	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
U.K.																						
2	M	Beecham Ltd.	31,900	£ 933,100,000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	×	-	-	-
2	M	I.C.I.Ltd	125,000	£ 4,135,000	-	-	-	-	-	-	-	-	-	×	×	-	-	-	×	×	-	-
1	M	Glaxo Co.	29,781	£ 539,056,000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	×	-	-	-
2	M	Wellcome Foundation Ltd.	1,700	£ 32,000,000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	NF	N.R.D.C.	Research development and licensing organisation		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1	NF	Sec. of State for Defence	?	?	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1	None	T.P.Whitehead	none	none	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Key

N = National } Manufacturing base
M = Multinational }
None = Indicates patents held by an individual
NF= None found

established leader in amino-acid production in the postwar period and is now a dominant force in this area.

Comments on the E.E.C.Patents Table 3.14

Generally the companies involved in patenting are multi-nationals, Novo is reputed to be the world's largest producer of bulk enzymes and it is one of the two companies that appears to have developed many patents around industrial enzymes e.g. the patents of glucose isomerase.

The German patents obtained by Bayer, were for the use of asparaginase - in anti-leukemia treatment, whilst the main Miles Patents are again concerned with producing glucose isomerase.

Boehringer Mannheim is a leader in diagnostic enzymes and this is reflected in the numbers and variety of patents it has taken out, in this field.

Apart from these companies, patent activity is low, although a large proportion of these companies are established pharmaceutical manufacturers.

There appear to be more patents taken out as a result of German Government Aided Applied Research than emerge from any other member states. France manages 1 patent, and the U.K. none. However 2 patents emerge from the work of the N.R.D.C. which serves to facilitate the patenting of inventions that emerge from university or private research in the U.K.

Other Countries

I have not bothered to analyse these patents as there were so few of them.

Summary of Observations on Patenting by Companies and by Category. Table 3.14

(i) General

1. The major companies innovating in enzyme technology in the U.S. and the EEC have a multinational production basis; the companies innovating in enzyme technology in Japan use a national basis for manufacture.

2. In size Japanese companies (or trading units) appear smaller than their U.S. or EEC counterparts. However, those that belong to any of the new company grouping, are linked into a network of banks, finance houses and expertise in industry and government that are unrivalled in the West.

(ii) The Food Companies

Judging by patent activity above, the largest development in enzyme technology appears to have taken place in the U.S. within the corn processing industry. No such equivalent development has occurred in the EEC, but then the EEC is a net exporter of sugar and the pressure to find alternative substitutes for sugar is lower, in part governed by market focus and in part modified by a system of levies and their quotas on production. Japanese advances in this field are also prompted by their need to find sugar substitutes, as a consequence Japanese companies appear to have developed a variety of possible alternatives. The long tradition of using fermented foods provided the Japanese with a domestic market into which it was possible to innovate a variety of fermented food products or additives. An equivalent fermentation based industrial sector is missing in the West, here

colourants, flavours and fragrances have been developed as speciality chemicals.

(iii) The Pharmaceutical Companies

There has been a general interest in patenting enzymes in the world's pharmaceutical industry. This is because, at present, enzymes are most suited to bring about specific transformations within complex macromolecules which are difficult to achieve by chemical means, as illustrated by the discussion of the use of penicillin amidase in chapter 8. This area is developing rapidly judging by the numbers of patents taken out within the EEC. Generally European companies trading in the health care sector appear to be at least holding their own, if indeed they are not ahead in the development of this technology.

The U.S. and EEC pharmaceutical companies are larger than the Japanese companies. It must be remembered that pharmaceutical companies operate in a highly specialised market, and within precise and well defined legal limits. They form a special group as far as this study is concerned. Their behaviour within the enzyme field reflects this for example "co-operation" in patent cross licensing and the division of markets by agreement are known to occur. Fermentation knowhow and expertise makes for easier technology transfer between the production of antibodies and the production of enzymes. A notable example is the Danish company NOVO Industri A/S which was and still is a major insulin producer but since the 1960's has been recognised as a leading bulk enzyme producer, steroid modifications, semi-synthetic penicillins, therapeutic enzymes, all seem to be

natural developments for pharmaceutical companies and fermentation knowhow.

(iv) The Chemical Companies

The chemical companies seem to be more interested in supplying resins or assistance with general purification technology than actually getting involved in enzyme purification, processing and marketing of enzyme products. These are inevitable exceptions.

The Japanese developments that have no counterparts in the EEC or U.S. have traded in the areas of food additives, vitamins and fine chemicals. The world consumption of these products has been increasing as indicated by the world tonnage statistics. This market has been satisfactorily supplied by the Japanese producers, e.g. amino acids, especially monosodium glutamate. The other area of development that has no counterpart in the West has been the development of Japanese co-enzyme or cofactor patents (a spin off the development of DNA based food flavour technology!). Within the scientific literature there has been evidence of the use of these products in regeneration schemes coupled to syntheses with enzymes, a current commercial use is as therapuetic drugs in counteracting such things as liver disorders, and a process for producing L-alanine by the German Company Degussa.

(v) Summary

Both in the areas concerning food additives and vitamin production Japan appears to have more patents than any other

country. Such additives come under strict legislation, but their introduction has been hampered more by conservative Western tastes; a situation that is beginning to change. The EEC companies most suited to take up the challenge would be those already in the food processing industry, as they have the advantage of marketing know-how and distribution, or from any of the companies which are involved in fermentation. Perhaps joint venture companies may help reduce the cost of initial investment and reduce the risk of such ventures.

Synthesis has long been the established route in the chemical industry. Enzyme linked systems to cofactors could enable developments to take place that could compete with chemical routes for synthesis of some complex chemicals. But we found no patents in our data base for using enzymes in this way. However, alternative pathways starting off with a complex molecule, re-arranging it or breaking it down should not be overlooked as possibilities. There appears to be a little activity in re-arranging molecules, particularly the large steroids, by enzyme use, within the pharmaceutical sector.

Analysis of enzymes by IUB categories mentioned in patents

An analysis of enzymes on pages 103-5 gives the following breakdown representing the number of enzymes with at least one mention in the patents by IUB categories.

Table 3.15. Analysis of enzymes by IUB Categories found in the Appendix

IUB Categories	No. of Enzymes mentioned at least once
Oxidoreductases	30
Transferases	12
Hydrolases	36
Lyases	17
Isomerases	2

These figures are distorted by the following:

1. some general patents may cite many enzymes;
2. the Hydrolases are grossly underestimated due to their early separation prior to processing:

Nevertheless these figures tend to highlight categories of enzymes receiving most commercial attention indicating perceived potential utilisation of enzymes by patentees.

3.3.8. Case F - Summary and Analysis of R.P.van de Kasteele's Report.

3.3.8.1. The Report

This is the second report of the series edited by Martin Terpstra. It was written by R.P.van de Kasteele and made for the Commission of European Communities Directorate General XIII, "Scientific and Technical Information and Information Management" Patents Division, Luxemburg. As before, the sources are patent applications and patents, published by the industrialised countries during the period 1970-79. No differentiation has been made between patents and patent applications, both were designated as patents.

This report is called "Application of Enzymes" and is divided into the following four chapters:

Chapter 1 - Introduction

Chapters 2-4 - Deal with the patent analyses under the following headings:

Chapter 2 - Technologies (generally applicable technologies)

Chapter 3 - Analytical applications
Cosmetics applications
Pharmaceutical applications
(including production technologies for specific enzymes)

Chapter 4 - Therapeutic applications
(including production technologies for specific enzymes)

This report concentrates in general on the medical and fine chemical aspects of enzymes. The review mentions around 1150 patents published in 16 countries, of these 540 main patents are discussed, the rest are corresponding patents - (Patent family members), "of which 90% owned 70% of the patents" (i.e. 1-3

patents/company), "10% owned 30% of the patents" (more than 3 patents/company). This situation is similar to the one I found in Case E. (see Figs. 3.11-3.25.)

The report further states that no one company has a really dominant position.

As R.P.van de Kasteele had carried out quite extensive analyses on this data we propose to present his data findings and proceed with a further analysis to highlight national differences, using van de Kasteele's own classification found in Chapters 2, 3 and 4. However, I have made slight alterations reclassifying certain enzyme patents where needed.

3.3.8.2. Chapter 2. Technologies - Abstract of van de Kasteele's findings.

Van de Kasteele encountered difficulties in carrying out an acceptable classification of the various technologies because of

a) Lack of uniformity in standard or unit processes.

b) Unit operations are common but occur in different sequences in processes.

c) Technological keywords in patent titles can be extremely misleading and may have very little relation to the subject of the text or the claim of the patent.

He resolved this problem by creating an idealised process and assigning patents to its components. (Not all patents listed in the tables were mentioned in van de Kasteele's text.)

Table 3.16 A comparison of van de Kasteele's data with information contained in the text

GENERAL PROCESS TECHNIQUES		PATENTS FROM TABLE	FROM TEXT
Specific Processes	Raw material extraction	4	4
	Precipitation	8	8
	Oesalting	4	4
	Purification	17	15
	Oehydration	3	3
Specific process techniques sub-total		36	34
Enzyme Stabilization		20	17
Confectioning		17	15
General process techniques total		73	66

Van de Kasteele stressed that :

a) such terms as absorption, concentration, dissolving, and separation are not used because they are included in the majority of the cited basic process steps.

b) solid-liquid separation of the raw extract is not cited, because there were no patents submitted on this subject, i.e. conventional separation by centrifugation, filtration or sedimentation.

If the relative importance of these groups is indicated by the number of patents in each group then it follows that most proprietory interest concerns just three processing stages, enzyme purification, enzyme stabilization and confectioning. (see Table 3.16.)

Table 3.17 Analysis of "Chapter 2" to highlight national strengths.

Processes or Techniques \ Country	United States	Japan	E.E.C.								E.E.C. Totals	From analysis of Text	From Table presented in Chap.2.
			Belgium	Denmark	France	Germany	Ireland	Italy	Netherlands	U.K.			
Specific Processes	I O	I O	I O	I O	I O	I O	I O	I O	I O	I O	I O		
Raw materials extract	1 -	- -	- -	- -	- -	2 1	- -	- -	- -	- -	2 1	4	4
Precipitation	1 2	- -	- -	- -	- 1	- 4	- -	- -	- -	- -	- 5	8	8
Desalting	- 2	- -	- -	- -	1 -	- 1	- -	- -	- -	- -	1 1	4	4
Purification	1 5	- 4	- -	- -	- 1	- 1	- -	- -	- -	3 -	3 2	15	17
Dehydration	1 -	- -	- -	- 1	- -	- 1	- -	- -	- -	- -	0 2	3	3
Process Techniques Total	4 9	- 4	- -	- 1	1 2	2 8	- -	- -	- -	3 -	6 11	34	36
Stabilization	2 5	- -	- -	- -	3 1	2 3	- -	- -	- 1	- -	5 5	17	20
Confectioning	- 4	- 3	- -	- 1	- 2	- 2	- -	- -	- 1	- 2	0 8	15	17
General Processes Total	5 18	- 7	- -	- 2	4 5	4 13	- -	- -	- 2	3 2	11 24	66	73

Key: I = Patents assigned to individuals, O = Patents assigned to Organizations

Analysis of Chapter 2 to highlight national strengths (see Table 3.17)

Comment

On the whole the match between tabular and textual information is acceptable. As the total discrepancy between the two is 9.6% I have continued with the analysis. There appears to be a large number of process patents in the U.S. (24) and FDR (17). Also the numbers of patents obtained by individuals is noteworthy, 6 in the U.S., 3 in France, 2 in Germany and 3 in U.K. but none in Japan. Research in Japan appears to be team orientated. Japan

has few patents in the "general process techniques" category - and of those 4 concern purification and 3 confectioning of the enzyme product.

Against the backcloth of industrial activity discussed in chapter 2 these results appear to be contradictory. However a possible explanation may be that many of the processes in Japan use immobilised cells, to catalyse reactions or effect biotransformations. This is because they are cheaper to prepare and use on an industrial scale than enzymes. In which case use of the term biocatalyst could be more appropriate since it should include intracellular enzyme catalysed processes as well. Unfortunately this term has not entered the data bases and in this case would be of little help.

The bulk of the EEC patent effort seems to come from Germany (17) then France (9) and then the U.K. (5).

There is no activity from the Other Countries group.

3.3.8.4. Chapter 3. Applications

It should be remembered that most of the enzymes application discussed by van de Kasteel are in the medical and research field.

Van de Kasteele divided Applications into 4 categories:

Approximate	No.of Patents	% of Total
a) Analytical applications	21	5
b) Cosmetic applications	17	4
c) Pharmaceutical	69	17
d) Therapeutic	304	74
Total	411	100

The latter applications were given a separate chapter which formed about half the patents under review.

Van de Kasteele observed that :

(i) The number of patents produced over 20 years turned out to be too small to discern any significant trend. The only exception being enzymes such as:

(i) Asparaginase - (1968-73 28 patents)

(ii) Urokinase - (1970-78 34 patents)

(ii) The number of specific enzymes used in the application field under review is surprisingly small in comparison with the total number of EC* identified enzymes.

Table 3.18 Known numbers of enzymes by classes, compared with patents received from 'Applications of Enzymes' by R.P.van de Kasteele p.13.

E.C.* Main Class	EC* coded	% of Class	Review	% of Class	% of Review as EC
1. Oxidoreductases	481	26.5	16	20.5	3.3
2. Transferases	484	26.6	12	15.4	2.5
3. Hydrolases	458	25.2	43	55.2	9.4
4. Lysases	230	12.7	7	8.9	3.0
5. Isomerases	87	4.8	-	-	-
6. Ligases	77	4.2	-	-	-
Total	1817	100	78	100	4.3

* EC = Enzyme classification.

Van de Kasteele also states "It is unknown", at least to him, "how many of the 1817 enzymes coded are only laboratory curiosities, but whatever the reason, the difference is striking. Table 3.18 shows furthermore that in the field under review the hydrolases are again by far the most widely patented enzymes and that the less common classes are as yet not used at all".

There is a small number of therapeutic enzymes patents, but 50% of those are applications of the ten most used enzymes.

The patent literature reviewed supports the idea that the use of enzymes is still in its infancy in this area.

Of the large number of claims presented the majority still have to be proved in practice, either with regard to their therapeutic properties or efficiency, their production in suitable quantities, and at an acceptable price.[2]

I have reanalysed chapter 3 to highlight national strengths, largely following the format adopted by van de Kasteele.

Chapter 3 Applications

1. Non Therapeutic Applications
   a. Analytical applications
   b. Cosmetic applications
   c. Pharmaceutical applications

2. Therapeutic Applications
   d. (subdivided into 7 sections)
      (i) Blood Group
      (ii) Anti-Tumour Group
      (iii) Digestive Group
      (iv) Skin Group
      (v) Anti Inflammatory Group
      (vi) General Therapeutic Group
      (vii) General Enzyme Group

Non-Therapeutic Applications - Reanalysed to show national strengths

a. Analytical Applications

Table 3.19 Analysis of 'Analytical Applications' by patent controlling country

Applications \ Country	U.S. I	U.S. O	Japan I	Japan O	Belgium I	Belgium O	Denmark I	Denmark O	France I	France O	Germany I	Germany O	Greece I	Greece O	Ireland I	Ireland O	Italy I	Italy O	N'lands I	N'lands O	U.K. I	U.K. O	USSR I	USSR O	Panama I	Panama O	Australia I	Australia O	Total
(i) Analytical Reagents	-	6	-	-	-	-	-	-	1	-	10	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	18
(ii) Diagnostic Reagents	1,1	-	-	-	-	-	-	-	1,1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4
Total	1,7	-	-	-	-	-	-	-	1,1	1,11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	22

Key * = Individuals O = Organisations

Analytical Applications

This is the small research market, and a major effort appears to come from Germany and the U.S. The diagnostic market appears to be small, however some of these enzymes may appear in the general category.

b. Cosmetic Applications

Table 3.20 Analysis of 'Cosmetic Applications' by patent controlling country

Applications \ Country	* U.S. I	U.S. O	Japan I	Japan O	Belgium I	Belgium O	Denmark I	Denmark O	France I	France O	Germany I	Germany O	Greece I	Greece O	Ireland I	Ireland O	Italy I	Italy O	N'lands I	N'lands O	U.K. I	U.K. O	USSR I	USSR O	Panama I	Panama O	Australia I	Australia O	Total
(i) Mouth Hygiene	-	3	-	3	-	-	-	-	1,1	-	-	-	-	-	-	-	-	-	-	-	1,1	-	-	-	-	-	-	1	1,1
(ii) Skin Hygiene	-	-	-	-	-	-	-	-	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6
Total	-	3	-	3	-	-	-	-	7,1	-	-	-	-	-	-	-	-	-	-	-	1,1	-	-	-	-	-	-	1	1,7

Cosmetic Applications

These include a variety of tooth paste formulations, and anti-bacterial enzymes.

Key: * I = Individuals O = Organisations

c. Pharmaceutical Applications

Van de Kasteele distinguished between pharmaceutical and therapeutic applications according to the following rules. Patents having pharmaceutical characteristics are :

(i) Applications that use enzymes for the production and preparation of chemicals with pharmaceutical characteristics.

(ii) Therapeutic products in which an enzyme or enzymes are a minor constituent.

(iii) Enzyme inhibitors.

Table 3.21 Analysis of 'Pharmaceutical Applications' by patent controlling country.

Applications \ Country	*	U.S.		Japan		Belgium		France		Germany		Ireland		Italy		N'lands		U.K.		Panama		Sweden		Total	
		I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O
Processes		-	4	-	2	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	1	-	-	9	
Products		-	2	-	1	-	-	-	5	2,2	-	-	-	1	-	-	-	-	-	-	-	-	-	-	13
Inhibitors - Wide Ranging		-	-	-	6	-	-	-	1	-	5	-	-	-	-	-	-	-	-	-	1	-		1,12	
Inhibitors - Specific		1,4		-	3	-	-	-	1	1,8		-	-	-	-	-	-	-	-	-	-	-	-	18	
Total		1,10		-	12	-	-	-	7	3,17		-	-	1		-	-	-	-	-	1,1	-		1,52	

Pharmaceutical Applications Comments

There are 16 patents unaccounted for in the text that appears in the analysis i.e. 23% of the total. In the inhibitors section of this analysis Bayer (of Germany) and Zaidan (of Japan) seem to be by far the most active in patenting this form of therapy. Once again there appears to be only minor interest in this area.

Key * I = Individuals O = Organisations

Enzymes feature in product applications particularly strongly in the EEC.

d. The Therapeutic Applications

The Report.

This section contains some 325 patents which were divided by van de Kasteele according to the following categories in

(i) diseases

(ii) product characteristics -

P specific

E, S wider

L very wide

(iii) Raw materials used

(iv) Mode of administrations.

The final divisions of products and patents are shown as follows:

Blood

Digestive

Skin

General Inflammatory

Various

And an unknown category.

Table 3.22 Table 4.1 from p.24 of van de Kasteele's study 'Applications of Enzymes.

Prod.group:	P PR-PA	E PR-PA	S PR-PA	L PR-PA	Total PR-PA	%
Blood	11-91	2-2	2-4	1-1	16-98	(35%)
Digestive	13-36	2-20	3-16	5-5	23-77	(28%)
Skin	11-37	----	----	---	11-37	(14%)
Gen.Inflam.	7-15	3-19	----	1-1	11-35	(13%)
Various	7-9	2-3	2-2	1-1	12-15	(5%)
X	9-10	----	1-1	4-4	14-15	(5%)
Total	58-198	9-44	8-23	12-12	87-277	(100%)

Patenting by product group and type of patent protection sought

KEY PR - Product (roughly equivalent to an enzyme)
PA - Patents
P,E,S,L- see page 65

These data show that the Blood group : with 35% of the patents is by far the most important, with the Digestive group with 28% a good second.

Some seven enzymes have dominated the patenting activity. The range of patenting per enzyme product is shown in the table below.

Table 3.23 Table 4.2 from page 30 of the van de Kasteele study 'Application of Enzymes'.

Number of Patents	25 PR-PA	10-24 PR-PA	5-9 PR-PA	3-4 PR-PA	2 PR-PA	2 PR-PA	Total PR-PA
Blood	2-59	1-12	2-13	2-6	1-2	8	16-98
Digestive	----	2-25	3-20	5-16	3-6	10	23-77
Skin	----	1-19	----	3-10	1-2	6	11-37
Gen.Inflam.	----	1-16	1-5	2-6	1-2	6	11-35
Various	----	----	----	----	3-6	9	12-15
X	----	----	----	----	1-2	13	14-15
Total	2-59	5-72	6-38	12-38	10-20	52	87-277
in %	2-21	6-26	7-14	14-14	11-7	60-18	100-100

Patenting by product group and number of patents.

These tables show that there are only 7 products with more than 10 patents, which represents 47% of the total patents, and even 60% of the patent total is derived from 15 products. The remaining 74 enzyme products, representing 39% of the patents, can only have received slight development.

Van de Kasteele also mentioned the problem of classifying enzymes having more than one use, particularly if they had applications in an unrelated field e.g. therapy and food preservation. Mixtures of enzymes also caused classification problems and these were grouped under a "Various" Category As to the real use of enzymes in medicine van de Kasteele claims that "of the 135 enzymes cited in the text only 31, or less than 25%, are listed in Martindale 'The Extra Pharmacopeocia' or the list of the Royal Netherlands Pharmaceutical Society as approved for therapeutic use. The most interesting enzyme, urokinase, seems as yet not approved,* at least is not cited as such in the above lists." (1982)

In the reanalysis of this data I reclassified those enzymes from the "various sections" and re-allocated them where possible. I have also created a new section dealing with anti-tumour activity.

My analysis of the therapeutic group is divided as per Table 3.24.

* Urokinase has been available for parenteral use in some countries since 1965, Japan represents the largest market - in excess of $70,000,000 in 1976.

Table 3.24 Therapeutic Enzyme Applications by Categories

Sub-Section	Category
d(i)	Blood Group
d(ii)	Anti-Tumour Group
d(iii)	Digestive Group
d(iv)	Skin Group
d(v)	Anti-Inflammatory Group
d(vi)	General Therapeutic Group General Enzyme Group

d(i) Comments on Blood Group Table 3.25 - Blood Group Enzymes

Of these enzymes urokinase is receiving most attention. Most of the patents are concerned with achieving improved purification and cheaper production methods, problems none of the patents seem to have resolved, although the enzyme is in use in the U.S. and Japan. Streptokinase, which has a longer history than urokinase receives less attention. French companies hold a number of patents in this area but they appear to be concerned with developing new alternatives. It should be noted that heparin appears in the text as a substance possessing anti-coagulant activity but as it is not an enzyme it has been excluded in our analysis, although van de Kasteele included it in his report.

d'1 Therapeutic Enzymes - Reanalysed.

d'1(i) Blood Group

Table 3.25 Analysis of Blood Groups by patent controlling country.

Enzymes \ Country	U.S.		Japan		Belgium		Denmark		France		Germany		Greece		Ireland		Italy		N'lands.		U.K.		USSR		Switzer.		Sweden		Canada		Total
*	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O	
Fibrinolytic																															
Urokinase	11	-	16	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	31
Streptokinase	-	1	-	-	-	-	-	-	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
Aspergillopeptidase	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	3
Streptomyces peptidase	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Plasmin	-	1	-	-	-	-	1	-	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	5
Creatinekinase	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Subtilopeptidase	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Armillaria melia peptidase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	1
Anticoagulants																															
Thrombin	-	3	-	-	-	-	-	-	12	-	-	-	-	-	-	-	1	-	-	-	2	-	-	-	-	-	-	-	-	-	18
Succinyl CoA Synthetase*	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Hypotensives																															
Kallikrein	-	-	-	4	-	-	-	-	-	-	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	11
Anti Cancer Activity																															
Anti Leukemia activity	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
L-Asparaginase	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
Tresolase (Modified Tyrosinase)	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Arteriosclerosis																															
Anti-arteriosclerosis Factor	-	-	-	1	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
Glucoronoglycosamino-glycom hyaluronate lynase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	4
Uremic Enzymes	-	-	-	-	-	-	-	-	-	-	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6
Total	1,22	-	21	-	-	-	1,	2,	20	-	10	-	-	-	-	-	1	-	-	-	7	-	-	-	1,	2,	1	-	1		90

* Key I = Patents assigned to Individuals

O = Patents assigned to Organisations

d1. Therapeutic Enzymes cont'd.

d1(ii) Anti-tumour

Table 3.26 Analysis of Anti-tumour Group by patent controlling country.

Enzyme \ Country	* U.S. I	U.S. O	Japan I	Japan O	Belgium I	Belgium O	Denmark I	Denmark O	France I	France O	Germany I	Germany O	Greece I	Greece O	Ireland I	Ireland O	Italy I	Italy O	N'lands. I	N'lands. O	U.K. I	U.K. O	USSR I	USSR O	Switzer. I	Switzer. O	Sweden I	Sweden O	Canada I	Canada O	Tot.
-Asparaginase	-	2	-	2	-	-	-	-	-	1	-	6	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	1	-	-	14
-Asparaginase erivatives	1	4	-	-	-	-	-	-	-	-	-	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10
onuclease	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
lucose oxidase	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
sine oxygenase	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
nticancer lytic enzyme	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
ational Total	1	6	-	2	-	-	-	-	-	1	2	13	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	1	-	-	28

* Key I = Patents assigned to Individuals

O = Patents assigned to Organisations

d(ii) Comments on Anti-tumour activity Table 3.26 - Anti-tumour Enzymes

L-Asparaginase and its derivatives were considered to have anti-tumour activity. Certain neoplasms (cancers) lack the ability to synthesise asparagine, one of the amino acid constitents of proteins. In the body normal cells maintain cancer cells by supplying them with asparagine, so that dietary therapy is ineffective, therefore L-Asparagine needs to be continuously removed from the body fluids. This approach has been tried and found to be selective in the treatment of acute lymphocytic leukemias which are blood related cancers but less so for neoplasms rooted in body tissues or cancers which have the ability to synthesise L-asparagine. The other enzymes used for treatment of cancers seek to destroy other vital metabolites, or destroy the DNA of the cells. Targeting of these preparations to the cancer has proved to be mosk difficult.

d) Therapeutic Enzymes cont'd

d(i)(ii) Digestive Enzyme Group

Table 3.27 Analysis of Digestive Group by patent controlling country.

Enzyme / Country	U.S.	Belgium	Denmark	France	Germany	Greece	Ireland	Italy	Netherlands	U.K.	USSR	Switzerland	Yugoslavia	Sweden	Total
	I O	I O	I O	I O	I O	I O	I O	I O	I O	I O	I O	I O	I O	I O	I O
Pepsin	- 4	- -	- -	- -	- 1	- -	- -	- -	- -	- -	- -	- 1	- -	- -	- 6
Trypsin	- 1	- -	- 1	- -	1 -	- 3	- -	- -	- -	- -	- 1	- -	- 2	- -	1,8
Papain	- 1	- -	- -	- -	- -	1 -	- -	- -	- -	- -	- -	- -	- -	- -	- 2
Chymotrypsin	- -	- -	- -	- -	- 1	- -	- -	- -	- -	- -	- -	- -	- -	- -	- 1
Papain	- 4	- -	- -	- -	- 2	- -	- -	- -	- -	- -	- -	- -	- -	- -	- 6
Rennin	- 2	- -	- -	- 1	1 -	- -	- -	- -	- -	- -	- -	- -	1 -	- -	1,4
Bromelain	1 3	- -	- -	- 1	- -	- -	- -	- -	- -	- -	- -	- 1	- -	- -	2,4
Lipase	- 2	- -	- -	- 2	- 4	- -	- -	- -	- -	- -	- -	- -	- -	- -	- 8
Galactosidase/(Lactase)	- 1	- 1	- -	- -	- 1	- -	- -	- -	- -	- -	- -	- -	- -	- -	- 3
Other Enzymes	- -	- 3	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- 3
Other Extract	- -	- -	- -	- 1	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- 1
Amylate cyclase	- -	- -	- -	- 1	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- 1
Fibrinolytic Product	- -	- -	- -	- 1	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- 1
Alkalase	- 1	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- 1
Proline aminopeptidase	- -	- -	- -	- 1	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- 1
Diphatase	- -	- -	- -	- 1	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	1 -
Enzyme Complex	- -	- -	- -	- 1	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- 1
Citrate lyase	- -	- -	- -	- 1	- 1	- -	- -	- -	- -	- -	- -	- -	- -	- -	1,1
Totals	1,10 -	4 -	1 -	- -	5,10 -	12 -	- -	- -	- -	- -	- -	1 -	1 -	3 -	1 - - 5,5
Useful Enzyme Patents															
Panic 1	- -	1 -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	1 -
Adrenal Extract	- -	- -	- -	- -	- 1	- -	- -	- -	- -	- -	- -	- -	- -	- -	1 -
Total Totals inclusive of Doubtful Enzyme Patents	1 10	1 4	- 1	- -	6 10	- 12	- -	- -	- -	- -	- -	1 -	1 -	3 -	1 - - 2 5

Key I = Patents assigned to Individuals

O = Patents assigned to Organisations

d(iii) Comment on Table 3.27 - Digestive Enzymes

The use of enzymes as digestive aids is a very old one. The digestive group enzyme analysis reveals that there is still considerable patent activity in this area, in the U.S. (9), Germany (10) and France (10). Most enzymes have animal or microbial origins but the U.S. (6), France (3) and Switzerland (1) have taken out patents for plant enzymes Bromelain and Papain.

Japan's only entry is unique in that it alone holds patents in the novel field of liver treatment, which is a complex biochem

France, the country which originated the use of enzyme extracts as digestive aids (Boudalt's "pepsine") and continues to have a higher level of patent activity by individuals than any other country in this form of therapy.

d. Therapeutic Enzymes con'td

d9(iv) The Skin Group

Table 3.28: Analysis of "Skin Group" by patent controlling country.

Enzyme \ Country	U.S. I	U.S. O	Japan I	Japan O	Belgium I	Belgium O	Denmark I	Denmark O	France I	France O	Germany I	Germany O	Greece I	Greece O	Ireland I	Ireland O	Italy I	Italy O	N'lands I	N'lands O	U.K. I	U.K. O	Canada I	Canada O	World Total I	World Total O
1.Lysozyme Products	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	3	-	-	-	-	-	-	-	1	3
Purification	-	-	-	1	-	-	-	-	-	1	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	3
Stabilisation	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Derivatives	-	-	-	1	-	-	-	-	-	1	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	6
Compositions	-	1	-	-	-	-	-	-	1	1	-	-	-	-	-	-	1	-	-	-	-	-	-	-	1	3
Lysozyme	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Total Lysozyme	-	1	-	4	-	-	-	-	1	3	1	-	-	-	-	-	9	-	-	-	-	-	-	-	2	17
2.Collagenase	-	2	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
3.Hyaluronidase	-	1	-	1	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	3
4.Wound treatment																										
DNAase	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Protease	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
Trypsin and anti-biotics	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Total Wound treatment	2	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	3
5.Hæmorrhoids	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
6.Poison Ivy, (Treatment for)	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
7.Lysome Inhibitor	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
8.Antibloat control	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
National Totals	3	8	-	5	-	-	-	-	3	5	1	1	-	-	-	-	9	-	-	-	-	-	-	-	7	28

d(iv) Comment on Table 3.28 - The "Skin Group"

Most of the patent activity centres around Lysozyme, the enzyme that hydrolyses the polysaccharide backbone of the bacterial wall in Gram positive bacteria.

Collagenase is used for dissolving white collagen fibres, e.g. in collapsed interverterbral discs, or in scar tissues during healing.

Hyaluronidase is used to dissolve the cement in between cells facilitating the dispersion of drugs into the muscles and tissues.

The Italian Company 'Societa di Prodotti Antibiotici', holds very many Lysezyme patents, and as such both the U.S.(1), and Japan (4), have only a few. France holds four patents increasing the strength of the EEC in this area. The patenting of enzymes for wound debridement with or without drugs seems to be restricted to the U.S.

d. Therapeutic Enzymes cont'd

d (v) The General Anti-Inflammatory Group - This group contains patents whose applications are less clearly defined, but all claim a common anti-inflammatory activity.

Table 3.29 Analysis of the 'General Anti-Inflammatory Group' by patent controlling country.

Anti-inflammatory enzymes \ Country	U.S.		Japan		Belgium		Denmark		France		Germany		Greece		Ireland		Italy		N'lands		U.K.		Israel		Total	
*	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O
Elastase	1	-	-	4	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	5
Chymopapain	-	1	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	2
Neuraminidase	-	1	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1	-	3
Proteases	-	-	-	2	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	3
Proteolytic	-	-	-	1	-	-	-	-	-	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	3
Cerase	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
Amino oxidase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Histaminase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	1	-
Retikinonase	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
"Osaminidases" ?	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
Total	2,2		-	8	-	-	-	-	1,7		-	3	-	-	-	-	-	-	1	-	-	-	-	1	4,21	

We have been unable to identify "Osaminidases".

Key I = Patents assigned to Individuals

O = Patents assigned to Organisations

d. Therapeutic Enzymes cont'd

(vi) The General Group - General patents on enzymes or their preparation

Table 3.30 Analysis of "General" Group by patent controlling country.

Enzyme \ Country	U.S.		Japan		Belgium		Denmark		France		Germany		Greece		Ireland		Italy		N'lands		U.K.		Canada		Total	
	*I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O
Category -																										
Amino acid Oxidase 1.4.3.3.	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Carnitine acetyl transferase 2.3.1.7.	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Tyrosine amino transferase 2.6.1.5.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1
Thymidine kinase 2.7.1.21	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Polynucleotide phosphorylase 2.7.7.8	-	1	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	2
Carboxypeptidase 3.4.12.3	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Modified Enzymes	-	1	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	1	-	-	-	-	-	-	-	3
Proteases	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
Enzyme Complexes	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Totals	-	3	1	-	-	-	-	1	-	1	-	5	-	-	-	-	-	1	-	-	-	-	-	1	-	13

* Key I = Patents assigned to Individuals
O = Patents assigned to Organisations.

d(vi) Comment on General Anti-Inflammatory Group and General Therapeutic Group and General Enzyme Group

Patent activity is low, restricted in general to France, Germany, Japan and the U.S. However, the Japanese patenting appears to be characterised by more concentrated patent activity on few products whereas France, and the U.S. are characterised by patenting over a wide range of enzymes.

3.3.8.5 Summary of reanalysed data in van de Kasteele study

Prescribed use of enzymes for therapeutic use aside from blood therapy and digestive therapy is still very low. Problems appear to be concerned with the targeting of the enzymes, and problems dealing with the prolonged stability of enzymes within the body.

The use of Antihæmophilic Factors which don't appear in the patents surveyed is judged to be the largest market worth 44 million $ in the U.S. (see Table 3.31) Alimentary canal treatments account for 16 million $. Wound debriding agents account for approximately 8 million $. Thrombolytic's account for less than 2 million $. However the Japanese market for Urokinase appears to be valued at 70 million $. * The rest are valued at less than 1 million $, include Hyaluronidase as an absorption promoter, the use of Chymotrypsin in Opthalmic surgery, Asparaginase in Leukemia chemotherapy, and Penicillinase in Penicillin shock allergic drug reactants.

Two new areas that appear to have some patent activity are Hypotensive and Antiarteriosclerosis enzyme treatments.

* L.Terminiello and A.Lesuk p.120, Enzymes as Parenteral Therapeutic Agents in Enzymes The Interface between Technology and Economics. Edited by J.P.Danehy and B.Wolnak. Marcel Dekker Inc. N.York and Basel.

Table 3.31 Ethical (Prescription) Enzymatic Products Currently Employed in the United States

Therapeutic Category	Enzymes Involved	Source	Indications	Approximate Mfrs. Sales
Blood Clotting Factors:				
Antihemophilic Factor (AHF)	Factor VIII	Donor	Hemophilia A	$40 million
Plasma Thromboplastin Component	" IX	plasma	Hemophilia B	4
Gastrointestinal Digestive:				
Combinations - including e.g. phenobarb. simethecone &/or bile salts	Pepsin	Animal organs	"Nervous" or other indigestion	11
Single source	Lipase, trypsin, amylase	Procine pancreas	Inadequate fat digestion: Cycstic fibroses	5
Wound Debriding Agents:				
Ointment or powder w. or w.o. antibiotic	Chymotrypsin	Bovine pancrease	"Possibly effective" for epistiotomy	2
	Bromelains	Pineapple		
	Papain	Papaya	"	3
Ointment w. or w.o. antibiotic	Fibrinolysin & Deoxyribonuclease	Bovine plasma & pancreas	Removal of purulent exudates ' escher	4
	Trypsin	Mammalian pancrease	"	2
	Subtilisins	Bacillus subtilus	"	
	Collagenase	Clostridium histolyticum	"	< 1
Topical, intramuscular & oral	Streptokinase & Streptodornase	Group C strepto-cocci	Clot Lysis, reduction of oedema ' inflammation	1
Thrombolytic:				
	Urokinase	Hemolytic strep. Urine or tissue cult.	Lysis of intravascular blood clots	< 2
	Fibrinolysin (plasmin)	Donor plasma	" "	
Absorbtion Promoter	Hyaluronidase	Bovine testicles	Rarely for IM or SC inj.	< 1
Ophthalmic Surgery		Bovine pancrease	Cataract removal	< 1
Cancer Chemotherapy	Asparaginase	Escherichia coli	Leukemia	< 1
Allergic Drug Reaction	Penicillinase	Bacillus cereus	Destroy penicillin	< 1

3.3.9 Summary and Analysis of M. Sigmonds Report

This case study is again based on a D.G.XIII report for Scientific and Technical Information Management, Patents Division, Luxemburg, compiled by M. Sigmond, edited by M.Terpstra and called 'Immobilisation of Enzymes". The report was intended to be exclusively informative in character. As the chemical substrates and methods used in immobilising enzymes were so numerous, the author stated that clear and well-organised classification would exceed the limits of the report. As for the other reports patent families were identified and listed as an appendix.

The report also provided a limited statistical analysis summarised by the following table.

Table 3.32. Patenting by Nations

	Country	No. of Patentees
	U.S.	57
	Japan	19
E.E.C.	Belgium/Lux	1
	Denmark	2
	France	16
	FDR	18
	Ireland	2
	Italy	1
	Netherlands	5
	U.K.	13
	E.E.C. Total	58
OTHERS	Switzerland	6
	Sweden	4
	Hungary	3
	Israel	2
	Czechoslovakia	1
	DDR	1
	South Africa	1
	N.Zealand	1
	Australia	1
	Others Total	20

Patents issued to individuals were separated from those assigned to companies. From our experience these patents were seldom taken up industrially. Licensing arrangements that may have tied them in with companies were not pursued further in this analysis. (N.B. The group holding 10 patents were P.Duncan, G. Dean and C.R. Lowe, concerned co-enzyme requiring enzyme reactions. H.P. Gregor bonded enzymes to ultrafiltration membrane and holds 3 patents. The rest of the individuals hold a patent apiece.)

The reworked analysis was as follows:

Table 3.33 Companies assigned to nation on the basis of the registered nationality of the parent or holding company

Countries		Companies
U.S.		46
Japan		18
E.E.C	Belgium	1
	Denmark	1
	France	10
	Germany	13
	Ireland	2
	Italy	1
	Netherlands	5
	U.K.	11
E.E.C. Total		42
Others	Australia	1
	Bermuda	1
	Czechosklovakia	1
	Hungary	2
	New Zealand	1
	Panama	3
	Sweden	2
	Switzerland	2
	Israel	2
Total		15

Table 3.34 Patenting by countries from Sigmond's data.

Country	No. of originating patents
U.S.	168
Japan	33
F.D.Germany	53
Great Britain	34
France	24

Five companies had more than 10 patents (see Table 3.35)

Table 3.35. Patenting by Company

Patenting by Company	
Corning Glass Works	20 patents
Monsanto	14 "
Bayer A.G.	13 "
SNAM	13 "
Boehringer Mannheim	11 "

This analysis was useful as far as it goes. The comprehensive appendix of patents, and the abstracts in the text enabled us to recalculate the data and restructure it. It became clear to me that Sigmond had grouped the companies by countries, by their geographical location rather than by the

nationality of the parent or holding company. I have used the latter since the parent company has access to these patents by rights of ultimate ownership. I have also grouped subsidiary organisations together e.g. in France, Rhone Poulenc controls the Dutch company Rhone-Progil, and thus the patents assigned to Rhone-Progil are controlled by the Rhone Poulenc group. Similarly the French companies' Institute Merieux, Inst. Francais d'Immunologie are all controlled by Rhone Poulenc so the patents assigned to them are ultimately controlled by Rhone Poulenc, and were assigned to the parent.

In order to focus on the patenting activity in the field of immobilised enzymes by companies and the nature of the immobilisation being developed I analysed the information available by companies and form of immobilisation adopted.

By U.S. owned companies & corporations

PATENTEES	TOTAL PATENTS	ENTRAPMENT: ULTRA FILTERS	ENTRAPMENT: MATRIX/ FIBRE	ENTRAPMENT: ENCAPSU LATION	ADSORP TION	IONIC BONDING	LINZYME LIGAND CARRIER	CROSS LINKING	COENZYMES	SEPARATION AND PURIFICATION	NOVEL CARRIE
Corning Glass Works	18	-	-	-	8	-	9	1	-	-	-
Monsanto	14	1	-	-	1	-	10	1	-	1	-
U.S.D.A	9	-	2	-	-	-	4	3	-		-
Research Corp.	8	-	1	1	-	1	4	1	-	-	-
Nat.Pat.Dev.Corp.	7	-	1	-	-	-	-	4	2	-	-
American Cyanamid	7	-	1	1	-	-	4	1	-	-	-
Owens-Illinois	7	1	-	-	-	-	3	3	-	-	-
Baxter	5	-	1	-	-	1	2	1	-	-	-
C.P.C.	5	-	-	-	5	-	-	-	-	-	-
Merck & Co.	5	-	1	-	-	-	2	-	-	2	-
Purdue Research Found.	4	-	1	-	1	-	1	1	-	-	-
Gulf R.D.& Co.	3	-	-	-	-	-	3	-	-	-	-
Proctor & Gamble	3	-	-	-	-	-	-	3	-	-	-
Standard Brands	3	-	-	-	-	1	-	-	-	2	-
Exxon	2	-	-	-	-	-	2	-	-	-	-
Pennick & Ford	2	-	1	-	-	1	-	-	-	-	-
Abbott Labs	1	-	-	-	-	-	1	-	-	-	-
Amerace	1	-	-	-	-	-	1	-	-	-	-
American Hosp.Supply Corpn	1	-	-	-	-	-	-	-	-	-	1
Battelle	1	-	-	-	-	-	1	-	-	-	-
Beckman Inst.	1	-	-	-	-	-	1	-	-	-	-
Biol.Developments	1	-	-	-	-	-	-	-	-	1	-
Cancer Research	1	-	-	-	-	-	1	-	-	-	-
Carborundum	1	-	-	-	1	-	-	-	-	-	-
C.C.I. Life Systems	1	1	-	-	-	-	-	-	-	-	-
Damon	1	-	-	1	-	-	-	-	-	-	-

PATENTEES	TOTAL PATENTS	ULTRA FILTERS	ENTRAPMENT MATRIX	ENTRAPMENT ENCAPSULATION	ADSORPTION	IONIC BONDING	ENZYME LIGAND CARRIER	CROSS LINKING	COENZYMES	SEPARATION AND PURIFICATION	CARRIE
Dorr Oliver	1	-	-	1	-	-	-	-	-	-	-
Dow Chemical Corpn.	1	-	-	-	-	-	1	-	-	-	-
Ferro	1	-	-	-	-	-	-	1	-	-	-
Gore	1	-	-	-	-	-	-	-	-	1	-
Marine Colloids	1	-	-	-	-	1	-	-	-	-	-
Midwest Res.Inst.	1	-	-	-	-	-	-	1	-	-	-
Millipore	1	1	-	-	-	-	-	-	-	-	-
Nelson R&D Co.	1	-	-	-	-	-	-	1	-	-	-
Ohio State Res.Found.	1	-	-	-	-	-	-	-	-	1	-
Polymeric Enzymes Inc	1	-	1	-	-	-	-	-	-	-	-
Research Triangle Inst.	1	-	-	-	-	-	-	-	-	1	-
Reynolds Tobacco	1	-	-	-	-	1	-	-	-	-	-
Rohn & Haas	1	-	-	-	-	-	1	-	-	-	-
Smith Kleine Inst.Inc.	1	-	-	-	-	-	-	1	-	-	-
Temple University	1	-	1	-	-	-	-	-	-	-	-
University of Utah	1	-	-	-	1	-	-	-	-	-	-
UOP	1	-	-	-	-	-	1	-	-	-	-
Wisconsin Alumini	1	-	-	-	1	-	-	-	-	-	-
Total	130	4	11	4	18	6	52	23	2	9	1
% of Total	100%	3.1	8.4	3.1	13.7	4.6	40.5	17.6	1.5	6.8	0.7

Comments on Table 3.36 - The U.S. Analysis

1. Chemical and Petrochemical Corporations, Research Organisations and a Glass Company hold the most patents on immobilisation in this field.

2. Corning Glass Works has made a concerted effort in enzyme immobilisation technology using a variety of porous glass and other supports, and as such stands out as a leader in these technological developments.

3. The Petrochemical and Chemical Corporations and Research Institutions also hold many patents. Almost inevitably they have made use of covalent linkages between enzymes and enzymes, or enzymes and carriers. A reflection of their expertise in covalent chemistry and partly from the drive to secure new markets by developing existing chemical products as enzyme carriers.

4. The only enzymes that have developed cheap immobilisation techniques suitable for large scale industrial usage are those for the use of the corn wet milling industry.

5. The Pharmaceutical companies have looked at immobilisation of enzymes and have concentrated on covalent linkage also securing enzymes to supports by the strongest bond possible. They are used for making semisynthetic drugs, or directly as therapeutics in analytical processes or diagnostic agents.

6. There has been little development in immobilisation technology from companies which are in the business of supplying bulk enzymes on the open market, however some of the large corn

processing organisations have opted to develop immobilisation and enzyme "knowhow" inhouse.

7. There is a noticeably low number of patents obtained using entrapment techniques.

8. The low number of patents obtained by companies within the field of diagnostics.

9. The interesting development of membrane reactors by a corporation producing what have been to date ultrafiltration membranes, i.e. Millipore (recently applied by Degussa.)

by Japanese owned companies

PATENTEES	TOTAL PATENTS	MEMBRANE	MATRIX	CAPSULE	ADSORPTION	IONIC BONDING	LIGAND CARRIER	CROSS LINKING	COENZYMES AND PURIFICATION	CARRIER
Tanabe Seiyaku	5	-	2	1	-	-	1	-	-	1
Mitsubishi Chemical Ind.	4	-	-	-	-	2	-	-	1	1
Agency of Ind.Science & Tech.	3	-	1	-	-	-	-	1	-	-
JERI *	3	-	-	-	3	-	-	-	-	-
Asahi Kasei Kogyo KK	2	-	1	-	1	-	-	-	-	-
Fuji Photo	2	-	-	2	-	-	-	-	-	-
National Food Res. Inst.	2	-	2	-	-	-	-	-	-	-
Kansai Paint Co.Ltd.	2	-	2	-	-	-	-	-	-	-
Sumitomo Chemical Co.	2	-	-	-	-	1	-	1	-	-
Ajinomoto	1	-	-	-	-	-	1	-	-	-
Dainippon	1	-	-	-	-	-	1	-	-	-
Denkai	1	-	-	-	-	1	-	-	-	-
Green Cross Corp.	1	-	-	-	-	-	-	1	-	-
Ono	1	-	-	-	-	-	-	-	1	-
Teijin	1	-	-	-	-	-	1	-	-	-
Toyo Jozo	1	-	-	-	-	-	-	-	1	-
Zaidan	1	-	-	-	-	-	1	-	-	-
K.Seiju	1	-	-	-	-	-	1	-	-	-
Total Patents	34		8	3	4	4	6	3	2	2
%	100		23.5	8.9	11.	11.7	17.6	8.9	5.9	6.9

* Atomic Energy Research Institute.

Comment on Table 3.37 - The Japanese analysis

1. The Chemical, Biochemical, Pharmaceutical Companies and Research Organisations hold most of the patents in this field.

2. The emphasis is on entrapment adsorption and ionic immobilisation of enzymes. Being cheaper they are therefore more applicable for industrial processes. Cells are often immobilised containing the required enzyme, this takes away the need for the disruption of the cell and stabilisation of the required enzyme particularly as the enzyme is fixed within the cell; other enzymes that may interfere with the desired reaction can be inactivated e.g. by heat or chemical treatments.

   Immobilised cells also have very long half lives and less stringent operating factors.

3. There are few patents which use covalent linkage and of these the diazo method appears to be the preferred system of attachment.

by the countries comprising the E.E.C. Patentees	TOTAL PATENTS	MEMBRANE	FIBRE/ MATRIX	CAPSULE	ADSORP TION	IONIC BONDING	LIGAND CARRIER	CROSS LINKING	COENZYMES	SEPARATION AND PURIFICATION	CARRIER
BELGIUM											
Kali-Chemie A.G.	2	-	-	-	1	-	-	-	-	-	1
Total	2	-	-	-	1	-	-	-	-	-	1
%	100%	-	-	-	50%	-	-	-	-	-	50%
DENMARK											
NOVO	4	-	-	-	2	-	-	2	-	-	-
Total	4	-	-	-	2	-	-	2	-	-	-
%	100%	-	-	-	50%	-	-	50%	-	-	-
FRANCE											
Rhone-Poulenc	9	-	-	-	-	3	4	-	-	2	-
ANVAR	5	-	-	-	-	2	-	1	-	1	1
ELF	4	-	-	-	1	1	-	-	-	2	-
L'Air Liquide	2	-	-	-	-	-	-	1	-	1	-
Sogeras	2	-	-	-	-	-	-	-	-	-	2
Centre National Dela Recherche Scientifique	1	-	-	-	-	-	-	1	-	-	-
Inserm	1	-	-	-	-	-	-	-	-	1	-
Mar-Pha	1	-	-	-	-	-	-	1	-	-	-
Roquette Freres	1	-	-	-	-	-	-	1	-	-	-
Societe Nationale des Petroles D'Aquitaine	1	-	-	-	-	1	-	-	-	-	-
Total	27	-	-	-	1	7	4	5	-	7	3
%	100%	-	-	-	3.7%	25.9%	14.8%	18.6%	-	25.9%	11.1%

PATENTEES	TOTAL PATENTS	MEMBRANE	FIBRE/ MATRIX	CAPSULE	ADSORP TION	IONIC BONDING	LIGAND CARRIER	CROSS LINKING		AND PURIFICATION	CARRIER
GERMANY											
Bayer/Miles	17	-	-	1	1	1	12	-	-	2	-
Boehringer Mannheim	14	-	-	-	-	-	10	3	-	1	-
B.A.S.F./Rohm	5	-	2	-	-	-	3	-	-	-	-
Hoechst Behringwerke	3	-	-	-	-	-	1	1	-	1	-
Hoechst Aktiengeslschaft	3	-	-	-	-	-	3	-	-	-	-
Freidrich Flick Industrie Werwaltung K.G.A.	2	-	-	-	-	-	2	-	-	-	-
Dynamit Nobel	2	-	-	-	-	-	2	-	-	-	-
W.R.Grace	5	-	-	-	-	-	4	-	-	1	-
Boehringer Ingelheim (Sohn)	1	-	-	-	-	-	-	1	-	-	-
Forschung Inst.	1	-	-	-	-	-	-	-	-	1	
Max Plankgeselschaft	1	-	-	-	-	-	1	-	-	-	-
Blendaxwerke	1	-	1	-	-	-	-	-	-	-	-
Ind. Herman G.	1	-	-	-	-	-	-	-	-	1	-
Pentapharm An	1	-	-	-	-	-	1	-	-	-	-
Total	55	-	3	1	1	1	37	5	-	7	-
%	100%	-	5.5%	1.8%	1.8%	1.8%	67.3%	9.1%	-	12.7%	-
IRELAND											
A.Guinness	2	-	-	-	-	-	-	2	-	-	-
Inst. of Res. and Stand.	1	-	-	-	1	-	-	-	-	-	-
Total	3	-	-	-	1	-	-	2	-	-	-
%	100%	-	-	-	33.3%	-	-	66.1%	-	-	-

PATENTEES	TOTAL PATENTS	MEMBRANE	FIBRE/ MATRIX	CAPSULE	ADSORP TION	IONIC BONDING	LIGAND CARRIER	CROSS LINKING	COENZYMES	PURIFICATION	CARRI
ITALY											
Snam - Progretti	13	-	12	-	-	-	-	-	1	-	-
Total	13	-	12	-	-	-	-	-	1	-	-
%	100%	-	92.3%	-	-	-	-	-	8.7%	-	-
NETHERLANDS											
Gist Brocades	2	-	1	-	-	1	-	-	-	-	-
Kngs	2	-	2	-	-	-	-	-	-	-	-
Dsm-N.V.(Stamicarbon)	1	-	-	-	1	-	-	-	-	-	-
Unilever	1	-	1	-	-	-	-	-	-	-	-
Avebe/Stader AB	1	-	-	-	1	-	-	-	-	-	-
Total	7	-	4	-	2	1	-	-	-	-	-
%	100%	-	57.1%	-	28.6%	14.3%	-	-	-	-	-
U.K.											
Koch Light Laboratory	7	-	-	-	-	-	6	-	-	-	1
Beecham Group	6	-	-	-	-	6	-	-	-	-	-
N.R.D.C.	5	-	-	-	-	2	-	-	2	-	1
R.H.M.	5	-	1	-	-	1	3	-	-	-	-
I.C.I.	4	-	-	-	-	3	-	-	-	-	1
U.K.A.E.A.	3	-	-	-	1	-	-	1	-	1	-
A.B.M.	1	-	-	-	-		1	-	-	-	-
Glaxo	1	-	-	-	-	-	1	-	-	-	-
Royal Cancer Hospital	1	-	-	-	-	-	1	-	-	-	-
Radiochemical Centres	1	-	1	-	-	-	-	-	-	-	-
Unisearch	1	-	-	-	-	-	1	-	-	-	-
Total	35	-	2	-	1	12	13	1	2	1	3
	100%	-	5.8%	-	2.8%	34.3%	37.1%	2.8%	5.8%	2.8%	8.6%

E.E.C. Summary

E.E.C. Summary PATENTEES	TOTAL PATENTS	MEMBRANE	FIBRE/ MATRIX	CAPSULE	ADSORP TION	IONIC BONDING	LIGAND CARRIER	CROSS LINKING	COENZYMES	AND PURIFICATION	CARRI
BELGIUM	2	-	-	-	1	-	-	-	-	-	1
DENMARK	4	-	-	-	2	-	-	2	-	-	-
FRANCE	27	-	-	-	1	7	4	5	-	7	3
GERMANY	55	-	3	1	1	1	37	5	-	7	-
IRELAND	3	-	-	-	1	-	-	2	-	-	-
ITALY	13	-	12	-	-	-	-	-	1	-	-
NETHERLANDS	7		4	-	2	1	-	-	-	-	-
U.K.	35	-	2	-	1	12	13	1	2	1	3
Total	146	-	21	1	9	21	54	15	3	15	7
%	100%	-	14.4%	0.7%	6.2%	14.4%	36.9%	10.3%	2.0%	10.3%	4.8%

Comment on Table 3.38 - The EEC nations and 3.39 - The EEC as a whole

Certain national trends manifest themselves :

Belgium - Kalichemie A.G. a subsidiary of Solvay holds patents in adsorption and carrier development.

Denmark - NOVO a major bulk enzyme producer holds only a few immobilisation patents utilising adsorption and cross linking techniques - and these refer to Glucose isomerase.

France - Quite a major effort has been made in immobilisation technology chiefly by the company Rhone-Poulenc a major chemical producer.

The remaining companies, that hold patents are the Petrochemical and Chemical companies and Research Organisation. The emphasis appears to be in developing immobilised enzymes on ionic carriers e.g. ion exchange resins, purification of enzymes and immobilisation by cross linking.

Germany - The major patent holding organisations within the Community are German. They are Pharmaceutical or Fine chemical manufacturers, with a few patents held by Research Institutes. The pharmaceutical companies hold patents for diagnostic and therapeutic uses of enzymes. Boehringer Mannheim has many patents dealing with immobilised enzymes, an indication of its strong position in the diagnostic field.

Italy - Snam-Progretti holds many patents in entrapment immobilisation of enzymes i.e. with potential

Italy (cont'd) - industrial applications, which will be discussed in the immobilisation case study.

Netherlands - They hold a few patents, the largest number controlled by Gist-Brocades - involve matrix and ion immobilisation.

The U.K. - The U.K. companies that hold patents include Pharmaceutical, Biochemical, Chemical, Food and Research Organisations. A lot of patents involve ionic bondings that are used for drug modifications on an industrial scale. The covalently linked enzymes are developed by the biochemical companies serving the research market and as more stable catalyst in the pharmaceutical sector.

The EEC overall - There appears to be a high level of patenting activity in the pharmaceutical and diagnostic field. Four EEC member countries appear to have taken out more than ten patents involving aspects of immobilisation technology. Ranked they are as follows: Germany (with 53 patents), the U.K. (with 35 patents), France (with 27 patents) and Italy (with 13 patents). Covalent methods of attachment account for 67.2% of the total German patent effort. In the U.K. two immobilisation techniques appear to have extensive patents: ionic and covalent bondings with 34.3% and 37.1% of the total U.K. effort. The French patents cover four areas. Ionic bonding and separation and purification stages in the technology both account for 26% each. Covalent bonding by either immobilisation enzymes onto

a carrier, or by cross linking 15% and 18.5% respectively.

The Italian situation is less diverse in that 92% of their patents fall into the entrapment category. This is somewhat distorted by the fact that only one Italian company has entered this field to date.

The low number of coenzyme patents indicates that none of the EEC countries have begun to develop immobilisation technology involving this aspect of enzyme action.

Nations Category. PATENTEES	TOTAL PATENTS	MEMBRANE	MATRIX	CAPSULE	ADSORP-TION	IONIC BONDING	CARRIER	LINKING	COENZYMES	PURIFICATION	CAPI
AUSTRIALIA											
Aspro-Nicholas Ltd.	1	-	-	-	-	1	-	-	-	-	-
BERMUDA											
Engelhard Minerals & Chemical Corporation	1	-	-	-	-	-	-	1	-	-	-
CZECHOSLOVAKIA											
Ceskolovenska Ackademie Ves.	6	-	-	-	-	-	4	-	-	2	-
HUNGARY											
Chinoin Gyoggszeres Vegyeszeti Terme gyam Rt.	1	-	-	-	-	-	1	-	-	-	-
Muanyagipari Kutato Intezat	1	-	-	-	-	-	-	1	-	-	-
ISRAEL											
Research Products Rehovost Ltd	1	-	-	-	-	-	-	1	-	-	-
Yeda R&D Co.Ltd.	5	-	-	-	-	-	5	-	-	-	-
NEW ZEALAND											
Development Finance Corp.of NZ	1	-	-	-	-	-	-	-	-	-	1
PANAMA											
Syntex & Syva	10	-	-	-	-	-	10	-	-	-	-

PATENTEES	TOTAL PATENTS	MEMBRANE	MATRIX	CAPSULE	ADSORPTION	IONIC BONDING	ENZYME LIGAND CARRIER	CROSS LINKING	COENZYMES COENZYMES	SEPARATION AND PURIFICATION	CAPS
SWEDEN											
Astra A.B.	2	-	-	-	-	-	-	-	-	2	-
Exploaterings A.B.	3	-	-	-	-	-	2	-	-	1	-
Pharmacia A.B.	3	-	-	-	-	-	3	-	-	-	-
Stadex A.B.	1	-	-	-	1	-	-	-	-	-	-
SWITZERLAND											
Nestlé S.A.	6	-	-	-	-	-	5	1	-	-	-
TOTAL	42	-	-	-	1	1	31	3	-	5	1
%	100%	-	-	-	2.4%	2.4%	73.8%	7.1%	-	11.9%	2.4

Comment on Table 3.40 - The Other Nations

The bulk of the activity is concerned with covalent immobilisation of enzymes, but overall this figure is low. With the exception of the Syntex Corporation of Panama (10), Nestle of Switzerland (6) and the few Swedish pharmaceutical companies holding (3-2) patents the other companies have one patent apiece. The Syntex subsidiary Syva Research Institute Palo Alto, U.S.A., has ten patents assigned to it for homogeneous enzyme immuno assays, and restricted to the assay of low molecular weight substances or haptens. These are used to determine drugs of abuse and therapeutic drug levels in patents.

The development of heterogeneous assays has taken the advantages of this method of assay and combined them with advantages of radiochemical and fluorescent techniques. The role of the enzyme remains, as in the case of homogeneous assays, essentially that of an amplifier in the detection of these substances. No mention of heterogeneous techniques appear in any of the patents examined. We have identified the following companies selling heterogeneous kits.

In the U.S.	-	Abbott Laboratories
		Cordis Laboratories
		Microbiological Associates
In the EEC	-	
France		Laboratoire Dynatech.
F.D.R.		Boehringer Mannheim
The Netherlands		Organon Teknika. Subsidiary of Akxo.

patents 1970-79

PATENTEES	TOTAL PATENTS	MEMBRANE/ FILTER	MATRIX	CAPSULE	ADSORP TION	IONIC BONDING	ENZYME LIGAND CARRIER	CROSS LINKING	COENZYMES	SEPARATION AND PURIFICATION	NE CARR DEVE
The U.S.	131	4	11	4	18	6	53	23	2	9	
Japan	34	-	8	3	4	4	6	3	2	2	
E.E.C.	146	-	21	1	9	21	54	15	3	15	
Others	42	-	-	-	1	1	31	3	-	5	
Total	353	4	40	8	32	32	144	44	7	31	1

Table 3.42- Immobilised enzyme patenting activity expressed as a percentage total of world activity period 1970-79

		MECHANICAL RETENTION			COVALENT BONDING						
PATENTEES	TOTAL PATENTS	MEMBRANE/ FILTER	MATRIX	CAPSULE	ADSORP TION	IONIC BONDING	ENZYME LIGAND CARRIER	CROSS LINKING	COENZYMES	SEPARATION AND PURIFICATION	CA DEVE
The U.S.	37.1	1.1	3.2	1.1	5.1	1.6	15.1	6.5	0.6	2.5	
Japan	9.6	-	2.3	0.9	1.1	1.1	1.6	0.8	0.6	0.6	
E.E.C.	41.4	-	5.9	0.3	2.5	5.9	15.3	4.3	0.9	4.3	
Others	11.9	-	-	-	0.3	0.3	8.7	0.9	-	1.4	
Total	100	1.1	11.4	2.3	9.0	8.9	40.7	12.5	2.1	8.8	

Table 3.43 - Immobilised enzyme patenting activity expressed as % of total world patenting per immobilisation method group

	MECHANICAL RETENTION			ADSORPTION	IONIC	ENZYME LIGAND CARRIER	CROSS LINKING	COENZYMES	SEPARATION AND PURIFICATION	NEW CARRIER DEVELOPMENTS
	MEMBRANE	MATRIX	CAPSULE							
The U.S.	100	27.4	50.0	56.25	18.75	36.8	52.3	28.3	29.0	0.1
Japan	-	20.4	37.5	12.5	12.5	4.2	6.8	28.6	6.5	18.2
E.E.C.	-	52.2	12.5	28.125	65.625	37.5	34.1	42.8	48.4	63.6
Others	-	-	-	3.125	3.125	21.5	6.8	-	16.1	9.1
Total	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%

Comment on Tables 3.41, 3.42 and 3.43

1. The EEC countries together hold 42% of the total world patents on immobilised enzymes and form the largest group.

2. The U.S. is the next largest holding 37.1%. Thus together with the EEC they account for nearly 80% of the patents.

3. Japan holds a mere 9.6% and forms the smallest group.

4. Covalent bonding is the most patented form of activity accounting for over 50% of the patents. The remaining patents are distributed evenly through the next 3 groups, approximately 10% each. These are Matrix, Adsorption and Ionic Bonding.

   The separation and purification category also accounts for the next 10%.

Considering the Japanese strengths in the previous analysis it is strange to see them last in this category. One aspect of Japanese marketing strategy is to go for high volume low cost production. One answer may be that at present immobilised enzyme technology does not compete on a cost basis with the alternative process of immobilising cells to carry out the required reaction. At present the payoff for research into immobilised cells would appear to be potentially greater for industrially produced products.

The following general observations can be made on this data.

The U.S. 1. Large chemical companies have developed extensive utilising covalently coupled enzymes or covalent carriers.

2. Only the companies that have an interest in utilising the enzymes in industrial scale process e.g. isoglucose have developed the cheaper methods of immobilisation of enzymes.

The pharmaceutical companies have adopted various immobilisation techniques, the companies patenting the most, use enzymes for diagnostic purposes.

Japan Most of the patents taken out by Japanese companies have industrial applications emphasising on entrapment techniques although some diazo couplings of enzymes were developed.

The EEC There are a considerable number of patents concerned with the use of immobilised enzymes, with the main effort being directed towards covalently immobilised systems. Ionic immobilisation using ionic bonds is also well developed. However, apart from the work carried out by SNAM, there are hardly any developments using physical retention. The EEC countries' efforts seem to have been largely directed towards immobilisation of high purity products, useful for technical and analytical works.

Other Nations The Other Nations fare badly in all categories of immobilisation except that involving enzyme ligand carrier linkages which are taken out by tax avoiding corporations e.g. Syntax, The Swiss Company Nestle, two Swedish pharmaceutical companies and the Yeda R & D Co. from Israel.

Table 3.44. Companies active in the manufacture, immobilisation and distribution of enzymes in the U.K.

Bulk industrial producers

A.B.M.C. Food Division (Dalgetty-Spillers)

Glaxochem

J.E.Sturge (R.T.Z.)

I.C.I.

Agents. Cambrian Chemicals - Worthington

Bulk suppliers

Grinsted Products Ltd.
Novo
G.B.

Immobilisation

Fisons
Pilkington Glass
Tate & Lyle
I.C.I.

Maltsters

Biddle & Sawyer
Munton & Fison
Powell & Scholefield

Inhouse

Unilever
Beecham Pharmaceuticals
Allied Breweries

Factors

Biocatalysts (Grand Met.Biotech Ltd.).
Murphy & Son (bulk papain)
Biomass Int.
Unichem Ltd.

Rennet producers (Foreign Subsids.)

Christian Hansen Laboratories

Research

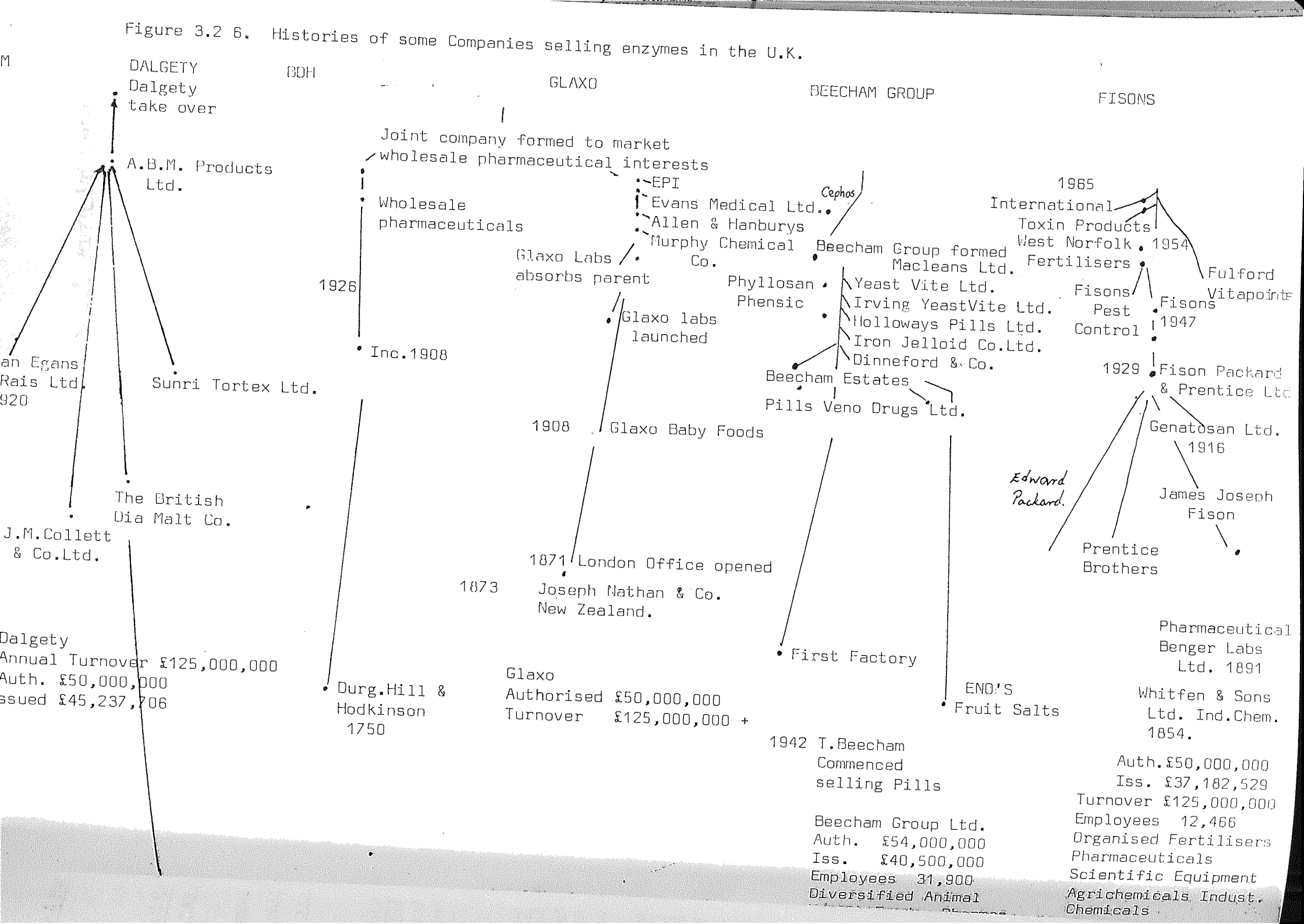
B.D.H. (Agents for Merck)
Biorad.
Biozyme Labs.
International Biochemicals Ltd.
Unichem
Cambridge Biotechnology Labs. (Unichem)
Genzyme Biochemicals Ltd. (Subsid. of Genzyme Corp.USA)
Hughes and Hughes (Enzymes) Ltd.
Windsor Laboratories Ltd.
Miles - Sigma.

Diagnostics

I.Q.Bio
AB-AG Labs Ltd.
EAB
Cambridge Life Sciences.

Source : Technology Policy Unit Company Data Base compiled by Z. Towalski.

Figure 3.2 6. Histories of some Companies selling enzymes in the U.K.



3.4. Breakdown of the U.K. industry - analysis of company information

This analysis is based on information obtained from a number of trade directories, such as Kompas,[28] and company lists especially those provided by Wiseman,[21] Godfrey and Reichelt,[22] Towalski, Rothman and Parkinson[23] and the D.T.I.[24]

It is possible to classify the companies by their sector of activity. (See Table 3.44). There appear to be a number of companies which produce enzymes in bulk. These include the specialist enzyme producers such as Maltsters and Rennet makers. Many of the companies are old established concerns, some like Glaxo and I.C.I. are more recent entrants. Competition from other European bulk producers of enzymes, namely Novo and Gist Brocades is quite intensive. It is interesting to note that one U.K. company also produces its enzymes inhouse (Beecham) and Unilever has the capability should it choose to do so.

There are three companies interested in utilising enzymes through the use of immobilisation technology, of which Tate & Lyle is the most active.

A small number of Agents and factors are also found who distribute enzymes for foreign producers.

By far the largest group in terms of numbers of companies active in the sale of enzymes is the group of companies supplying research biochemicals and specialist enzymes for genetic engineering technology. A small number of small companies have also been established to produce enzyme diagnostic kits.

The company histories of 5 enzyme companies are shown in Fig.3.26.

It shows that these companies have entered enzyme production from a number of routes, especially through acquisition and merger.

Many of the U.K. companies involved in the production of enzymes do not appear to be patenting their products.

3.5. Market for enzymes

Reliable and accurate data on the current size of enzyme markets is difficult to obtain for reasons of commercial secrecy. I have summarised some published estimates which I believe give on the evidence available a fair indication of market sizes and market trends.

(a) Bernard Wolnak and Associates.[25] Table 3.45 represents estimated markets for enzymes: Wolnak makes the point that by 1960 sales of enzymes had reached the level of about $25 million (based on the manufacturer's level of production.) Since then the U.S. market has approximately doubled and doubled yet again. The world market sales have also grown in real terms in the period 1972-1980. So Wolnak's statement that the world wide market is about double the U.S. market is upheld. He states that the major markets for enzymes are Europe and Japan with much variation with respect to sales of individual enzymes. However for 1960 the world total is just about $250,000,000. Even this value may be 25% higher since much of the starch converting enzymes are produced for captive use by starch processors, and are not recorded.

(b) The data produced by Godfrey,[26] estimated the world production (excluding Soviet Block) to be 53,000 tonnes with a value of £180,000,000 in 1979. These figures correspond roughly to those

Table 3.45

ESTIMATED MARKETS FOR ENZYMES (UNITED STATES ONLY) BY SALES IN $ MILLIONS.

Based on estimates produced by B.Wolnak and Associates. various publications

AMYLOLYTIC ENZYMES	1972	1975	1977	1980	1985
Alpha Amylases	6.61	5.5	10.0	11.6	14.8
Beta Amylases		2.5	2.8	3.2	4.1
Amyloglucosidase	1.7	6.0	12.0	14.3	19.1
Invertase	0.1	0.3	0.3	0.3	0.3
Cellulase	0.1	0.3	0.3	0.4	0.5
Xylose/Glucose Isomerase	1.0	15.0	40.0	50.0	65.0
Pectinase	1.56	2.0	2.3	2.7	3.6
Glucose Oxidase	0.35	0.7	0.8	1.1	1.3
PROTEOLYTIC ENZYMES					
Rennins	7.5	14.9	16.7	19.9	26.7
Pepsins	2.75	3.5	3.8	4.5	5.8
Pancreatins	0.8	4.6	5.1	5.9	7.5
Bacterial Proteases	1.83	4.7	5.2	6.2	8.2
Fungal Proteases	0.76	0.9	1.0	1.1	1.4
Bromelain	0.3	1.0	1.1	1.3	1.6
Papain	3.58	10.1	11.8	14.9	21.9
Others 1972 only	0.82				
LIPOLYTIC ENZYMES					
Lipase	N.A	0.5	0.6	0.8	1.3
MEDICAL AND DIAGNOSTIC					
Various	5.5	7.3	9.8	77.0 *	?
Total U.S.Market	35.26	79.8	123.6	215.2	(183.1)
Total World production ≙ 2 x U.S.	70.52	159.6	247.2	430.4	(366.2)
CORRECTIONS FOR $ EQUIVALENTS BASED ON THE 1972 VALUE. (Total U.S.sales)	35.26	60.91	80.51	118.26	

* Source OTA Impacts of Applied Genetic Statement p. 96 vol. II - 55% of $ sales are produced from processing human blood - they are used to treat haemophiliacs. These figures are very likely to be missing from Wolnak's estimates.

Table 3.46 · Industrial Enzymes - Distribution 1979: based on information supplied by T.Godfrey NOVO (personnel communication)

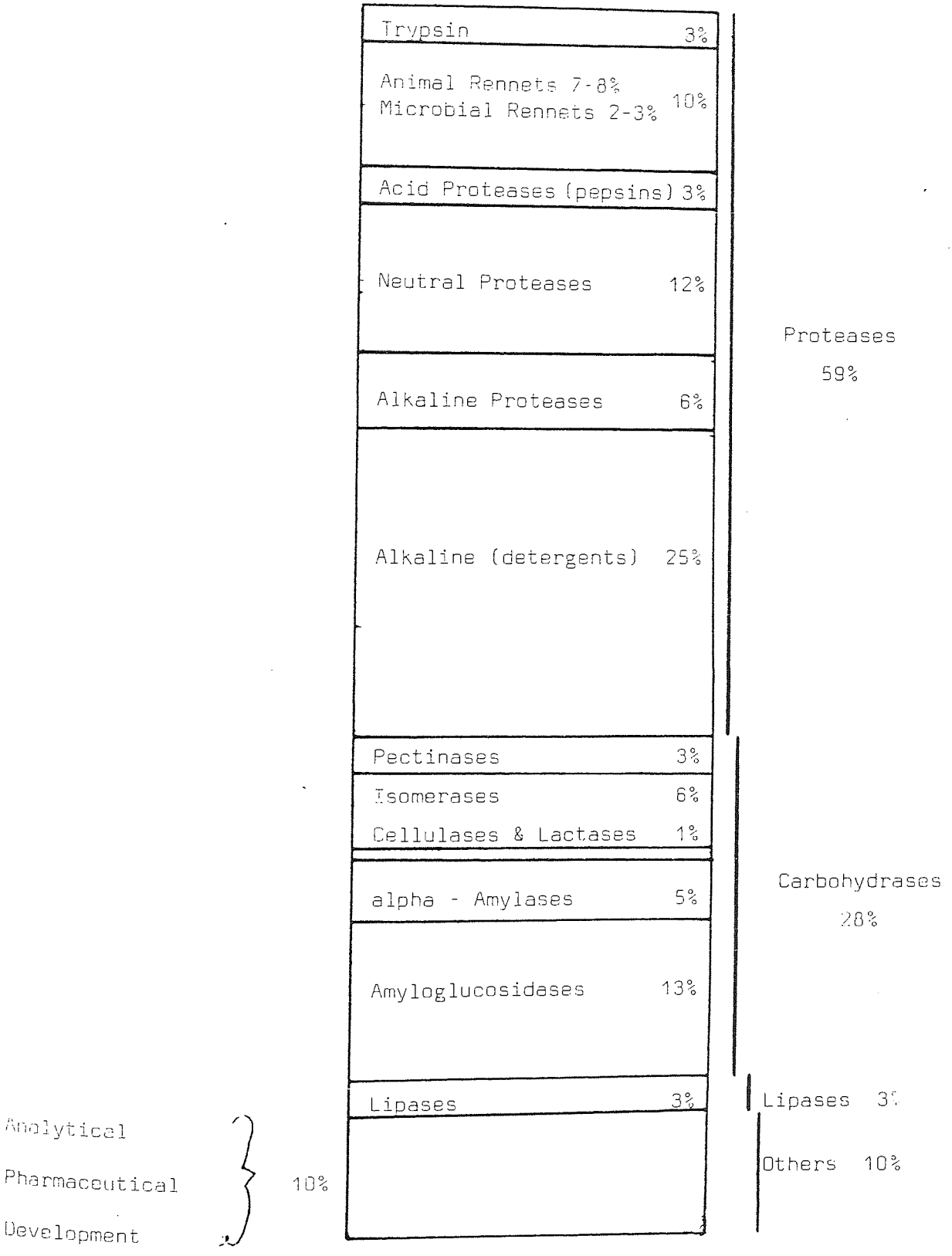


Table 3.47 Production of industrial enzymes by tonnage[1] within the free world based on Table 3.46

Nation		Tonnage (tonnes)	%
U.S.		6,360	12
Japan		4,240[3]	8
E.E.C.	Denmark	24,910	47
	France	1,590	3
	Germany W.	3,180	6
	Netherlands	10,070	19
	U.K.	1,060	2
E.E.C. sub total[2]		40,810	77
Switzerland		1,060	2
Others		530	1
Total		53,000	100

1. Personal Communication from T. Godfrey.
2. May include some countries in "others" category but this is only 1%
3. This figure does not match up with the estimate produced by K.Yamada[(29)] for 1977 of 12,750 tons.

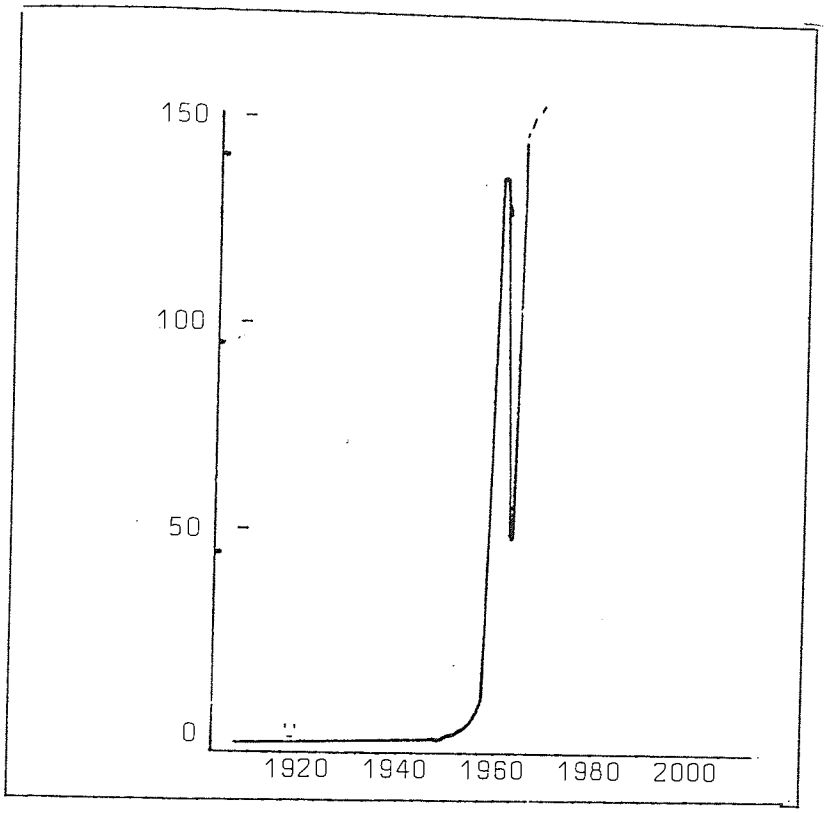


Fig.3·27 Microbial enzyme sales world-wide.

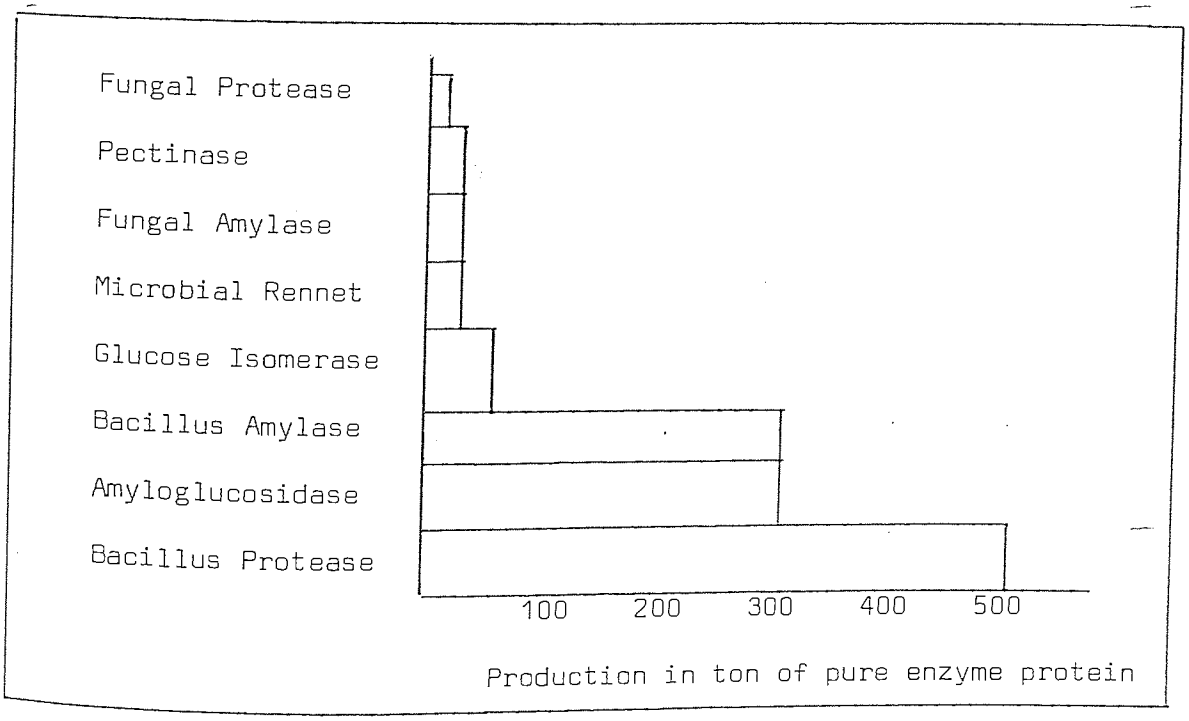


Fig.3·28 World production of individual enzymes

estimates produced by Wolnak for 1980, i.e. 2 x $138,000,000. Godfrey also produced a distribution of world tonnages[27] of industrial enzymes based on import/export data, and a breakdown of production by nation is included. This is presented as Tables 3.45 and 3.46. (These figures are based on the output estimates of 6 major companies and 10 minor ones).

(c) Aunstrup's [28] data in Fig 3.27 show that the fall in enzymes sales attributable to reduced protease sales is equivalent to some 70-80 million dollars in the period 1969-80. This was a consequence of the public outcry that followed the appearance of allergy reactions. They also show that there has been a recovery.

The data presented by Aunstrup on global enzyme productions (Fig 3.28) seem low compared to the estimates of Wolnak and Godfrey, but this could be explained in part by the nature of the Aunstrup data, i.e. an estimate of enzymes derived from microbial production alone.

(d) Hepner and Male [30] in their study stated "Western Europe runs ahead of the U.S., the value of those markets being $150,000,000, approximately 50% of the world market total". This study did not include speciality enzyme preparations or the Japanese bulk enzyme market (said to be of minor significance compared to Western Europe and the U.S.). The main outlet for enzymes in Japan, they claim, is in pharmaceutical applications. They forecast a high growth rate of 14% in enzymes within the U.S., a 5% growth rate for Western Europe and 6% for the rest of the world. The growth rate in the U.S. will be led by developments in HFCS sweeteners, where according to Bernard

Wolnak's data, it already accounts for more than 25% of the U.S. market, and gasohol which depends on the U.S. Government's will to encourage the blending of petrol with the alcohol ethanol.

The growth and development of the enzyme market is polarised around two distinct areas : the high volume, industrial grade enzyme products, and the low volume, high purity enzyme products with analytical, diagnostic or therapeutic uses. Within these groupings the growth rate of individual enzyme types varies greatly.

The high growth rate of the bulk industrial enzyme sector has been sustained by three developments: the growth of the alkaline proteases for detergent preparations in the '60's, the growth of glucose isomerase for the production of high fructose syrups in the '70's, and the growth of the amylases for the saccharification of starches for alcohol fermentations.

The medical/technical grade enzyme market is smaller but requires that the enzymes are produced to much higher standards of purity. The production of blood clotting factors VIII and IX is receiving a lot of attention from new start-up molecular DNA firms and pharmaceutical manufacturers. Further developments are likely to occur in this area. The development of enzyme diagnostics has also been rapid, and is likely to grow further. Some screening will be carried out by monoclonal antibody systems which may use enzymes as amplifiers so a period of readjustment in the market for diagnostics is likely to take place in the future.

There is, therefore, an as yet unexploited niche in the market for enzyme products in the intermediate range of purity and volume,

this is for enzymes used for: diagnostics; detoxification; and waste conversion. None of the established enzyme companies dominate the niche.

In chapter 1 I have discussed some of the technological limitations that influence the development of enzyme technology. Some of these limitations may be resolved technically but will be uneconomic to implement because the market may be too small to warrant the initial investment of time, effort and capital. Innovations that are most likely to occur will be in areas which will make the highest returns on capital, and where risks are relatively low. Progress is most likely to continue most rapidly at the high value added product range dominated by the pharmaceutical speciality chemical and research product business sectors.

I should point out that the industrial enzyme market, however, is also burdened with a certain amount of inertia. The catalyst market is not a free market in the sense that one enzyme catalyst can substitute for another. Choice of catalysts are made on the ability to meet quite narrow process specifications of a particular reaction and then the process is designed around the catalyst. The introduction of a new catalyst may consequently require considerable process redesign and alterations to existing plant and hence incur additional costs. This is particularly so for processes already utilizing immobilised enzymes in their production. The system's introduction of a new catalyst may substitute for an existing catalyst only if it is compatible with an existing process or its advantage is sufficient to merit the cost of a complete process change.

TABLE 3.48 Some of the types of waste produced by various industrial sectors in the U.K.

	Type of waste		
	Carbohydrate	Protein	Oils or Fats
Abattoirs	0	✓	✓
Breweries	✓	low	0
Butchers	0	✓	✓
Distilleries	✓	low	0
Food Processors			
a. Bakeries	✓	0	0
b. Cereal Food Producers	✓	✓	0
c. Confectioners	✓	low	0
d. Dairy	✓	low	0
e. Finished Whole Food	✓	0	0
f. Fish Processors	0	✓	0
g. Flour Millers	✓	0	0
h. Food stabilisers and thickener producers.	✓	0	0
i. Gelatin users	0	✓	0
j. Meat processors	0	✓	0
k. Poultry processors	0	✓	0
l. Sweetener producers	✓	0	0
Leather Manufacturers	0	✓	0
Oil Seed Processors	0	✓	0
Paper and Adhesive Manufacturers	✓	0	0
Textile Manufacturers	✓	0	0

Key: 0 = very little or none

low = low amount present, may need removal

✓ = high amount present and useful potential feedstock

When considering the very long term use of enzyme catalysts, one must also have an eye for the developments that are taking place within the wider field of catalysis. These developments will also have a marked influence on the success of enzyme technology since competition between them will readjust the market share amongst them. Recent advances in the understanding of these processes are taking catalysis away from empiricism, along the path of purposeful design. Many advances are being promised, from areas that include the development of heterogeneous catalysts, metal cluster catalysts, and photocatalytic processes.

New Market Opportunities

So far I have discussed only market trends for existing enzymes. New opportunities are more difficult to identify. One way is by carrying out an audit of the available feedstocks that can be upgraded into useful products by enzymes. Industrial wastes are likely candidates and even a simple survey reveals that large quantities of suitable materials are being generated by a number of industries (see Table 3.48).

The firm's size will influence how it will deal with this waste. Small firms find the costs of recovery and upgrading too costly; if treatment of effluents is necessary, where feasible, sale of wastes is attempted. Medium and large firms attempt recovery and reuse of wastes but only for specialised products. As most effluent treatments downgrade waste materials and as these wastes have high chemical oxidation values they require efficient systems for reducing this to reduce the oxygen demand of biological waste water purifying systems. Such systems are costly to install; consequently high polluting industries

aim to site their plants where untreated discharge of waste is possible, e.g. directly into an estuary or the sea. For plants already situated inland this is unrealistic and creates an additional financial burden in the form of a water charge, thus today such waste producing processes aim to minimise waste by treatment on site or opt for recovery of wastes for conserving of energy and materials. Some choose to upgrade the waste into a product for sale.

Enzymes are already available for all of these uses. However, there is a case for further development of other enzyme systems, e.g. faster acting amylases, more specific proteases, lipases, esterases, oxidases and transferases as well as enzymes capable of rendering harmless toxic wastes.

Another approach to the evaluation of market opportunities is to identify growing markets and to talk to industry about their problems and check if they may be resolved by the application of enzyme technology. It is likely that in such an audit companies will mention the need for better feedstocks, the need to reduce pollution, or the cost of toxicological testing by in vitro monitoring. However although pollution is biologically damaging the levels at which it is tolerated are determined politically and socially.

Social and Political Influences

Social and political influences are more difficult to predict. In the past social pressures have influenced the diffusion of enzyme products. The French Government introduced a system of legislation in the 18th century to control the production of "pepsine" after a spate of illnesses attributable to contaminated preparations, and we

have indicated in Fig.27 the size of the consumer 'backlash' against dusty enzyme detergents. Legislation has also been introduced to control the standards of products for use in food manufacture. Safety evaluation has to be paid for and the balance between protecting the public and increasing the costs of goods is delicate and varies from society to society.

More recently we have seen that political decisions can be used to override techno-economic advances that threaten the vested interests of groups with political or economic influence. Thus when threatened with a cheaper mid-invert sugar substitute produced by enzyme technology, a large farming sugar beet lobby was organised within the EEC that sought to control and retard the diffusion of this technology. They achieved their objective. The EEC suspended the manufacturers subsidy and curtailed production of high fructose syrups by fixing quotas.

In spite of such setbacks, within the existing markets, on economic grounds alone, it is possible to foresee that the field of enzyme technology is likely to continue to expand. The number of processes in which enzymes are used is also likely to continue to grow.

3.6. Summary of the U.K's enzyme science, technology and industry complex.

On the basis of these findings it is possible to make the following comments :

1. That enzyme research leading to the discovery of new enzymes appears to be declining and seems to be following a logistic curve. That activity had reached a peak for

4 groups of enzymes, oxidoreductases, transferases, hydrolases and lyases. That a peak occurred in isomerase research around 1960 and a later peak occurred around 1969.

2. That the enzyme industry is made up of a complex mix of companies serving many enzyme users.

3. That the production of bulk enzymes has many of the characteristics of an oligopoly.

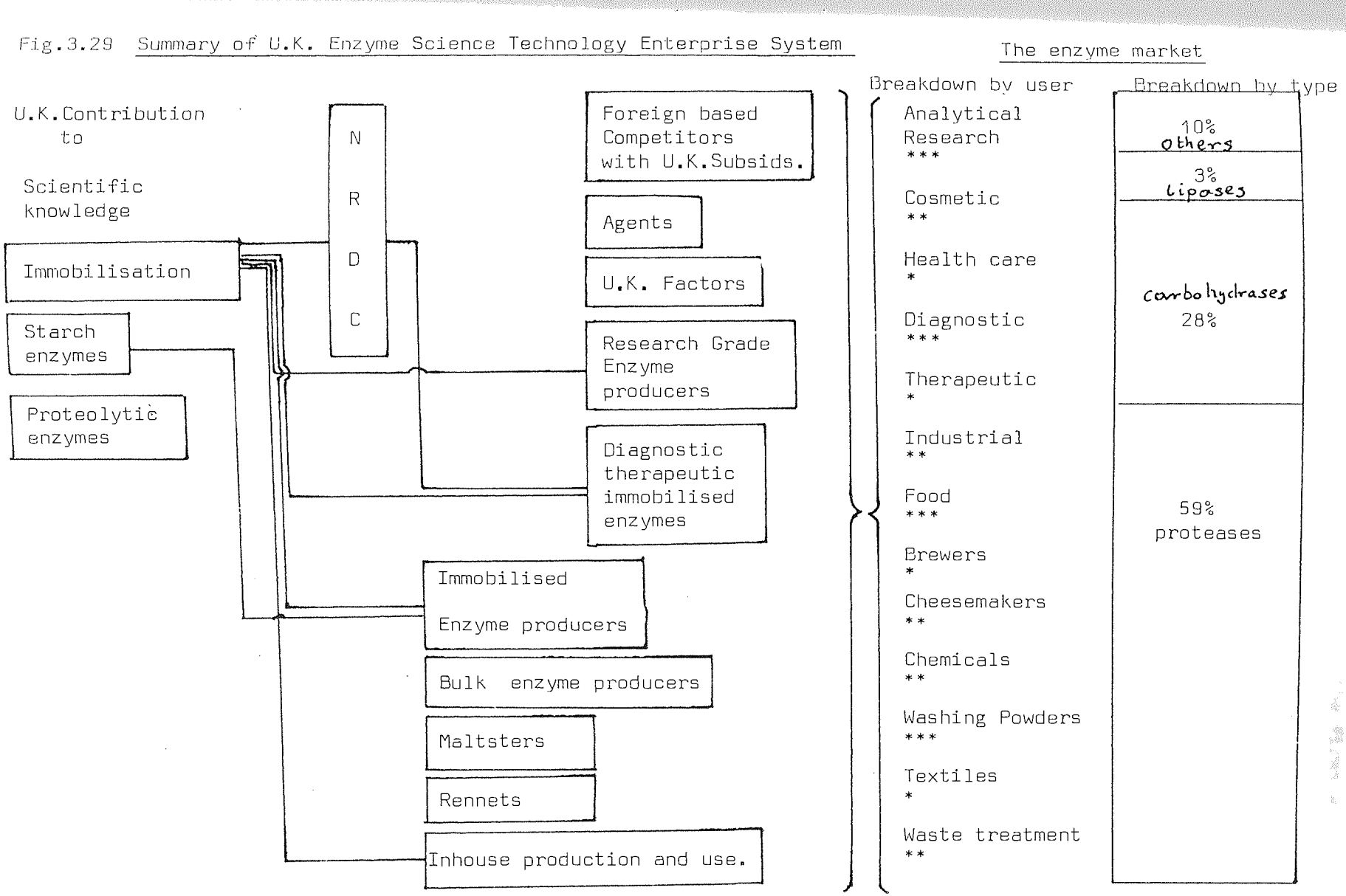
4. That large companies appear to patent more heavily than the small companies and that innovation in the production of bulk enzymes appears to be mainly accessible to companies that have sufficient resources and expertise to develop the technology. Many companies appear to have the capability of inventing in the area but few actually innovate. A difference perhaps attributable to the operation of strong selective market forces.

5. In general the U.K. industrial enzyme sector appears to be less inventive and less innovative than its competitors.

6. That growth is also taking place in the industry through the development of small high technology companies serving specialised sectors of the enzyme market especially in those sectors concerned with the production of research grade purified enzymes and diagnostic enzyme products.

7. That some companies and potential enzyme end users, have opted to develop their own enzyme technology e.g. pharmaceutical companies and food processors.

Fig.3.29 Summary of U.K. Enzyme Science Technology Enterprise System



Key : * slow or static growth ** moderate growth *** expansive growth

8. That the gaps in inventive activity not only reflect the amount of R & D being carried out by the firms working in this sector but also by gaps in the industrial profile of the enzyme product sector. (see Table 3.10).

9. That in the U.K. most inventive activity rested with pharmaceutical companies, one of which is a bulk enzyme producer, a chemical company and the N.R.D.C. It is worrying to note the absence of inventive and innovative activity by other bulk enzyme producers in the U.K.

10. On the basis of these findings I have been able to make a model of the U.K.Enzyme Science Technology Enterprise System. (See Fig.3.29).

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CHAPTER 4 : A RETROSPECTIVE ANALYSIS OF SOME ENZYME INNOVATIONS

4.1. Introduction

The purpose of this chapter is to identify long term trends and developments in enzyme technology that may be overlooked when examining recent information in considerable detail but over a much shorter period of time. As ever the main problem facing the researcher's limited information to carry out a comprehensive analysis. The history of enzyme science has been compiled and reviewed by a number of historians of science. Reviews by Fruton,[1] Teich,[2] Dixon,[3] Peters,[4] Kohler[5] and Florkin[6] are particularly useful. Literature describing aspects of the technology are more difficult to come by. The following are particularly useful - Effront and Prescott,[7] Smyth and Obold,[8] Tauber,[9] Prescott and Dunn,[10] Rainbow and Rose,[11] and Smith and Berry.[12] Patents are also another useful source of information especially those associated with Boidin and Effront[13] Takamine,[14] Wallerstein[15] and Underkofler.[16] More recent reviews of the technology are covered by Peppler,[17] Perlman,[18] and Wiseman.[19] Much of this information is still in need of systematic analysis and documentation, a task outside the scope of this thesis. The economic, social and political information has been obtained from various other sources, chiefly economic histories of the chemical industry, where references are made to prevailing market forces; and politico-economic conditions which are likely to influence this industry too.

4.2. Method of analysis

A number of authors have used chronologies to summarise the

developments of the different aspects of this field of knowledge. Understanding of science in terms of a series of dates can limit understanding of the process. However placing events in a chronological scale has an advantage when examining information from several different fields, since this enables links and patterns of development to be established. In this chapter I shall present a summary of these contemporaneous events as they occurred in the development of the science, the technology and the industry.

I have collected and placed scientific, technological and commercial events associated with innovations in a chronological framework. Within this framework I have been able to identify a number of events where the interaction between the science and the market appears to have had a profound influence on the development of enzyme technology (See Fig.4.1.).

In Fig.4.1. I shall present them for the sake of clarity, as a sequence of separate events (reading from top to bottom), although many of them overlap and run into one another:

1. A period of craft process development, characterised by empiricism and long periods of training of labour force. (A in Fig.4.1.)
2. The period of centralisation of the craft processes and the introduction of a system of production. Centralisation occurs at different times and at different rates, for different crafts (B in Fig.4.1.)

   The changes of organisation and scale, created a knowledge crises, which the application of scientific method solved. Technical improvements in rennet production led to the establishment of rennet manufacturers.

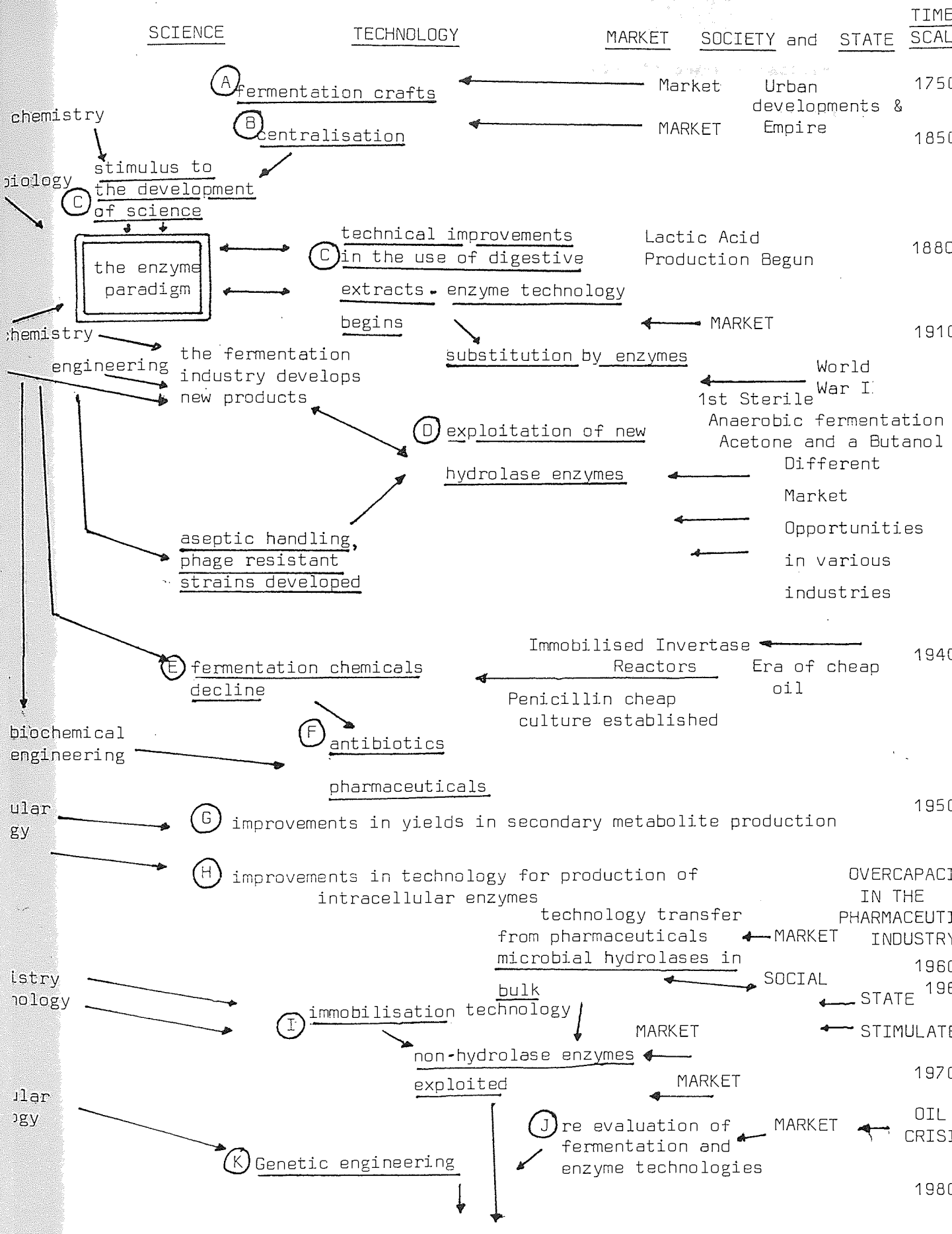

Fig.4.1. A chronological framework relating developments in science, technology and the economic, social and political environment.

3. The value of science and its contributions to society facilitated science becoming institutionalised. Workers in Research Orientated Institutions help the development of the enzyme paradigm which leads to the birth of enzyme technology proper. Enzymes begin to be substituted for crude extracts. Era of ethanol production systems based on enzymic degradation of starch - the Amylo process is developed on the continent. (C in Fig.4.1.)

4. Advances in other areas of science microbiology and fermentation provide alternative methods for the supply of organic solvents, acetone/butanol. A supply crisis during World War I prompts their adoption. Phage damage of cultures leads to the development of certain resistant strains and evolution of aseptic culture techniques. (D in Fig.4.1.) New hydrolase enzymes develop as new market opportunities arise.

5. The rise and sectorial demise of the fermentation industries during the era of cheap oil. The growth of antibiotic fermentation technology within the pharmaceutical industry, its effect on the scientific and engineering research and development and the evolution of deep fermentation. (E in Fig.4.1)

6. Technology transfer from the antibiotic industry faced with over capacity to bulk enzyme production. (F in Fig.4.1.)

7. The development of a theory of gene action and control of enzyme synthesis within the cells. (G in Fig.4.1.)

8. The development of the technology for the extraction of intracellular enzymes. (H in Fig.4.1.)

9. The development of immobilisation technology and the extension of the way enzyme catalysts can be used. (I in Fig.4.1.)

10. The re-evaluation of the fermentation industries and enzyme technology in the light of rising energy costs. (J in Fig.4.1)

11. The promise of genetic engineering with respect to enzyme production, and the impact that restriction enzyme research has had on other biotechnologies. (K in Fig.4.1)

Although I have researched each of these events in some detail, lack of space prohibits me from describing more than one in any detail here. I shall discuss the development of the enzyme paradigm and examine those innovations that led to novel industrial applications of enzymes before during and after its inception.

4.3. The development of the enzyme paradigm. (C in Fig.4.1.)

4.3.1. Introduction

The development of the enzyme theory came from the synthesis of a number of independent avenues of investigation, almost likened to threads which began to come together as independent lines of investigation on seemingly different problems, unravelled the life processes and led to the understanding of the workings of living organisms.

Today these findings are unified or integrated within the concept of the enzyme. Yet to contemporary workers these investigations appeared as unrelated activities, since they were directed at quite distinct problems.

The synthesis that led to the development of enzymes can be discussed in terms of major contributions that arose from investigations concerned with the nature of digestion; the

problem of fermentation; the nature of life and the cellular theory, and the nature of protoplasm and its constituents. It drew upon and unified a variety of other investigations whose relevance could be understood once the idea of the nature of an enzyme and its mode of action had gained more general acceptance.

4.3.2. The contribution from the investigation into the nature of digestion

In the 16th century the nature of digestion was debated as being either a process of the mechanical trituration of food, or a process of dissolution. As a subject it was a matter of considerable conjecture. In 1792 Reaumur[20] fed hawks with metal tubes filled with meat and starchy substances to show that dissolution occurred and Spallanzini[21] in 1793 was able to demonstrate that the gastric juice of hawks caused meat to dissolve 'in vitro'. In 1827 Tiedman and Gmelen[22] showed that gastric juices and saliva were different and that the former was secreted in 'peptogen cells' whilst in 1831 Leuchs[23] reported the diastatic action of ptylin. Bouchardat and Sandros reported also on the diastatic activity of pancreatic juice, and in 1834 Eberle[24] prepared an 'artificial' gastric juice by macerating pieces of stomach in slightly acidified solutions. He considered that the active principle causing this solution was in the mucus lining. Schwann[25] forecast the existence of an active principle as an entity similar to that found in malt and named it in anticipation of its discovery as pepsin. Wassman[26] isolated this principle three years later in 1839. Pancreatic enzymes were shown to be present in pancreatic juice by Bernard in 1856,

and named pancreatin by Corvisart.[27] By 1862 Danilewsky[28] identified and isolated three enzymes by absorbing the proteases on colloidion. Kuhne[29] worked on one of these proteases and named this enzyme trypsin in 1867, however its activity remained erratic. Pawlow and Schepowalnikow[30] discovered that intestinal juice increased the activity of pancreatic juice an observation that led to the isolation of the trypsin activator enterokinase in 1904.

The existence of a proteolytic enzyme in the small intestine was anticipated by Capparelli[31] in 1889 and was named Erepsin where it was isolated by Cohnheim[32] in 1901.

The isolation of the gastric proteases helped to support the enzyme theory but did little to develop it directly, the main development emerged from investigations that were addressed towards a better understanding of the process of fermentation.

4.3.3. Commercial uses of digestive enzymes

In 1855, 21 years after Eberle published his results on the macerates of calf stomachs, Corvisart recommended the use of pepsin as a therapeutic agent. Another Frenchman Boudault[33] developed that proposal, and in 1857 brought out a commercial preparation, purified by the technique developed by Eberle. Boudault's powder became popular in Paris and as is often the case it had its imitators. Many of these preparations were inexpertly made, they were inactive, unclean and dangerous. A number of lawsuits were brought against the manufacturers.

As a response to this a commission was formed in France by the Society of Pharmacists who began to regulate the making of pepsin from 1859. However, even these minimal criteria were not followed and pharmacies continued to produce preparations of doubtful value.

The ability to produce enzymes by the processing of animal tissues appears to have occurred independently in different times. On the American continent Emil Sheffer[34] isolated a saccharated product and communicated its production in 1872. It is interesting that the major users of this enzyme were also pharmacists. Haynes[35] states whilst reviewing the History of Oifco Labs Inc. that this laboratory of Digestive Ferments Co. as it was formerly known, was probably the principal manufacturer of pepsin in the U.S. Digestive Ferments Co. changed its name to Dickinson Chemical Co. and Rag Chemical Co. in 1895 and into Digestive Ferments Corporation hence (Difco) in 1913. It concentrated on the manufacture of pepsin, pancreatin, trypsin and bile salts, mainly to supply the pharmaceutical industry and research laboratories, and only later did it become a major producer and distributor of microbiological materials.

Other manufacturers of pepsin came into this field by diversifying out of the butchery businesses. Slaughterers and meat packers in the U.S. were able to use the offal provided by the carcases as sources of enzyme from which low cost preparations could be obtained as a by product of their main business, e.g. Cudahy Packing Co. Nebraska, U.S.A.

The production of pepsin is limited by demand. It has found some limited commercial use in cheese manufacture when mixed with rennet, (giving an important saving on the price of pure animal rennet) as well as chillproofing of beer and in the production of wine. Its main use has remained clinical as a digestive aid, although it was tried for the chemotherapy of cancer but its clinical use was surrounded by controversy. At present most of the major enzyme companies deal in this enzyme as it is relatively easy to prepare and purify. Companies such as Difco continue to purify and utilise pepsin in-house, to produce peptones, which are an important source of nitrogen in culture media used by microbiologists.

4.3.4. The Threads concerned with brewing

The move to understand fermentation began with Boerhaave's attempt to clarify its definition. Hitherto fermentation was loosely applied to a wide range of physical, chemical and biological phenomena. In 1735 he produced a restrictive definition in terms of materials which he listed as yeasts, malt liquor, dough or the leaven of bakers. He did not report on the work of a fellow Leiden scientist who had examined yeast and reported seeing globules.(36)

The determination of the chemical nature of fermentation was attempted both by Lavoiser, and Gay-Lussac.)37) Their work led to the generally accepted equation for alcoholic fermentation and opened the way for the chemists of the day to hypothesise on the chemical nature of the process.

In 1811 Kirchoff showed that starch could be converted into sugar by boiling it up with dilute sulphuric acid, and noted that the same effect could be produced by an aqueous extract of malt, although he wrongly attributed this to its gluten components.

The next significant development to the understanding of the process appears to have been made by Payen and Persoz[38] who isolated a component that liquified starch by alcoholic precipitation of an aqueous extract of germinating barley. They named this substance "diastase" from the Greek diastasis to make a breach. The existence of this active component was noted previously by Dubraunfaut but was not followed up.

The chemist Liebig[39] failed to unravel the complexities of living things, believed that they were under the control of a 'vital force' and that once deprived of this kept their properties only by inertia. He therefore equated decomposition and putrefaction with fermentation as process of slow combustion of living matter with oxygen. This view was challenged in 1838 by Cagniard-Latour[40] who on the basis of microscopic investigations showed that alcoholic fermentation depended upon yeast. Independently Schwann[41] again showed that infusoria did not arise spontaneously, that putrefaction was caused by micro-organisms and that fermentation was due to an organism he called 'Zuckerpiltz' or sugar fungus which still remains the German word for 'yeast'.

This conflict between the chemists and biologists can be regarded as the result of two quite distinct and separate branches

of knowledge, biology and chemistry attempting to investigate living processes using different techniques. These differences were difficult to reconcile as exemplified by the fact that yeast was not considered to be a living thing by many chemists but a product of putrefaction, e.g. by Mitscherlich in 1841 and by Berzelius not until 1848.[42]

In 1861 Pasteur had established that there was a specificity between the microbe and the type of fermentation that was produced, and he was able to bring about fermentation to produce lactic, tartaric, acetic and butyric acid fermentations by identifying cultures of the appropriate microbe. These experiments led to the redefinition of fermentation into two categories, organised ferments such as yeast, and disorganised ferments such as pepsin and typsin.

4.3.5. Contributions from the cellular theory of life

Based on extensive microscopic observations Schwann[43] published in 1839 a cellular theory that insisted on a common cellular origin for tissues. A cell was no longer considered to be the empty space described by Van Leuwenhoek but a layer of substance around the nucleus that he called the Zellenschicte. Virchow built on this idea and used this to extend it to the biologist's maxim of 'Omnis cellula e cellula'.

The workings of the protoplasm were treated rather as a black box by contemporary investigators. Dujardin considered protoplasm to be a simple biological entity and whose properties were lost once it had suffered chemical or mechanical damage.

Molecules of food or oxygen were incorporated into a huge complex or 'biogen' and lost their properties. This view was not universal. Traube in 1858 considered that molecules were present in yeast capable of accomplishing fermentation. In 1860 Bertholet[44] who isolated invertase from macerated yeast by precipitation and alcoholic extraction, supported the idea put forward by Traube of intracellular catalysts, but limitations of experimental technique were such that no unambiguous instance of an intracellular catalyst was demonstrable.

Attempts were made to reconcile these various findings. For example Pfluger in 1895 attempted to explain the findings of chemists with that of the 'biogen' theoreticians by ascribing its activity to unstable chemical groups and Hoppe-Seyler[45] attempted to integrate the concept of unorganised ferments with the 'biogen' theory by stating that all vital reactions were hydrations or dehydrations mediated by unorganised ferments.

4.3.6. The origin of the term enzyme

Kuhne[46] (1878) attacked Hoppe-Seyler's speculations as adventurous and coined the word 'enzyme' to distinguish between such agents as yeast and micro-organisms, and unorganised ferments. He also postulated that enzymes may be present in more complex organisms.

The criterion for calling an active component an enzyme was that its catalytic action could be demonstrated outside of the cell. This term became adopted rapidly in Germany and England but in France the older term 'diastase' lingered on.

Kuhne's definition of the term also helped to define the problem in terms of the need to show catalytic activity within a cell. It also enabled adherents to this hypothesis to identify cases of the presence of biocatalysts in other areas, (such as the digestive enzymes).

The crucial demonstration that the enzyme theory was essentially correct came about when Buchner[47] discovered cell free fermentation by accident whilst preserving yeast press juices with glucose, as part of a research programme looking at immunological characteristics. This discovery extended the definition of enzyme, and resolved the Liebig Pasteur controversy regarding the nature of fermentation proving both scientists partially correct. It opened the door to other workers wishing to investigate the workings of the cell, and provided a technique for preparing soluble extracts of intracellular catalysts.

The modern notion of an enzyme as a biochemical catalyst with unique properties emerged over many years. I shall now describe these events in sequence as they occurred.

° Enzyme - Catalysis

In 1837 Berzelius described catalysis as a concept and identified it as a distinct force from previous forces known. In explaining his definition he used examples of fermentation, and the decomposition of hydrogen peroxide by fibrin. However the quantitative verifications of this came from the work of Oswald in 1893, when he noted the difference between the transfer of heat or electrical energy and the transfer of chemical energy.[48]

° Specificity of enzymes

In 1894 Fischer[49] began a series of investigations which laid the foundations of the widely held belief that enzymes are highly specific. By means of projection formulae he was able to describe the configurations of hexoses and pentoses about the asymetric carbon atoms, and reported that some of these were dextrorotatory whereas fructose was levorotatory. On the case with which certain sugars were fermented he was able to conclude that yeast cells ferment those sugars whose symetry is similar to that of glucose. He extended his studies to include an aqueous extract of malt (diastase) and emulsin. On the basis of these observations he concluded that each enzyme and substrate fit one another and used the lock and key analogy to describe the interaction.

° Enzyme cofactors

Bertrand[50] worked to resolve the problem of the formation of black lacquer. He guessed that an enzyme was present in the latex that was associated with the uptake of oxygen and the production of carbon dioxide gas during the process. He named this enzyme 'laccase'. He further noticed that there was a higher than usual concentration of manganese in the ash of his preparations, and that the formation of the lacquer was enhanced by the addition of manganese salts. He thus established a link between enzymes and metals. He coined the word 'coenzyme', however he wrongly attributed the enzyme action to the manganese on the basis that manganese dioxide functions as a chemical catalyst. Thus to Bertrand the

active 'enzyme' component was the manganese and the 'co-enzyme' the protein. The modern use of the term was developed by Harden, who began investigating the anomolous nature of inhibition of yeast protease by blood serum. An early observation that in the presence of serum 60-80% more sugar was formed from the oxidation of glycogen than without serum. In 1905 in a joint paper Harden and Young.[51] reported that the coenzyme was dialysable, precipitated by alcohol, and necessary for the action of zymase. They later identified phosphates as also being necessary for improving the enzymes which they considered to be a second cofactor.

° The reversible nature of enzymatic reactions

In 1898 Croft-Hill[52] discovered that enzyme catalysed hydrolysis was reversible, when working with enzyme maltase. This enzyme splits maltase into two molecules of glucose. When starting with high glucose concentrations (40%) and maltase, maltase could be detected in the medium.

° The effect of pH on enzyme action

Prior to 1909[53] many of the results obtained by different workers describing using enzyme catalysis appeared to be ambiguous. Although Pasteur had shown the influence of pH on the process of fermentation its effects on enzyme action was not investigated. After Sørensen's investigations the idea that enzymes have pH optima explained the ambiguous results obtained by earlier workers. In future scientists investigating enzyme action, and users of enzymes in an industrial environment were able to control and define enzyme activity precisely.

° The determination of enzyme kinetics

This began with the work of Brown at the British School of Malting and Brewing, Birmingham. He noticed that the rate of hydrolysis of cane sugar by saccharase was independent of the sugar concentration over a wide range of concentrations but dependent on the concentration of enzyme. Brown[54] suggested that the enzyme and sugar formed a compound that broke down into the enzyme and products. Thus enzyme catalysed actions were different from chemical reactions in that the rate increased to a limiting value when the enzyme becomes saturated. Henri[55] working in Paris reached similar conclusions and produced a mathematical model. Michaelis[56] took up these ideas and published a paper in 1913 developing the enzyme substrate theory based on examination of the initial velocities of the hydrolysis of sucrose in varying sucrose solutions. In 1925 however Michaelis kinetics were superseded by Briggs and Haldane[57] kinetics and Michaelis kinetics became a limiting case of the Briggs-Haldane kinetics. The study of enzyme kinetics gave the study of enzymes considerable impetus. Kinetics also provided a valuable tool for use by engineers concerned with the design of enzymatic processes.

° The proteinacuous nature of enzymes

The term 'protein' was introduced by Mulder[58] in 1838 for a compound with an empirical formula he determined to be C_{40} H_{62} N_{10} O_{12} but modified by various amounts of sulphur and phosporous. These results were obtained after a systematic investigation of materials such as egg albumen. By 1882 Fischer had determined the composition of proteins and the

nature of the peptide bond. The problem of identifying the chemical nature of enzymes was not one of a lack of techniques, to analyse the enzyme but an inability to produce enzymes in pure enough states to determine their empirical formulae. Since chemical criteria of purity had to be met, the task of purifying enzymes was begun in the 1920s. Sumner[59] reported the purification of the enzyme urease and obtained it in a crystalline form. Unlike crystals of inorganic substances these materials were still impure with a 50% purity and care had to be exercised in their use. The crystallisation of other enzymes followed and enabled Northrop and Kunitz[60] to confirm their protein nature. In so doing they established new criteria of purity by determining the solubility of the protein in a constant amount of solvent and plotting the values against the protein in the supernatant. A straight line plot would indicate 1 protein whereas 2 or more lines indicated mixtures.

4.4. Some consequences of the development of the enzyme paradigm - Enzyme production

The knowledge about enzyme action and enzyme kinetics hastened the process of enzyme production and application throughout several industries. Whereas crude biologically active extracts were used as catalysts, purified sources of enzymes replaced them and improved the process by making it easier to control. What is striking however, is that the use and development of enzymes occurred over a considerable period of time. I shall discuss the production and application of some of these enzymes in the following section of this chapter.

Up to 1900 the production of purified enzymes had been practiced on a small scale mainly for research purposes or as scientific curiosities. The major source of enzymes were plants and animals such as the diastases from malt and digestive enzymes from the alimentary tract and its associated organs.

Pioneering work in utilising microbes as sources of enzymes began with the work of Takamine using fungi in the U.S. around 1900,[61] and Boidin and Effront[62] on fungi and bacteria in France in 1891. This formed the basis of the industrial culture of bacteria for enzymes. The culture apparatus contained many trays to allow the development of the film. Wallerstein[63] reports adopting this method for producing Bacillus organisms in the 40's with modifications. Takamine's method used the traditional 'Koji' method of inocculating a suitable substrate (in his case moist bran) and growing a culture of Aspergillus on it. The fungus produces a wide range of enzymes which it releases into the medium from which they can be extracted.

4.5. Enzyme innovation into the alcohol producing industry (D in Fig.4.1.)

This was an early industry to develop and a popular source of taxes levied by the exchequers in many countries.

Initially the industry was concerned with the production of potable alcohol only. By the 1850's[64] the industry began to be divided into two groups, that producing potable alcoholic beverages and that producing industrial alcohol, as demand for alcohol began to increase. The fermentation of potable and industrial alcohol was carried out by the brewer who produced a beer which was later distilled.

Traditionally the brewer obtained the fermentable medium from the maltster whose role in the brewing process was to change the starch in grains which cannot be fermented by yeast into where it was mostly glucose, which the yeast can utilise. This practice had evolved empirically over centuries and as a process was carried out along traditional lines. The process involved inducing germination of the dry grain (barley) by steeping the grain in rectangular tanks and replacing the steepwater at intervals. Too much or too little steeping reduced the amount of sugar produced in the batch. The steeped grain was then moved onto floors, which were long rooms with low ceilings with whitewashed walls and small shuttered windows along the sides. Normally the green malt was delivered at one end and heaped into a ruck or couch. Thus the malting process consisted of controlling the germination of the grain during which hydrolysis of starch was carried out by the diastatic enzymes. Germination was controlled by controlling the temperature of the grain. Once the correct temperature had been reached the grain was spread out onto a floor in a bed, and turned at intervals by workers using wooden shovels. The temperature of the bed was controlled by the shutters in the malt house and by thickening or thinning the bed of germinating seeds or pieces. The pieces were moved along the floors from the steep tanks to the kilns where the germinating seed was roasted.

Early investigations into the study of this process by workers such as O'Sullivan, gave detailed information on the products formed. This investigation paved the way for the discovery of more than one amylase. Knowledge of the α and β -amylases was well established by 1890.

Malts produced by these methods were of varying quality and presented a problem from the point of view of the brewer because they were deficient in the saccharifying amylase. As brewers expanded their businesses so the incorporation of maltsters into brewing companies helped the larger brewers to regulate their malt supplies. Likewise maltsters in order to compete with and meet the needs of the larger brewers also began to amalgamate into large concerns, e.g. A.B.M. (Associated British Maltsters).

Distillation of fermented worts produces alcohol a number of sources of which are available (potato, wheat or maize) which can be hydrolysed to produce a fermentable wort. The economics of each process depend very much on the cost of the starch feedstock, since yeast is used to ferment the glucose in the wort into alcohol. The saccharification of starch to glucose can be obtained by the application of dilute acids (Kirchoff) or the use of enzymes. A process that speeded up the conversion of grain starch to glucose and its fermentation into alcohol was developed in France. Calmette (in 1894) isolated amylolytic molds from crude rice cakes and showed that many species of the genus Rhizopus made amylolytic enzymes. Calmette and Boidin established the Society d'Amylo in 1891, a maize distillery at Seclin near Lille.[65] The process used maize starch, which was saccharified into glucose by the action of enzyme released by a fungus Alomyces rouxii. The conversion of glucose to alcohol was achieved by a strain of Rhizopus delemar.[66] The amylo process was more efficient than the more traditional yeast fermentation.

In the period between the two world wars the Amylo process was popular in France and on the Continent and in the U.S.A. It also led

to the development of a Japanese amylo industrial counterpart. It was not adopted by the U.K. because of the practice of calculating alcohol yields prior to fermentation for the computation of excise taxes to be levied was already well established and hindered the introduction of the new technology. Consequently in the U.K. brewers and distillers continued to produce alcohol by traditional means and were effectively cushioned against competition by the import duty on alcohol.

In the U.S. saccharification by fungal enzymes had been tried earlier. Takamine as a Japanese student studying abroad was familiar with the industrial situation in Europe through his studies; on returning to Japan he worked on the preparation of koji and became familiar with Japanese fermentation industry. He visited the USA whilst working in the Japanese Department of Agriculture and Commerce and on his return to Japan set up the Tokyo Artificial Fertiliser Company, the first superphosphate manufacturing company in Japan. In 1890 he returned to the USA and started the Takamine Ferment Co. He obtained a series of patents to protect the process for making alcoholic liquors by the use of a koji type preparation and experimented with this enzyme preparation in Peoria.[67]

In 1892 he moved to Chicago where his Taka-diastase process was evaluated by a Canadian distillery. Takamine's process was not adopted because of the presence of certain off flavours in the alcohol. It is interesting to note that in 1907 the Woolner Distilling Co. of Peoria a company that also evaluated Takamine's enzyme patented a process for making alcohol from worts saccharified by a mix of diastases and Aspergillus enzyme type diastase added during the mashing process.

Leo Wallerstein also entered the enzyme producing industry by innovating into the brewing industry. The demand for chilled beers during the summer is particularly high in the USA due to its hot continental climate. Cooling the beer produced a proteinaceous tannin haze. In the U.K. beers are not normally chilled and any turbidity is removed by treating the beer with isinglass or finings (dried swimbladders of fish). Wallerstein's solution to this problem was to digest the protein by using a proteolytic enzyme. In 1911 he obtained a patent protecting his invention for clarifying beer by processes using papain, bromelain, pepsin and fungal enzymes. "Wallerisation" of beer became a success with brewers and their customers and established Wallerstein's company as a producer of enzymes. The company continued to diversify its enzyme producing interests by patenting enzyme preparations for bates and hides in 1929. In 1932 he patented a process for making chocolate syrups using bacterial amylases, and yeast invertase in 1933.[68] In 1937-8 he patented the use of enzymes in the removal of proteins from rubber latex, aimed at improving the dialectic properties of rubber for use in underwater insulation cables. Wallerstein also pioneered the use of bacterial enzymes with an updated and modified version of the apparatus patented by Boidin and Effront in 1909 and obtained patent protection for the use of the enzymes in the chillproofing of beers.

The next group of innovations appear to be stimulated by attempts to save malt during World War II. Wheats contain sufficient β-amylases but are deficient in α - amylases, therefore malt containing α-amylase (a thermostable enzyme) is often added to compensate for this deficiency. Ball and Tucker effected the extraction of additional α-amylase from

wheat by addition of Sodium sulphite to a slurry of ground wheat which activated the inactive from off α-amylase and aided the flocculation of gluten. This gluten fraction was allowed to settle and the supernatant fluid was added to help and saccharify the cooked mash. Some addition of malt was still needed to bring about the required level of saccharification. However the sulphite liquor caused problems with downstream processing of the ethanol, so an alternative route was examined.

In 1934 Underkofler,[69] began to investigate alternate methods of saccharification of starch and in 1939 demonstrated the production of higher than average yields of ethanol from corn mashes saccharified by mold bran. This process was patented in 1948 and assigned to the Chemical Foundation Inc. This work in turn led to industrial scale tests of mold bran in the alcohol plant of the Farm Crops Processing Corporation, and its adoption for the production of industrial ethanol. During the late 40's and 50's new petrochemicals technology began to be introduced, and cheap synthetic ethanol became available from the oxidation of ethane. The fermentation route to industrial alcohol became uncompetitive, because of higher capital and operating costs. The 60's saw the closing down of the remaining alcohol fermentation plants in the U.S. and in Europe. The rate of diffusion of this technology was slower in many developing countries, which continued to produce ethanol by fermentation. Active intervention in Brazil by the government for economic and political reasons, stimulated the development of fermentation technology, as a petrol substitute.

With the collapse of the enzyme market for the production of industrial alcohol the maltsters concentrated their efforts on supplying

diastatic enzymes to flour millers as flour improvers to improve the flow of dough or its machinability or to improve on the texture and softness of the finished bread.

A.B.M. a large U.K. maltster diversified into the production of fungal and bacterial enzymes, through the acquisition of a smaller specialty enzyme producer, Norman Evans and Rais.[70] Smaller maltsters e.g. Munton & Fison still produce a diastatic malt, the remainder have been merged and integrated within the brewing industry. The production of enzymes for a captive market effectively prevented the diversification of the companies into new areas.

4.6. Enzyme Innovation into the cotton spinning industry

Separate developments occurred in the U.S.A. and France. In the U.S. after the failure of Takamine Fermentation Co., Takamine took other employment and whilst working as a consultant for Parke Davis, he isolated adrenalin for which he obtained a Trade Mark in 1901. Adrenalin became a commercial success and has been in use since 31st October 1900. The adrenalin patent was assigned to Parke Davis.[71]

Takamine organised the Takamine Laboratories in 1914 with some $10,000 and incorporated it in 1915 in Clifton N.J. and from the first it specialised in the production of enzymes and complex specialties. World War I created a demand for textile products for the Army and Navy, and a surge in demand for the U.S. cotton industry. Desizing of textiles with the aqueous fungal amylase put the company on a firm commercial footing.

In France the development of desizing enzymes took somewhat

longer. Boidin and Effront patented a process for producing enzymes from bacteria notably Bacillus subtilis (Bacillus mesentencus) in 1909.[72] They determined the maximum lytic power of amylaceous substances of the enzymes in neutral or alkaline solutions and at high temperatures. In their patent of 1971 they claimed the medium needed to be rich in nitrogenous substances, and in their patent of 1924 claimed a process for freeing textile materials from size by the action of these enzymes. To do so however Boidin and Effront had to develop a culture apparatus to allow the growth of these bacteria in a culture vessel. In it bacteria grew on a series of perforated plates as a film. The plates were perforated to allow the culture to be aerated by bubbles of air. By the 1920's diastases were used in the textile industries to remove excess size from woven fabrics, for degumming silks, and in the canning industry to clarify jellies by dissolving out amylacuous materials introduced with the pectins.

The cotton industry was an important user of starch and early immitators into this area was Rohm and Haas and by 1940 the following companies were producing enzymes for the U.S. market -

United States - Enzyme for textile industry.

Activin	Activin Corpn.
Degomma	Rohm and Haas
Diastaf	Standard Brands
Exsize	Pabst - (Brewers)
Polidase	Schwarz Laboratories
Polyzyme	Takamine Laboratories
Rapidase	Wallerstein Laboratories
Serizyme	Woolen Chemical Co.
Diazyme	Wolf & Co.

The introduction of rayon and synthetic fibres saw a decline in the domestic use of starch as a size. Synthetic fibres are generally stronger and cheaper than cotton so cotton spinning began to decline.

Cotton spinning gradually became re-established in India and other countries of the third world, however when the domestic market for desizing enzymes declined, so enzyme companies began to supply the developing new markets abroad.

The production of desizing enzymes in the U.S.A. also typifies another aspect of the enzyme business. It shows how early innovators develop the technology e.g. Takamine and Boidin are later challenged for this sector of business by other enzyme producers such as brewers, with surplus malting production e.g. Pbast, or starch processors e.g. Standard Brands for a share of this market.

4.7. Enzyme innovation into the leather industry

Industrial output during the 1850's was largely based on the production of iron, starch, cotton and leather. Of these the largest users of chemicals were the cotton and leather industries. A number of innovations expanded the range of leathers and chemists continued to concern themselves with improving the process. Bating remained an art, and made use of decanted liquors from the faeces of dog and pigeon droppings. Eitner considered that the softening of the green hides by the bates was due to micro-organisms, with the dung acting as a source of nutrients. Popp and Becker[73] followed up the work of Eitner and isolated 3 types of bacteria and in 1916 took out a patent on their use as bates. Their commercial bate Erodin was based on Bacillus erodiens cultures adsorbed on sawdust and mixed with ammonium chloride to act as a nutrient. The process was not very successful. Wood was the first to show that the bating action of dung liquors was due to enzymes and isolated trypsin, pepsin, an amylase and a lipase.

Wood[74] improved on this bate and in 1898 obtained a patent for a bate using isolated trypsin.

In 1906 Rohm working in Germany began to look at alternatives to bating with dog faeces and in 1907[75] obtained a patent for a mixture of pancreatic extract and ammonium salts. In 1907 Rohm entered into partnership with Otta Haas a financier who had worked in the United States. Their bate 'Oropon' was technically superior to the bate based on excrements.[76] Within 2 years the company moved from its humbler premises in Essinger to more extensive premises in Darmstadt and opened an affiliated plant in Lyons. Haas returned to the U.S. in 1909, selling 'Oropon' in a modest way in Philadelphia.[77] By 1916 it had established a plant in Bristol, U.S. and diversified into the production of sodium hydrosulphite, and sodium oxylate chemicals used in the processing of textiles.[78] By 1912 the Company diversified into producing mordants for dyeing leathers, synthetic tanning materials, such as chrome salts and a range of leather finishing materials.[79] The first World War established Rohm and Haas with textile contacts, so that it also began to produce synthetic surfactants, based on aryl alcyl polyether alcohols (Tritons): urea formaldehyde resins (Rhonites), aqueous dispersions of acrylic resin (Rhoplexes) all of which were used in treating textiles. The company diversified its production base to include desizing enzymes for textile production, enzymes for modifications of paper starches and Pectins.[80] In the 1920's the company launched the production of methyl methacrylate resins - Plexiglas. Work which Rohm had researched for his Ph.D thesis in 1901.[81]

A number of companies entered this field pioneered by Rohm

producing bates either based on enzymes or cultures of micro-organisms (See note). Enzyme bates were more successful as their working conditions were easier to standardise, so that enzyme bates totally replaced traditional bates over the next forty years.

In 1958 Corden et al[82] examined the effect of microbial and other enzymes as Depilatory Agents and identified :

Takamine Laboratories	Well diversified
Pabst Laboratories	fungal bacterial plant
Wallerstein Co.Inc.	and animal enzymes.
Rohm Corp.	Pancreatin
Mann Research Laboratories	Pepsin
Nutritional Biochemicals Corp.	Trypsin and Pancreatin.

4.8. Innovation into the starch industry

Innovation on an industrial scale has coincided with the growth of the starch processing industry. I also include in this the more conventional starch milling of grains for baking.

4.8.1. The European Starch Industry

The production of starch for baking can be regarded as a separate industry going back in antiquity. In Europe flour production was already a major concern by the 11th century. Other uses for starch followed. The introduction of starch for stiffening or starching clothes became fashionable in the 14th century.[83] Coloured starches were fashionable as hair powders during the 15th and 16th centuries. The introduction of starch to the manufacturers of papers was also a 15th century innovation. A 17th century innovation introduced starch as a strengthening

agent for the warp threads in textile manufacture. The number of starch manufacturers began to increase. An early patent for its production is one obtained by S. Newton in 1707, Arrowroot starch was patented in 1797.[84] In 1841 Coleman patented a process to manufacture starch from rice by removing the proteinacuous gluten by fermentation, and Brown and Polson obtained patents for these products in 1854.[85]

Diversification of the industry into other product sectors began in 1811 with the work of Kirchoff who showed that glucose could be obtained from starch by the hydrolytic action of sulphuric acid. By the following year 4 plants were producing the sugars and by 1876 47 plants making glucose from starch were operating in Germany alone.[86]

The accidental production of roasted starch in England, led to the discovery and production of British Gum as a cheap adhesive in 1821.[87] As the demand for starch and starch products grew in Europe it was met by many potato starch processing plants in Germany, Holland, Poland, parts of Russia, France and Czechoslovakia. These plants grew to meet the needs created by expanding textile and paper industries. The fermentation method for the production of grain starches was most common, innovations such as the tabling of starch in 1893 made the process faster and more productive. The potato became the dominant source of most of the European starches. Potato, wheat and maize starches are all produced in Europe today.

4.8.2. The United States Starch Industry

In the U.S. wheat starch production was started by Gilbert and initially supplied most of the starch for industrial needs.

A rice starch plant was established in 1815 and by 1880 several of these were operating, however their impact on the wheat starch industry was small. The first potato starch plant began operating in 1820 and by 1895 64 plants were operational. Maize starch manufacture began in 1842 with the successful development of this process by Kingsford - by 1895 most of the wheat starch producing plants had turned over their production to maize and their number had fallen to 15. They served the rapidly developing technologies of textile sizing and dressing calico printing, paper making, adhesive and food manufacture. Large scale production with maize began in 1873. A series of innovations followed, a major innovation was the introduction of corn wet milling in 1881 enabling the recovery of gluten and oil by-products, which in turn led to the formation of an animal food-stuffs industry. 1908 saw the introduction of the continuous corn steeping system. The industry began to change also, a series of takeovers and amalgamations had produced the U.S. National Starch Company which led to the formation of C.P.C. the initials of the Corn Products Corporation.

Innovation into the starch processing industry occurred quite late in the industry's development. Enzymes were able to increase the Dextrose equivalent value of certain syrups and were consequently introduced into the processing during the 1950's.

4.8.3. Flour modification

Wheat flour contains a α-amylase, a protease and varying amounts of β- amylase, the amount of each depends on the variety of grain and the growing and harvesting conditions. The α- amylase content of flour increases in wet climates since this enzyme is used to mobilise the starch reserves during germination. The proteases are particularly evident in newly ground flour and produce a sticky dough which is difficult to work, and which produces a doughy loaf on baking.[88] Aging the flour inactivates the proteases and makes the flour stronger. (Chemical agents can be used to age flour artificially.)

The β-amylase content of flour is also important since its action releases the fermentable sugar maltose and controls the amount of gassing in the bread. However flours rich in α -amylase produce very sticky doughs which can clog up a modern bakery especially at the cutting stages. Over the last 30 years in the U.K. millers have been controlling the α-amylase content whereas in the U.S.A. this supplementation is carried out by the baker. Flours with a low α-amylase content have malt flour added to them (Malt flour contains α-amylase which is a heat stable enzyme) but they needed careful monitoring. More recently however millers have been using fungal α - amylase. Flours with a high α - amylase content are usually mixed with flours with a low α - amylase content to produce a balanced flour. Fungal α- amylases are more suitable for baking purposes than bacterial amylases because they are more easily inactivated by baking and prevent further enzyme action once the bread is worked.

4.9. Enzyme Innovation in the silk processing industry

Prior to the introduction of proteolytic enzymes for the degumming of silk fibres, this process had been carried out by using a hot soap wash, which caused the removal of sericin, the silk protein gum but caused the fibres to lose some of their fine texture. Degumming of silk by proteolytic enzymes imparts a light colour and a finer texture to the silk.[89]

4.10. Enzyme Innovation into Laundering and Dry Cleaning

O.Rohm[90] patented a process for the use of pancreatic enzymes in laundering at low temperatures. A concentration of 2 grams of pancreatin per 100 litres of water gave satisfactory results at low temperatures, Rohm also recommended the addition of pancreatin to bath and wash water for this purpose. He suggested the use of 0.5-1gm. of pancreatin to 100 litres of water. He manufactured the product, an enzymatic pre-soak called Burnus.

Although a number of later innovations were tried, e.g. in the U.S., Balantine produced a soap with diastase to improve its cleaning action and Attenberg suggested the addition of Ricinus lipase. These and other developments will be examined in more detail in chapter 6 below.

4.11. Enzyme Innovation into the hydrolysis of fats

Not all of the early innovations go into enzyme producing by present-day companies. The patents considered to be the key ones were those which enabled a company to produce glycerol and fatty acids by the enzymatic hydrolysis route. The production of lipase was based on

the extraction of castor oil seeds and its action produced a lightly coloured fatty acid and glycerol liquor. Use of the lipase avoided the use of fuel for steam generation and the use of chemical reagents. However the process relied on the use of unstable enzymes and working conditions that at the time were difficult to control. It also suffered from undue emulsification once the process was complete and there were organic water soluble impurities in the glycerol liquor upon completion. These enzymatic hydrolyses of fats by lipase ceased in the 1930's. No further mention of it has been made, until recently when a Japanese company took out a patent for its production.[91]

4.12. Enzymes for the meat tenderisation processing industry

The tenderisation of meat with the aid of papaya juice was reported by early travellers to the tropical regions of Central and Southern America. Papain is the powdered latex of Carica papaya. Latex is obtained by making 3-4 scratches in the green fruit when it is still hanging on the tree. The liquid latex coagulates on the surface and collected and dried. About 50% of the total mass is the protease. This product was stabilised by treating with hydrogent sulphides, sodium or potassium sulphites. The application of the papain produced is mainly used for tenderisation of meat. Commercial products are marketed usually with a bacteriostat for example dilute alcohol. These preparations are applied to the meat prior to its cooking. Some slight but definite digestion occurs of the connective tissues and muscle fibres, which softens the meat. Recently the use of papain has been extended to injection of the animal prior to slaughter. The major distributors of papain are its importers and as a result they

only trade in this enzyme and constitute a market for it.

4.13. Enzyme innovation in fruit juice production

By the 1930's enzyme producing companies were diversifying their product ranges into each others product. Many had small research and development departments which were looking for new enzyme applications.

In 1926 the fruit juice industry was fairly static but innovations into better processing and improved packaging for distribution by bottling the juice or canning made fruit juices more accessible to the public and the consumption of fruit juice in the United States began to increase rapidly. Rohm and Haas identified a market need that could be met by an enzyme. Willaman and Kertesz[92] obtained a patent for the production of pectinases, enzymes that digest the mucilaginous pectins found in fruit and vegetables. Rohm and Haas began the production of pectinase shortly after which they introduced on to the market Pectinol. The production of fruit juices was also begun on the continent and its production was taken up also by Grindsted a company specialising in the production of stabilisers and emulsifiers for the food industry.

4.14. General Conclusion on innovation in Enzyme Technology

From this analysis it is possible to make the following observations about innovation in enzyme technology.

1. That technology using crude preparations of enzymes had been developed using empirical methods of analysis. Two areas in particular were already sophisticated in their methods of working - rennet production for cheese

making and the production of malt from barley. These two sectors have continued to be dominated by the descendents of companies that developed this commercial niche.

2. That a number of different scientific disciplines contributed to the development of the enzyme science, and that the coming together of two or more branches of science continues to produce new scientific breakthroughs in the understanding, production and use of these catalysts.

3. That during the development of the enzyme paradigm, scientist entrepreneurs were developing enzyme preparations from animal and microbial sources, which successfully substituted for the use of crude biological extracts.

4. That entrepreneurs continued to be important in the early stages of the industry and were able to exploit different commercial niches for their enzyme products, e.g. Takamine - desizing cotton; Rohm, washing preparations and bates for leathers; Boidin and Effront, desizing cotton. Political factors such as World War I helped to establish these companies because of its effect on the State's demand for uniforms and boots. (See Fig.4.1. above)

5. Companies specialising in the supply of research grade chemicals also began to sell enzymes and to produce high grade enzyme preparations for therapeutic uses in response to public and private demand for improved

medical services in U.S.A. and Europe.

6. Once these opportunities to substitute enzymes for crude preparations were established the further opportunity to innovate in the enzyme sector came to depend either on the evolution rather than the identification of suitable new markets or on the identification of new ways of using these enzyme catalysts. For example, Rohm & Haas pioneered the production of pectinase enzymes for use as processing aids in the rapidly expanding fruit juice market in the U.S.A. Wallerstein pioneered the use of papain for chill proofing beers in the U.S. which produce a protein tannin complex on cooling.

7. Most of the early enzyme companies effectively diversified their product ranges so that they began to compete with one another in more than one product. Competition made the early enzyme producers more amenable to being taken over thus Takamine Laboratories, for example, were acquired by Miles, a fine chemicals and pharmaceutical producer. As a result such a company, (which continued to diversify,) now produces enzymes for all product sectors and their associated markets.

8. Companies have been able to enter enzyme production by diversifying their business interests to include the production of enzymes. Successful companies have had either control of a 'waste' feedstock which they can process to make enzymes or have been able to identify a new niche in the market and have exploited it, or both. Companies in the first category include meat packers such

as Cudahy Packing Co., Omaha, Nebraska, U.S.A., maltsters such as A.B.M. Food (U.K.) and Premier Matt Products (U.S.). End users have also been able to become enzyme producers, e.g. food processors such as Standard Brands Sales Co.(U.S.). In the second catagory are found pharmaceutical companies such as Chas. Pfizer, Armour Pharmaceutical, Glaxo, Novo and Gist-Brocades; chemical companies such as Fermo Laboratories (a subsidiary of Harshaw Chemicals Co.U.S.). Developments in the understanding of the regulatory mechanisms controlling cellular metabolism opened the way for the production of enzymes. As did knowledge of fermentation technology. Companies that have successfully combined the two include J.E.Sturge and the Finnish State Alcohol Monopoly which was able to enter the market by producing glucose oxidase from species Aspergillus.

9. Immobilisation research created opportunities of extending the use of enzyme catalysts and creating or extending markets. This created opportunities for established companies as well as a number of new companies to enter this field. Thus chemical companies such as I.C.I., Monsanto American Cyanamid, were already active in this area. Other companies on the other hand, were able to diversify out of tobacco e.g. Reynold Tobacco; out of specialist glass production e.g. Pilkingtons Glass plc and Corning Glass Corporation. However, food companies e.g. Tate & Lyle plc., all had limited successes in market exploitation with these catalysts. (Immobili-

sation technology will be discussed in greater detail in chapter 8 below).

10. Immobilisation research also enabled enzymes to be used in diagnostics but this market has been predominantly exploited by specialist chemical producers such as Boehringer Mannheim. Despite this a number of small companies have been formed by scientist entrepreneurs who have more specialised advantage in that they can exploit up-to-the-minute research findings and translate them into new value products for a variety of markets using immobilisation technology. The production of small high value specialist diagnostic kits with medical applications, provided an opportunity for several new companies to enter the market. Likewise the boom in genetic engineering has created a demand for specialist enzymes, the genetic engineer's 'scissors' and 'glue'. Both these scientific areas increasingly attracting commercial activity have involved scientist entrepreneurs. (These developments will also be discussed in detail in chapter 7 below).

Focusing on innovations and the innovation process by means of a chronological and analytic framework such as Fig.4.1. above confirms that many of the successful innovations originally involved science-based entrepreneurs, but that as the market has developed more companies have entered into it and the trend in innovation appears to have swung in favour of the larger organisations, findings confirmed by the analysis of patents in chapter 3 above. Thus it is the

established enzyme companies through their inhouse research and development which appear to be better suited and are economically prepared to exploit new enzyme developments to meet and match changing market needs.

Yet it is the close relationship and match between the enzyme user and the enzyme producer that enables enzyme producers, enzyme factors and agents to compete in this market, and this helps explain why perhaps potential competitors without such links have been less successful in exploiting new market opportunities.

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CHAPTER 5 - CASE STUDY : THE HIGH FRUCTOSE SYRUPS

5.1. Introduction

This case study examines an area of research identified during the literature survey by quantitative indicators such as co-word analysis and patents as an important area of research and inventive activity. This analysis aims to identify the factors which influenced the development of this technology and those which continue to influence its subsequent diffusion.

5.2. An opportunity for substitution

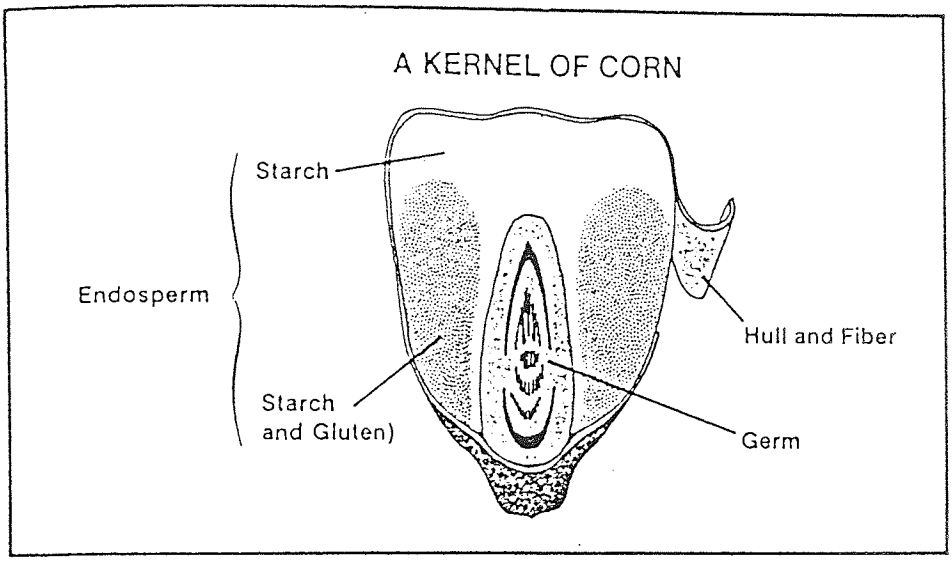
In an ideal market, i.e. where there is free competition, the incentive to substitute one product by another is driven by either the perceived or real profit or enhanced customer satisfaction or both. In the absence of any difference in quality between the two products then substitution is determined by the price difference alone. In this case study I shall discuss the substitution of one sweetener (sucrose) by another (high fructose syrup). Under certain conditions these two substances are chemically identical. In slightly acidic solutions sucrose and a 50% high fructose syrup made from starch are indistinguishable so that substitution of sugar by the syrup can occur on the basis of price alone. The production of both sweeteners involves the processing of agricultural producing crops, sugar cane or sugar beet in the case of sucrose and maize, wheat or potato for starch which is processed further to make a High Fructose Syrup.

Price differences between the two crops come about because of the biological differences between the crops, differences in their preferences for certain soil and climate types, with weather conditions, incidence

Table 5.1. The productivity of land using different crops.

Food Source	Column 1 Hectares of land to produce 1 million callories	Column 2 Calorie % of Product Ex Farm
Sugar	0.07	20%
Potatoes	0.20	20%
Corn (as corn meal)	0.40	85%
Wheat (as whole wheat flour)	0.54	85%
Pigs (pork lard)	0.90	100%
Whole milk	1.27	10%
Eggs	3.54	25%
Chickens	4.23	15%
Cattle	7.73	15%

Figure 5.1.



Source: Nutritive Sweeteners from corn.[2]

Table 5.2. By-products of corn wet milling

	% DRY SUBS. AS SOLD	TYPICAL ANALYSES		USES
			% DRY BASIS	
Steepwater	40	PROTEIN ASH	45 18	LIQUID FEED SUPPLEMENT
Germ	97	PROTEIN STARCH OIL	14 10 48	EXPELLED FOR OIL RECOVERY
Bran	30	PROTEIN STARCH	11 10	CATTLE FEED (LOCAL)
Gluten Meal (60%)	89	PROTEIN STARCH	70 14	CHICKEN FEED COMPONENT

HIS IS MINIMUM GUARANTEED PROTEIN ON COMMERCIAL BASIS.

Source: The Dimmitt Plant. Corn Products division. Amstor Corporation, Dimmitt, Texas.

of past damage, market considerations and political expediency. Column 1 of Table 5.1.[1] shows that sugar crop plants are efficient carbon converters of the food sources presented and make the most economic use of land. However, the second column indicates that the cost of extraction and refining is higher because maize and wheat seed stores are harvested in seed form, a more concentrated form of carbohydrate, than the stems of sugar cane or the roots of beet. In addition carbohydrate seed crops can produce valuable by-products if they are processed in a particular way. Thus the most completely utilised crop is maize (see Fig. 5.1.). The fruit coat contains protein and starch, the germ contains valuable oil and the starchy endosperm contains protein which is valuable as animal feed. Corn wet-milling separates the four components very efficiently and produces a clean starch which is available in the corn belt of the U.S.A. at around $110/tonne.[2] Even the steep water is sold as a valuable base for the fermentation industries (see Table 5.2.). The cost of sucrose in the U.S.A. (in 1983) was around $500/tonne so a sizable margin exists between these two prices which can absorb processing costs.[3] The U.S.A. produces vast amount of maize, over 90% of which is sold as animal feed.[4] It is stable when stored dry and therefore there are no problems of raw material supply to the corn (maize) wet millers and syrup producers. Although both beet and cane sugar are produced in the U.S.A. they compete with other crops, and large areas of crop land, the climate is not ideal for either, the winters are often colder than they should be under ideal growing conditions. Thus the American corn wet-millers had a strong commercial incentive to develop means of converting starch to glucose and then to convert it into High Fructose Corn Syrup. (H.F.C.S.)

Table 5.3. Major Companies involved in the Development of Isoglucose Production.

(Not intended to be an exhaustive list of manufacturers and suppliers).

A. THE WET MILLERS

Archer Daniel Midland (ADM) - the biggest producers of H.F.C. based in Iowa. Not an "R & D" company but very adventurous in introducing new technology. Major producers of power alcohol from corn starch. Traders in corn and soyabeans.

A.E.Staley - Illinois based cornstarch, syrup and H.F.C.S. manufacturers with soyabeans as another major interest. Do much of their own R & D but use outsiders' equipment and knowhow also. One-third shareholders of GR Amylum and Tunnel Refineries.

Clinton Corn Processing - The pioneers of isoglucose production developed widespread knowledge of enzyme technology. Acquired recently by Nabisco Brands and activities dispersed, H.F.C. syrup to AMD, enzyme expertise to Finn Sugar.

C.P.C. (Corn Products Corporation) - Multi-national producers of corn products (in U.K. they sell Mazola, Knorr Soups, Brown and Polson custards etc.) Were the originators of the concept of H.F.C. syrup produced enzymatically, but were very late into production, perhaps because they developed their own technology inhouse. Tend to be secretive but have recently announced an intention to enter the enzyme business as producers. Produce glucose syrups in U.K., not isoglucose.

Cargill - Another U.S. giant, primarily involved in soyabean trading, maize milling and wheat flour.

Anheuser-Busch - Major U.S. brewers, important in that they developed one of the best glucose isomerases and worked extensively on alkaline isomerisation, but have made little impact as isoglucose producers.

G.R. Amylum (Aalst, Belgium) and Tunnel Refineries, (Greenwich, U.K.) - Separate companies but both owned equally by A.E.Staley, Tate & Lyle and a Luxembourg company.

B. THE ENZYME COMPANIES

Novo Industries AS - Based in Copenhagen, the world's major producers of enzymes and the major suppliers of glucose isomerase. Probably the most successful "biotechnology" company with excellent R & D and process experience.

Gist Brocades - Based in Delft, Holland. A long established yeast and pharmaceuticals company with a good record in producing and marketing enzymes.

Table 5.3. (continued)

Miles - A multi-national biological company that tries hard, has impressive R & D but had made little impact on isoglucose manufacture, and has been purchased by the German Company, Bayer.

I.C.I. - The major U.K. chemical company, into enzymes competitively recently. Had their fingers burned in the disastrous KSH-Albion isoglucose venture, but may offer their enzyme again. The only U.K. manufacturer of glucose isomerase.

Finn Sugar - The very wealthy Finnish sugar state monopoly has entered into the enzyme industry with, among others, Clinton's expertise.(see A above)

At the time of this innovation being developed the starch processing industry was and has remained essentially an oligopoly with entry into the industry requiring high capital investment and familiarity with the technology. A description of the major companies within the industry and major enzyme innovators is provided in Table 5.3. above.

5.3. The development of enzyme technology

The industry was aware of possibilities of developing alternative sweeteners from glucose but an intense period of competition between corn wet-millers had led to cost cutting and the reduction of overheads such as R & D departments. Work on isomerisation was isolated. That isomerisation of glucose to fructose occurred when glucose was on heat in an alkaline solution of sodium hydroxide had been known since 1895 when Lobry de Bruyn and Alberda van Ekenstein described this alteration. Despite numerous attempts to make it work on a commercial scale, alkaline isomerisation proved difficult to manage because of uncontrolled side reactions which produced unwanted sugars and a coloured mixture of products that was difficult to purify. Many catalysts were tried and included alkalis, alkali earth carbonates, ammonia, alkaline organic compounds and alkaline ion exchange resins. This avenue of research continued to produce patents as late as 1974.[5] However, in all these cases product yields were low, about 20-30%, and the formation of the bi-products, maltose and psicose and brown colours made processing expensive and prevented the adoption of these alternative strategies for syrup production ever being developed on a commercial scale.

After the Second World War other changes in technology were afoot in the starch industry. Demand for syrups with higher DE (Dextrose Equivalent) values than those produced by acid hydrolysis led to the gradual adoption of enzymic hydrolysis of starch. New working skills associated with enzymes began to be developed within the industry. Meanwhile pure science research was producing evidence that isomerase enzyme did exist in a number of tissues as a number of independent investigations in enzymology began to identify this new class of enzyme activity. During the period 1952-3 these independent investigations showed that a range of bacteria and animal cells which were able to isomerise five carbon sugars, such as erythrose and D-xylose.[6] The existence of five carbon sugar isomerases prompted the research Director of Corn Processing Corporation, (CPC) to search for a six carbon sugar isomerase. In 1957 Marshall and Kooi demonstrated the conversion of D-glucose to D-fructose by a microbe Pseudomonas hydrophila.[7] The commercial potential of this method of converting glucose to fructose was protected by a U.S. patent which was filed on 1.9.59 and granted on 23.8.60.[8]

The process however required arsenate ions, had a low conversion ratio of glucose to fructose, i.e. around 33%, needed a high pH (8.5) and used expensive xylose in the substrate to induce the formation of the enzyme. All these factors mitigated against the commercial development of the process. C.P.C. protected this line of investigation with a blocking patent, (a patent that would require a licensing from C.P.C. or involvement in any later breakthroughs by any of its rivals). Having proceeded this far C.P.C. chose to abandon this line of investigation. However research on isomerisation of glucose to fructose continued in a number of institutional laboratories, chiefly

in Japan, and in 1958 Yamanaka reported sources of glucose isomerase from a number of Lactobacilli and Leuconostoc[9] species of microbes and in 1963[10] that Arsenate was not a metal requirement for this enzyme. The Japanese workers concentrated their efforts on screening and categorising a series of organisms and their isomerases. Tsumura and Takasaki[11] discussed the production of glucose isomerases from a number of organisms. In 1966 this line of investigation resulted in the grant of patents to the Japanese Bureau of Industrial Technics for Streptomyces bodiviae and Streptomyces flavovirens glucose isomerases. The work of the Japanese scientists was being monitored by the Clinton Corn Processing Corporation, (C.C.P.C.) a division of Standard Brands. The Director of Research had personally initiated a programme - to isomerise dextrose to fructose in 1964, a period of high market sugar prices. (see Figure 5.2.).

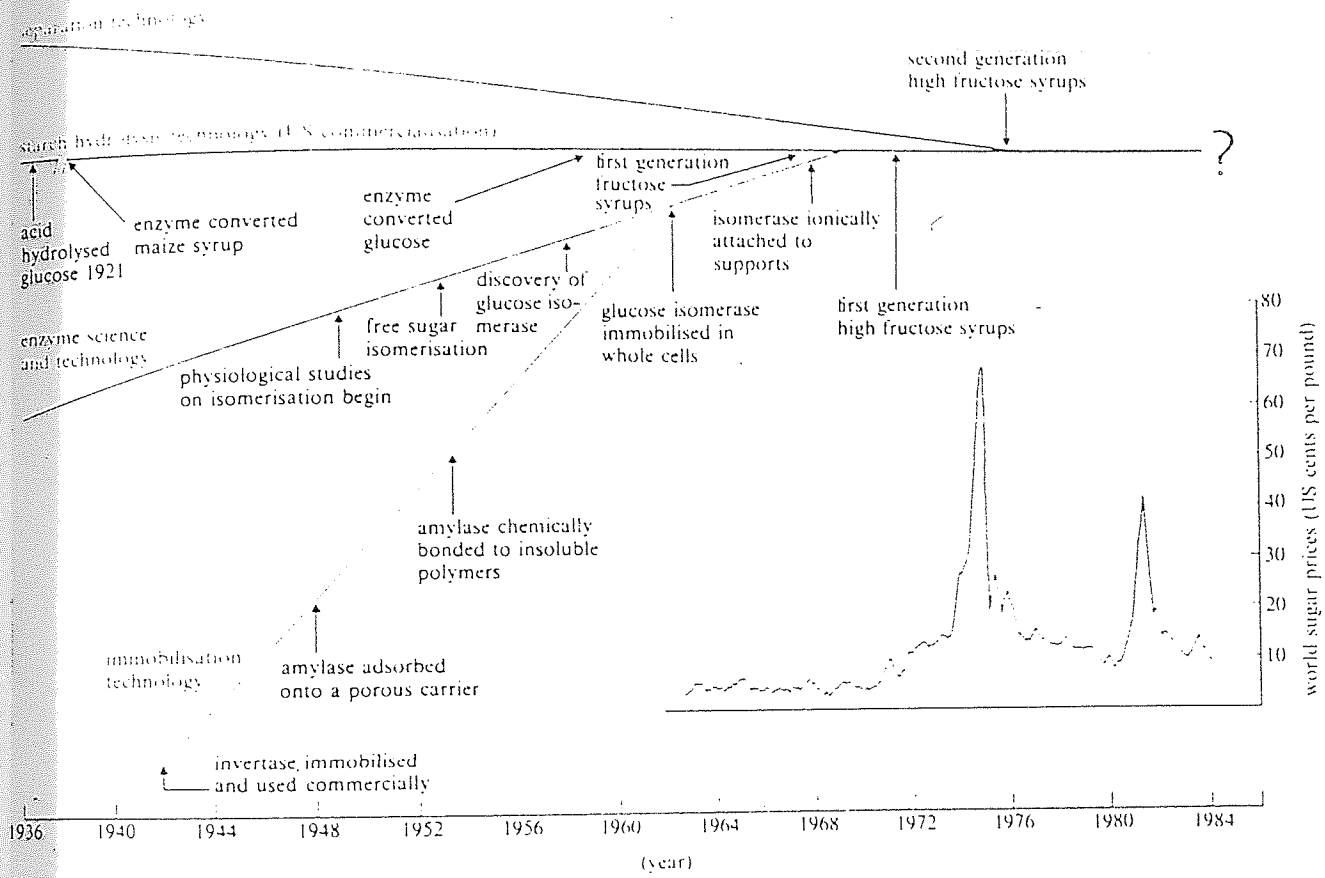


FIGURE 5.2A chronological summary of the major technological inputs to high fructose syrup production

Source : Towalski and Cohen 1985[1]

TABLE 5.4.

Heat Treatment of Cell and Confinement of Glucose Isomerase inside Cell

Temperature of Treatment °C	Time of Treatment minutes	Extracted G.I.* (A) (u/ml)	Total G.I. (B) ** (u/ml)	ratio (B-A)/B x 100 %
50	60	14.8	14.4	0
	120	14.1	14.1	0
	240	15.6	14.4	0
55	30	15.1	14.6	0
	60	14.1	15.8	8
60	10	3.6	15.0	76
	20	3.2	15.0	79
70	10	2.4	14.6	84
75	10	1.5	13.4	89
80	10	1.3	12.0	89
85	2	1.2	13.9	--
	5	3.3	8.8	--
	10	0.9	8.0	--
No heat treatment		15.6	15.0	0

* Glucose isomerase amount extracted, after the heat-treated cell had been incubated for 24 hrs under the conditions favourable to autolysis.

** Glucose isomerase amount extracted from the heat-treated cell by means of sonic oscilator.

Source : Takasaki, Y., Kosugi, Y. and Kamibayashi,A. in Fermentation advances. Perlman, D., Ed.561-589 Academic Press, New York 1969.

The company began the investigation of corn syrups containing fructose to increase their sweetness, and had managed to overcome the problem of crystallisation of high DE syrups during transportation by using heated and insulated tankers. In 1965 Dr. R.G.Dworshack, Head of Clintons Microbiology Section identified the developments in Japan's Fertilizer Research Institute, which he and Dr. R.V.McAllister (the Director of Research for Clinton) and Dr. L. Atkin, (Director of Research for Standard Brands) went to Japan to evaluate this R & D work.[12] This meeting led to an eventual agreement between Japanese Agency of Industrial Science and Technology and Clinton Corn Processing Corporation which allowed them to investigate the commercial potential of the enzyme. Ultimately this led to an agreement giving Clinton the exclusive U.S. rights to utilise the Streptomyces enzyme for the production of fructose syrups.[13]

The Japanese developed the technology to a level where commercial production using a batch process was possible. This involved contacting the dextrose with cells containing the isomerase enzyme, the enzyme being fixed in the cells by temperature treatment (see Table 5.4.) The cells containing the enzyme were separated from the product by filtration and centrifugation, a slow and costly process.[14] This technology was developed and commercialised in Japan in 1966 by Sanmutsu Kogyo. Clinton's scientists developed methods of extracting the enzyme[15] from the cells and patented two methods of immobilising the enzyme using pressure filters, and a system using diethyl amino ethyl (DEAE) cellulose, a weakly anionic material made by chemically modifying cellulose[16] which can be used as an insoluble enzyme support and make the separation of enzyme from product relatively simple. Immobilisation not only enabled the enzyme to be removed

TABLE 5.5. EQUILIBRIUM CONCENTRATION OF FRUCTOSE AT VARIOUS TEMPERATURES.

D glucose - D - fructose equilibrium temp.determined by analysis of glucose fructose composition at equilibrium.

	% fructose			
25°C	42.5 (44.7[a])	Takasaki (1967)		
30°C	46.5	Lloyd and Khaleluddin, K(1976)		
35°C				
40°C	47.9	Takasaki 1967		
45°C	48.2			
50°C				
55°C				
60°C	49.9	53.4 (50.0[a])	50.4	Havewala & Pitcher (74)
65°C	52.4	56.4		
70°C	53.1		51.4	
75°C		Clinton Corn Processing results		
80°C	54.2		52.3	
85°C	54.7			

Lloyd, N.E. and Khaleeluddin, K. 1976. Cereal Chem. 53, 270.

Takasaki, Y. 1966. Agric.Biol.Chem. 80, 1247-53.

Havewala, N.B., and Pitcher, W.H. (1974). Streptomyces sp. ATCC 21175

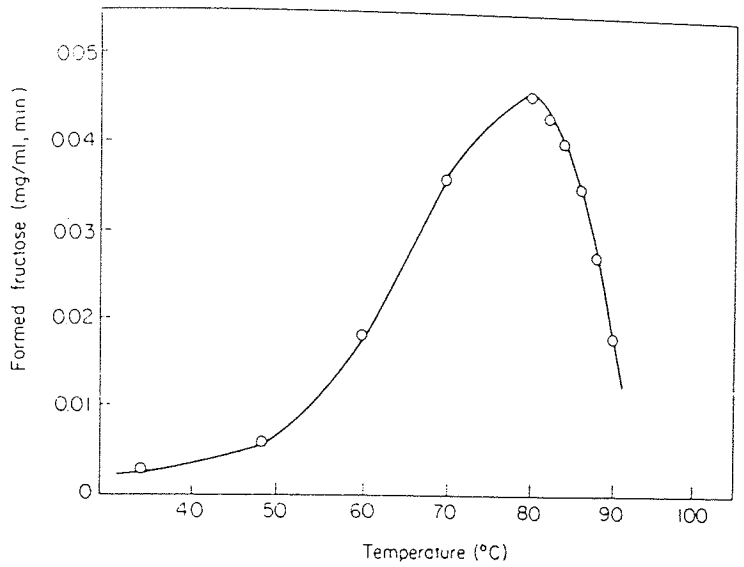


Figure 5.3 Effect of temperature. Ten minutes at respective temperatures. Phosphate buffer concentration 0.05 M; $MgSO_4$ concentration 0.01 M; glucose concentration 0.1 M; enzyme - ten minutes at respective temperature.

Source : Takasaki, Y., Kosugi, Y., and Konbayashi, A., Streptomyces Glucose Isomerase p.583 in Fermentation Advances Ed. Perlman, D., Academic Press, New York London 1969.

but also it had the advantage that the enzyme could now be reused, cutting the cost of enzyme per production cycle.

CCPC's first commercial fructose syrup was introduced in 1967, called Isomerase(R) 30, it contained 15% fructose and was made in a batch reactor using soluble glucose isomerase.(17) From 1968, production continued as a batch process but an immobilised glucose isomerase was used instead. In November 1972, the batch process was superceded by a continuous process which yielded a 42% fructose syrup, and marked the production of first generation fructose syrups.

The equlibrium constant of the Streptomyces glucose isomerase catalysed reactions is such that at higher temperatures, higher percentages of fructose are produced. (See Table 5.5) However the enzyme denatures more rapidly when worked at elevated temperatures. Therefore to minimise shutdown times to replace the catalyst the process operates at a reduced temperature around 70°C.(18) This process is illustrated in Figure 5.3.

Somewhat later the enzyme companies and some of those interested in producing "inhouse" syrup began R & D programmes by screening for glucose isomerase activity in microbes. The following microbes were selected : Bacillus coagulans (Novo Industry, Denmark), Actinoplanes missouriensis (R & D by Anheuser-Busch, manufactured by Gist Brocades, Holland), Streptomyces sp. (Corn Products Company), Arthrobacter sp. (Reynolds Tobacco research, developed and manufactured by I.C.I.). Of these companies Novo claims that they learnt a lot from published Japanese material in the 1960's and they were puzzled why the Japanese did not commercialise the technology. The patents literature describes many other glucose isomerase products but only those mentioned above

have found commercial use, in some cases only briefly. It is interesting to note that glucose (xylose) isomerase activity has now been widely reported in many species of microbe. (See Table 5.6.[19])

During the early 70's Clinton had a clear lead over competing corn wet-millers but, to share the risk of developing a new product and to give customers the reassurance of having an alternative source of supply, they sub-licensed the technology to their rivals A.E.Staley Inc.[20] Clinton and Staley reached full scale production in the early 70's and, most significantly, had high fructose corn syrup available in large quantities during the sucrose shortage of 1973-4 when sucrose prices reached record levels (see Figure 5.2.) (This was due to a combination of several factors including adverse climatic conditions and panic buying). It is generally recognised that Clinton and Staley made a "killing" in that period. This confluence of science, technology and market opportunity is summarised in Figure 5.2. above.

5.4. The diffusion of the technology

Not surprisingly other corn wet-millers were also keen to exploit this technology so the period 1973-5 was very active, both for the research and development departments of starch processors, the constructors of corn wet-mills and enzyme manufacturers. Very rapidly, all the major corn wet-millers and a few other organisations were building HFCS plants, but the range of capacities indicated a degree of caution. Plant sizes ranged from 22,000 ton/annum to 330,000 ton/annum.[21] The most committed of the wet-millers was Archer Daniels Midland Co., a company that does little or no research and development, but concentrates on management production and the benefits

TABLE 5.6 GLUCOSE ISOMERASE (D-XYLOSE ISOMERASE) PRODUCING ORGANISMS

Genus	Species
Streptomyces	bobilai, flavovirens, echinatus, achromogenes, chromogenes, fradiae, roseochromogenes, olivaceus, californicas, venuceus, virginial, olivochromogenes, venezulae, wedmorensis, griseolus, glaucescens, bikiniensis, albus, rubiginosus
Lactobacillus	brevis, mannitopoeus, pentoaceticus, gayonii, plantarum
Brevibacterium	pentoso-aminoacidicum, imperiale, incertum
Micrococcus	agilis
Pseudomonas	hydrophila
Leuconostoc	mesenteroides
Aerobacter	aerogenes, levanicum
Bacillus	coagulans, stearothermophilis
Escherichia	coli
Aspergillus	oryzae
Mycobacterium	
Nocardia	asteroides, dassonvillei, corallia
Micromonospora	rosea, rosea monnitrogenes
Microellabospora	flavea
Arthrobacter	
Actinoplanes	missouriensis
Thermopolyspora	
Pseudonocardia	
Streptosporangium	albus, vlugare
Curtobacterium	
Flavobacterium	devorans

Source : Antrim R.L., et al.[19]

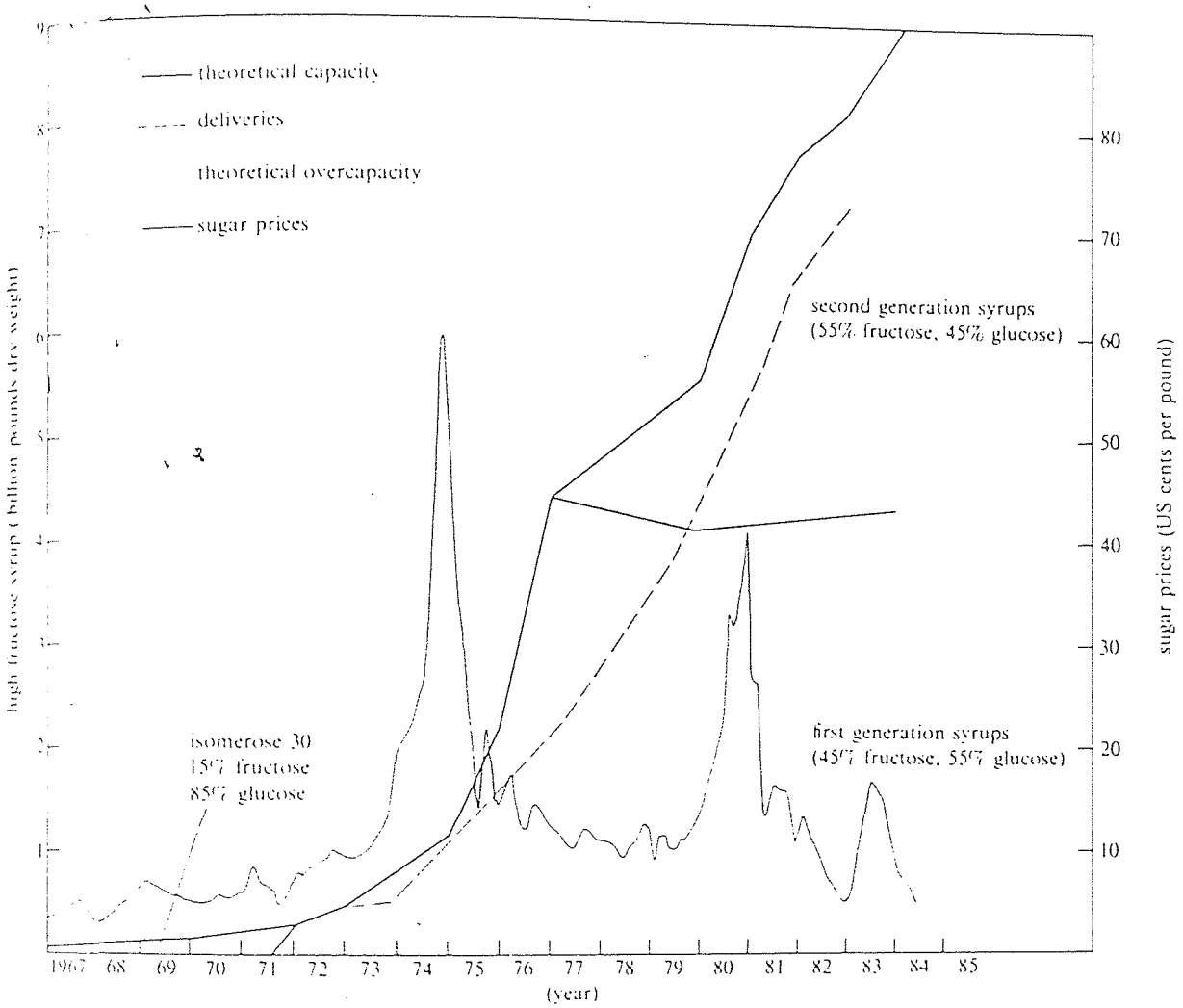


FIGURE 5.4 The diffusion of high fructose syrup technology into the United States from 1967 to 1983 showing the amounts produced and the production capacity available

Source : Towalski, Z. and Cohen, N.C.1986 (1)

of scale to complete. When it comes to new technology it puts its faith in the expertise and competence of its suppliers. This policy appears to have paid off and ADM now hold a very powerful position in the HFCS industry.[22] On the other hand Corn Products Co. who first thought of producing HFCS were one of the last companies in the U.S.A. to produce it. They were slow to develop the technology because they felt they had some claim to it by virtue of their patent but fell foul of the patents system. In an action between CPC and Standards Brands, their blocking patent was found to be invalid because the arsenate requiring enzyme isomerase specified in CPC's patent was shown to be a glucose phosphate isomerase and not a glucose isomerase. Since the enzyme glucose isomerase occurs in a range of organisms it gave enzyme manufacturers and HFCS producers the freedom to develop several alternative methods for producing their products. Later they chose to develop their own glucose isomerase, using a species of Streptomyces which was not as productive as some of their competitors' enzymes.

Other U.S. companies that invested significantly in HFCS plants were Cargill, Heinz, Amstar, Holly Sugar, American Maize and Anheuser Busch.[23] Inevitably such a large investment resulted in over-capacity and this, coupled with the fall in sugar prices by 1976, meant that the HFCS business was by no means as profitable as Clinton and Staley had found it. In 1977-79 plant utilisation levels for some companies were as low as 50% but this was more a consequence of the construction of new plant than of any fall in sales of HFCS. In fact sales of HFCS rose from 942,000te in 1977 to 1,543,000te in 1979. (see Figure 5.4.)

Over-capacity on this scale resulted in fierce competition between companies, and slight differences in manufacturing and

TABLE 5.7. MAJOR COMPONENTS OF U.S. HFCS VARIABLE PRODUCTION COSTS (1982)

CORN	50%
ENERGY	20%
LABOUR	10%
CHEMICALS	10%
ENZYMES	5%
MISCELLANEOUS	5%
TOTAL	100%

Source : Vuilleumier, S., F.O.Lichts International Sugar Report 1981.

distribution costs became significant. The roles of R & D departments and plant managers became more than usually important, and choice of catalysts and support materials was crucial. With improvements in enzyme preparations, larger scale plants and further developments in immobilisation technology enzyme costs fell to 5%. The relative sizes of the major components of the variable production costs of HFCS in the U.S.A. are summarised in Table 5.7.

The cost of corn is unlikely to vary greatly; less than 10% of U.S. maize is wet-milled for human consumption so there is little chance of there being periods of shortage of raw material. Transport costs are significant so the location of the wet mill in relation to the source of corn and to the major markets for the product is important. The efficiency of the wet mill is crucial, the process is energy intensive and it is most important that losses of corn oil and gluten are kept to a minimum because these are more valuable than starch. The cost of the enzymes (bacterial α-amylase, glucoamylase and glucose isomerase) is not large but the conditions under which they work and their performance influence energy, labour and chemical costs.

5.5. The process technology

Figure 5.5. summarises the stages in the production of HFCS from starch and indicates the conditions under which each of the enzymes operates. The starch slurry in water passes from the wet mill to the hydrolysis plants. The starch is in the form of grains and in this state it is insoluble and unavailable to hydrolysis by enzymes. Thus the grains need to be disrupted, and the starch rendered soluble hence more accessible to enzyme action. The starch

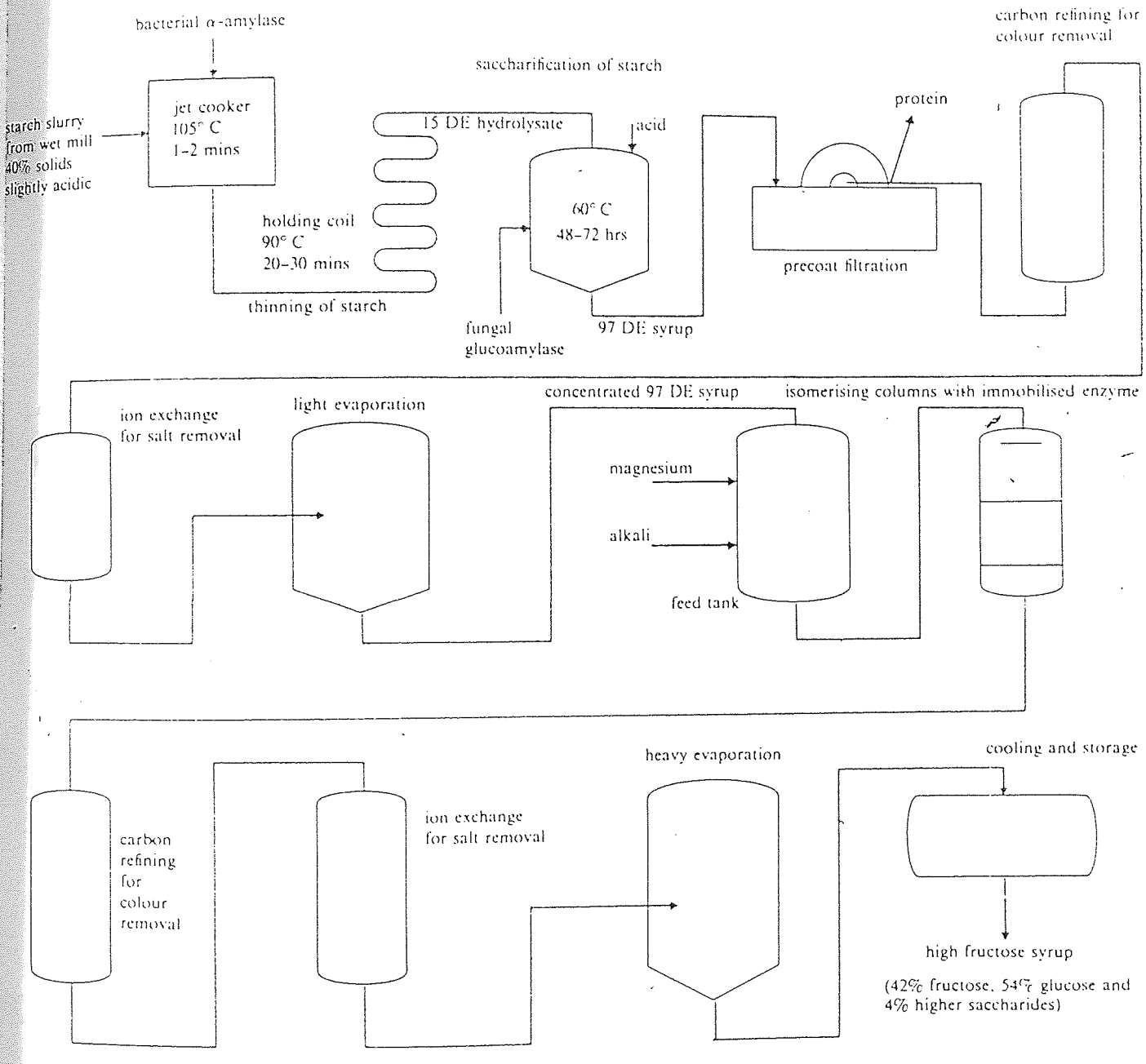
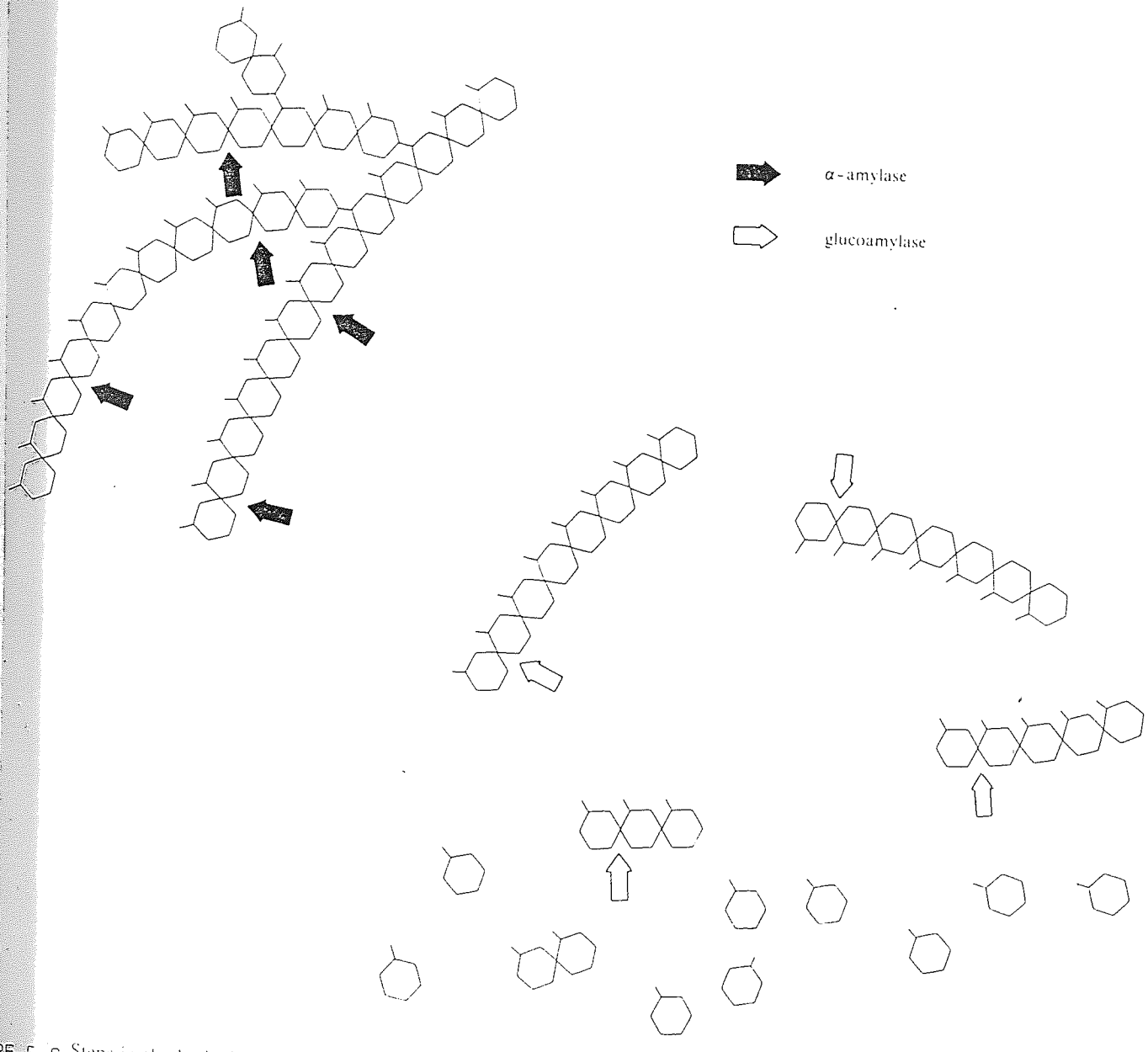


FIGURE 5.5 The processing of starch into (42%) high fructose syrup

Source : Towalski, Z., and Cohen, N. (1)



RE 5.6 Steps in the hydrolysis of starch by two enzymes: α-amylase and glucoamylase. Under industrial conditions where concentrations of starch are used, hydrolysis is incomplete, with some maltose and short chains of glucose remaining.

ources : Towalski, Z., and Cohen, N.[1]

slurry is pumped through a series of narrow tubes, surrounded by jackets through which super heated steam, (i.e. steam under pressure and therefore at a temperature above 100°C) is passed. The combination of heat and the sheer forces produced by passage of the slurry through the narrow tubes disrupts the starch grains and starts the dissolution of the starch molecules. In practise the bacterial alpha amylase which has a higher temperature stability of all the alpha amylases is mixed with the slurry before it passes through the jet cooker. The hydrolysis is allowed to continue in a holding coil in which the temperature is maintained at 90°C. The liquifaction of starch takes place rapidly, the enzyme being denatured slowly at this temperature. The starch slurry enters the coil with a DE of 1 or 2 and passes through it for 20-3- minutes leaving with a DE of 10-15. The DE value is defined as the reducing sugar content of the filtrate expressed as glucose and calculated as a percentage of the dry solids content of the hydrolysed liquors. In some cases a second jet cooker is used to heat the solution leaving the coil to 130°C in order to inactivate the bacterial alpha-amylase.

The hydrolysis of starch "saccharification" (meaning literally making into sugar) is completed by the enzyme glucoamylase (also known as amyloglucosidase) which releases monomeric glucose from the di-saccharides produced by bacterial alpha-amylase.[24] (see Figure 5.6.) Glucoamylase has a lower pH optimum and is less stable to temperature than bacterial alpha-amylase. The starch hydrolysis is run into large "saccharification tanks", the pH lowered to 4.5 and cooled to 60°C. Dissolved glucoamylase is stirred into the hydrolysate and the reaction is allowed to proceed for 48-72 hours.[25]

When low concentrations of starch are treated with glucoamylase high DE value syrups can be obtained but at the concentrations used industrially the maximum DE attained is 97, because these enzymes are unable to break the 1-6 glucose to glucose and non-standard glucose to other sugar linkages. In the final 97 DE product, the monomeric glucose content is about 94% of solids, the remainder consisting of maltose, isomaltose and larger oligosaccharides.[26]

The saccharification tanks are enormous. A plant producing 25,000 tonnes (solids) of 97 DE material per annum, requires at least four 600,000 litre saccharification tanks. If an immobilised glucoamylase could be used the volume of saccharification reactors could be reduced to around 4,000 litres with considerable savings in capital and running costs. However for reasons that are not completely understood it is not possible to obtain 97 DE material using immobilised glucoamylase. Syrups with a 94 DE value have been reported in the literature. Since 94 DE syrup is likely to have no more than 90% of its solids as monomeric glucose it is a less acceptable starting material than 97 DE syrup for the production of high fructose syrups.

The starch grains contain small amounts of protein which is released when the starch is hydrolysed and must be removed by filtration on fabric filter cloths aided by the use of inorganic, usually siliceous, filter aids. The filtrate is crystal clear but amber coloured and contains mineral salts, principally calcium. The colour is removed by treatment with activated carbon using similar equipment to that used in filtration and the ash is removed using columns of ion-exchange resins. The refined 97 DE glucose syrup is colourless translucent fluid.

Most glucose isomerases operate optimally at pH 8.0 or higher and require magnesium ions to maintain activity so small amounts (about 10mg's per litre) of magnesium sulphate are added to the glucose solution and its pH is increased using sodium hydroxide. Many producers concentrate the glucose syrup to 50% solids by evaporation before passing it through the glucose isomerase reactors. During the reaction a little colour is generated in the enzyme reactors and is removed by treatment with activated carbon and the salts (magnesium sulphate and the sodium chloride produced during pH adjustment) are removed using ion exchange resins. The very pure water white high fructose syrup is evaporated to 71% solids and supplied hot (at 30°C) to consumers in rail cars or road tankers. This is necessary since the solubility of glucose is low with decreasing temperature. If the solution is evaporated to 71% solids and the temperature is dropped then glucose will begin to crystallise out and accumulate as a sediment on the bottom.

Evaporation of water is the single most expensive process and is affected by the substrate concentration. However jet cooking and filtration require a certain amount of water. The actual concentrations used, range from 30-40% solids. Companies wishing to cut energy costs, favour the production of the somewhat higher DE glucose syrups with lower oligosaccharide contents, they also try to raise the temperature at which isomeration proceeds to increase yields. The usual lifespan of immobilised glucose isomerases operated at 60-70°C today is measured in months and the enzyme is at its most stable when a 50% solids glucose syrup is used as a substrate.

5.6. Role of R & D in the development of glucose isomerase and HFCS Production

It is difficult to obtain information about trends and patterns of research from companies directly. Much of the information presented in this section has been obtained from the careful analysis of glucose isomerase patents. There seem to be two schools of thought among the corn wet-millers as to the value of R & D. Clinton Corn Processing (Standard Brands), A.E.Staley and Corn Products Co. appear to carry out extensive R & D and apply for large numbers of patents whereas the others, strikingly ADM (see Chapter 3), let others get on with it and rely on suppliers. They then select the best technology available on the open market, often allowing, enzyme and equipment manufacturers, to test their products on ADM's premises. Cargil on the other hand teamed up with Miles to form a joint venture company Car-Mi using Miles technology. Most enzyme producers carry out extensive R & D although some of them now sell glucose isomerases initially developed by other organisations (See Table 5.8).

Analysis of the patents indicates that R & D departments of the other innovators had to solve a number of problems before they could use glucose isomerase as a commercially useful catalyst e.g. finding organisms which produced glucose isomerase naturally without the need for induction.

The results of the screening programme for glucose isomerase producing microbes had a major influence on the commercial acceptability of the enzyme.[68] A range of microbes were screened and selected but of these only Bacillus coagulans (Novo's selected microbe) had Generally Regarded As Safe status and hence initial FDA approval.[69,70] A problem

Table 5.8 Immobilized Glucose Isomerase Reactor Configurations

Company	Reactor configurations	Immobilized enzyme	References
	Batch	Fixed cells	Standard Brands Takasaki & Kamibayashi (1973) (27) Takasaki et al. (1969 [28
Baxter Labs	Batch	Adsorbed on anionic cellulose	Sipos (1971) Baxter Labs ([
Miles Labs	Batch	Glutaraldehyde cross-linked	Sipos (1973) Baxter " ([3 Zienty (1972) Miles " ([3 Zienty (1973) Miles " ([32
	Batch	Entrapped in cellular triacetate	Dinelli et al. (1973) SNAM (33)
	Batch	Whole cells adsorbed in $MgCO_2$	Heady and Jacaway (1974) CPC (34) Heady and Jacaway (1974) CPC (35)
	Batch	Fixed cells	Lamm et al. (1974) (36) Standard Brands
	Batch	Diazotized diamino cross-linked cells	Moskowitz (1974) (37)
	Batch/PBR	Immobilized in polyacrylamide	Kaetsu et al. (1975) [38
	Batch	Collagen-cellulose acetate membrane complex	Research Corporation (1975) (39)
Corning Glass	Batch	Tetrazotized benzidine cross-linked whole cells	Lartigue and Weetall (40) (1976) Rhone Poulenc
	PBR	Fixed cells	Takasaki et al. (1969 Standard Brands (41)
	PBR	Fixed Cells	Lloyd et al. (1972a) Standard Brands (42) Lloyd et al. (1974) (43)
	PBR	Glutaraldehyde cross-linked to nylon	Reynolds (1973) Monsanto (44)
	PBR	Fixed cells plus perlite	Takasaki and Kamibayashi (1973) (27)
	PBR (spiral wound module)	Collagen-enzyme complex	Wang and Veith (1973) [45 Vieth et al. (1973) [46
	PBR	Flocculated whole cells	Lee and Long (1974) Reynolds Tobacco (47)
	PBR (membrane)	Covalent attachment to polyethylene disks	Monsanto Company (1974) (48)
	PBR	Coupled to ZrO_2 coated glass	Tomb and Weetall (1974) Rhone Poulenc (49)
	PBR	Adsorbed on anionic carrier	Thompson et al. (1974) Standard Brands (50)
Corning Glass	PBR	Adsorbed on porous alumina	Messing (1975) Corning Glass (51)
Novo	PBR	Glutaraldehyde cross-linked lysed cells, Type S	Zittan et al. (1975) Nov Poulsen and Zittan (197 Novo (52)
	PBR	Adsorbed on cation-exchange resin	Bouniot and Guerineau[5 (1976) Rhone Poulenc (54)

C.P.C.	PBR	Adsorbed on basic $MgCO_2$	Heady (1976) CPC (55)
			Heady (1974) CPC (56)
	PBR	Cells plus nonporous inert granules	Heady (1974 CPC(57)
			Idaszak et al.(1976) CPC (58)
	PBR	Adsorbed on anion resin	Ishikawa et al.(1976)CPC
	PBR	Immobilized in resin	Ishimatsu and Kusai (1976)(59)
	PBR	Covalently bonded to glass	Lee et al.(1976) Reynolc Tobacco (60)
	PBR	Flocculated whole cells and hardener	Long (1976) Reynolds Tobacco (61)
	PBR	Flocculated cell-free enzyme	Nystrom (1976) Reynolds Tobacco (62)
	PBR	Glutaraldehyde cross-linked whole cells	Snell (1976) (63)
	PBR	Adsorbed on strong-base resin	Tamura et al. (1976) CPC (64)
	PBR	Adsorbed on DEAE-regenerated cellulose sponge	Brouillard (1977)Penick & Ford (65)
C.P.C.	Membrane	Retained by UF membrane	Walon (1971) CPC (66)
Novo	Expanded-bed	Glutaraldehyde cross-linked lysed cells. Type S	Zittan et al. (1975)NOVO (52)
	CSTR[b] and fluidized bed	Adsorbed on cationic carrier	Thompson et al (1975) Standard Brands (67)

a PBR - packed-bed reactor.

b CSTR - continuous stirred-tank reactor

that enzyme companies were familiar with and perhaps had anticipated prior to and included in their screening programme.

Another major production cost element is the amount of enzyme produced per unit of fermenter volume.[71] Here it appears that some luck played a part. CPC had to carry out extensive mutation and selection to increase the productivity of their Lactobacillus[72] and Streptomyces[73] sp strains licensed the immobilisation technology from Corning Glass whereas Gist Brocades, using Actinoplanes missourienses technology licensed from Anheuser Busch[74] appear to have had excellent productivity with their original strains, as apparently did Novo with Bacillus coagulans[75] and ICI who licensed and developed R.J.Reynolds Tobacco Arthrobacter[76] microbe glucose isomerases. It must be stressed that these comments are based on published information, and in fact it is likely that there has been extensive strain development behind the scenes. Yet another factor that influenced the adoption of a particular system was the ability of certain enzyme manufacturers, principally Gist Brocades and Novo, to give their customers strong biochemical engineering support particularly in the case of A.D.M.[77]

The development of glucose isomerase producing organisms, ran parallel to studies on the immobilisation of the glucose isomerase enzymes. Glucose isomerase is used on a commercial scale in an immobilised form. The reasons for immobilising enzymes are principally to reduce the operating cost of the enzyme and to improve the working properties of this catalyst. Exo-enzymes, that is enzymes secreted by the microbial cell are usually very stable and were the first to be exploited commercially. Cheap to produce they are almost

invariably discarded or inactivated when the desired conversion is complete, even though their catalytic ability still remains high. Enzyme cost is not significant because exo-enzymes are usually cheap to produce by fermentation. With more expensive to produce endo-enzymes this is not the case. Immobilisation overcomes this, by attaching the enzyme to an insoluble support, the enzyme can be separated readily from the product at completion of the reaction and re-used. Better still, if the enzyme is immobilised on a suitable support it may be used continuously in a column reactor and the substrate continuously passed through it. In such a reactor higher concentrations of enzyme may be used and thus residence times (the time the enzymes spends in contact with substrate) within reactors may be very brief. However other factors had to be considered also. In the event glucose isomerase was an ideal immobilised enzyme to catalyse this reaction because :

* it is an endo-enzyme and is expensive to extract.
* it is unstable outside cells internal environment and upon extraction denatures rapidly.
* the substrate stream is clean and free of particular matter which could cause severe problems in column reactors, such as clogging of the reactor bed or poisoning of the enzymes by impurities.
* the glucose substrate and fructose product are small molecules whose access to the enzyme molecules are not impaired when the enzyme within the disrupted cell is immobilised. Such diffusion restrictions cause problems when immobilised enzymes are needed to modify larger molecules such as proteins.

* the enzyme does not require the addition of expensive cofactors.

The various enzyme companies and corn wet-millers involved in the development of glucose isomerase took a variety of approaches to immobilising their enzymes. (See Table 5.8. above).

Clinton Corn Processing and A.E.Staley, the pioneers, used partly purified glucose isomerase bound ionically to DEAE cellulose. The flow characteristics of chemically modified cellulose as a support are poor, which meant that shallow trays of enzyme were used in packed bed reactors rather than more convenient narrow densely packed columns since the DEAE cellulose enzyme bed can only withstand a low pressure drop across it. Later glucose isomerase preparations used by Novo, Gist Brocades and ICI removed the need even for a purification stage since the preparation consisted of the cellular materials containing the enzyme. The cellular enzyme product could be used to make loosely compacted beds that could withstand a moderate pressure drop without becoming compacted or without developing channels.

Other manufacturers aimed to produce immobilised glucose isomerase with higher activities per unit volume by purifying an enzyme and linking it to a support material. This could reduce the capital costs of the reactor unit still further as done by CPC, Miles and Finn Sugar. However entrapment of the cells containing the enzyme, cross-linking them to one another using gluteraldehyde or some other supports produced a cheaper catalyst. Despite these differences it is difficult to make a decision between them based on the published information.

The most successful GI manufacturer is Novo whose position as the

largest enzyme company and experience in technical back-up producing pilot plants and basic process designs and they feel that they are unique in doing so. It appears to have given them a considerable advantage over the other enzyme companies whose GI preparations were equal or even superior to Novo's. Novo feel that there is little value added in the engineering part because in the main standard equipment is used. They don't favour any particular engineering firm though they have informal links with some.[77] Novo also appears to have benefited from having an established working relationship with the corn processors.

5.7. Second Generation High Fructose Syrups

In order to make sense of these developments it is necessary to digress slightly and explore the uses for HFS.

Industry uses liquid sugar as an ingredient where it can, since crystalisation involves the removal of water, which would be subsequently added by the user, it makes commercial sense to use liquid sugar. With a price advantage HFS might be expected therefore to gain most of the market occupied by liquid sucrose solutions, providing that HFS matched the liquid sugar in terms of quality. Table 5.9. shows the major markets for HFS in the U.S. for the years 1982/3. Since beverages use large quantities of liquid sugar they constitute the key market for HFS penetration.

The rapid development of the technology by the corn processing industry was based on the assumption that the "big two" corporations in the U.S. beverage industry, Coca Cola and Pepsi Cola would buy HFS

TABLE 5.9. 1982 and 1983 ESTIMATED INDUSTRIAL SUGAR AND HFCS DELIVERIES BY MARKET CATEGORY (Million dry pounds)

	Sugar		HFCS		HFCS % Sweetener Market		Long-Term Theoretical Penetration
	1982	1983	1982	1983	1982	1983	
Beverage	3,263	3,180	3,990	4,199	55	57	90% *
Baking	2,609	2,661	840	861	24	24	25%
Canning	460	410	725	791	61	66	70-75%
Processed Foods	410	350	385	460	48	57	60-65%
Dairy Produfts	810	800	320	378	31	32	35%
Confections	1,868	1,924	40	42	1	1	5%
Other Food & Non Food Uses	1,330	1,317	-	-	-	-	
Total	10,750	10,642	6,350	6,731	37	39	

* This figure would only be obtainable if The Coca-Cola Company and Pepsico were to authorize 100% replacement of sucrose with HFCS at some future date

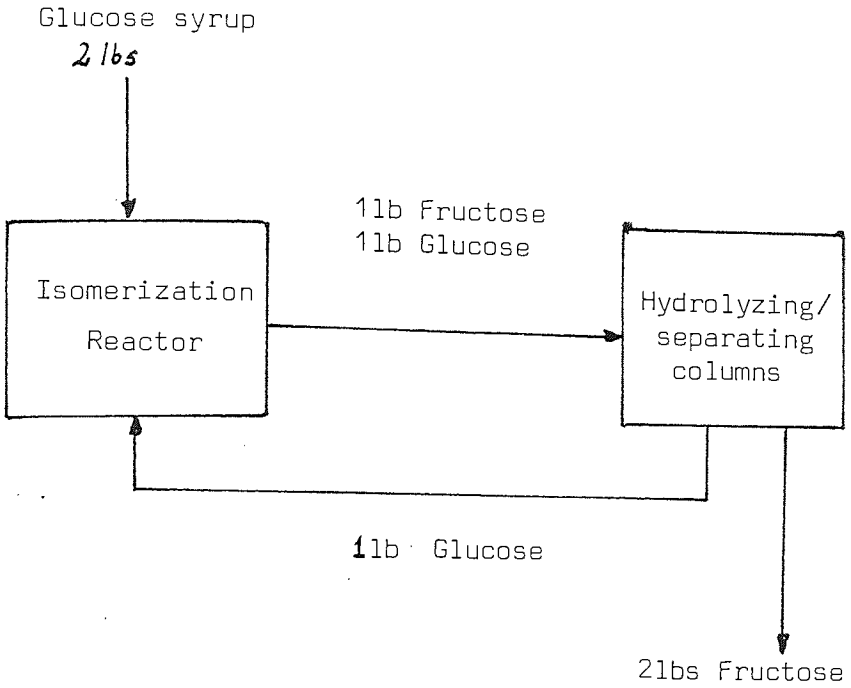
Source : S.Vuilleumier 1983 International Sweetener Colloquium.

in quantity. This did not happen with first generation syrups, because they did not match the sweetness quality of liquid sugar, when used in quality colas.[78] To be of use to Coca Cola and Pepsi-Cola HFS would have to contain 55% fructose. To produce a 55% HFS it is necessary to raise the fructose content above that of the 42% HFCS stream by some 8%. There were two ways that this could be achieved. Firstly to raise the working temperature of the enzyme (which was possible but on economic grounds impracticable) and secondly to separate the fructose from glucose and add this fructose to the 45% syrup. There were three possible ways that such a separation could be achieved.[79]

1. Oxidation of glucose to gluconic acid using the glucose oxidase catalase reactions followed by precipitation of gluconate leaving the fructose solution. This method has been practiced by Daw's Laboratories and had been the basis for obtaining crystalline fructose as a laboratory reagent for a number of years.

2. Fructose may be separated from the glucose by using a line process developed by the U.S. National Bureau of Standards in which a calcium fructosate is precipitated leaving behind the glucose. This method had once been used on an industrial scale but had since been abandoned as tedious and uneconomic..

Figure 5.7 Coupling of glucose isomerase process and the Boehringer process to produce 2lbs of fructose for every 2lbs. of glucose fed.



Source : Glucose Isomerase : A case study of enzyme catalysed Process Technology. Hamilton, B.K., Colton,C.K., and Cooney, C.L., in Immobilised Enzymes in Food and Microbrial Processes. Edit.Olson, G.C., and Cooney, C.L. Plenum Press, N.Y. 1974 (71)

3. The use of chromatography using columns packed with materials with which fructose interacts preferentially, e.g. sulphonated polystyrene cationic resins or anionic resins in their disulphite forms. Once it has been separated from the glucose it can be reccovered by adjusting the pH and eluting the fructose solution. From there on it can be added to the 42% syrup to produce the sweeter second generation syrup containing 55% of fructose; as suggested in Figure 5.7. or sold as a 95% fructose syrup with 1.7 times the sweetness value of sucrose.

Ion exchange resins and zeolites in their calcium forms are capable of binding fructose sufficiently to allow its separation from glucose, had been developed in the 1960's and were already in use in the mid-1970's for the production of crystalline fructose from sucrose by some fine chemicals companies and sugar refiners. These operations were carried out on a very small scale using technology unsuitable for the size of operation demanded by ADM and others. ADM approached UOP Processes Inc., a company skilled in the application of chromatography on a vast scale to the separation of petrochemicals using zeolites as absorbents.

UOP applied their knowledge to the separation of fructose from glucose using a single column of absorbent and a complex valve system that allows continuous production of a stream containing 90% fructose, 7% glucose and 3% higher saccharides. This is blended with 42% HFCS to produce the second generation material. Various other separation processes have been developed and all the major HFCS manufacturers now produce 55% fructose material.

In 1980, having thoroughly satisfied themselves that 55% HFCS is capable of matching sucrose and maintaining their own perceived high standards, Coca Cola gave approval for their bottlers to substitute up to 50% of their sucrose by 55% HFCS and Pepsi Cola responded by allowing 25% substitution. This helped to solve the immediate problem of over capacity and prompted further expansion by most of the large manufacturers. By late 1982 as new plants come on stream over capacity began once more to be serious but in early 1983 Pepsi Cola approved substitution of sucrose up to 50% by 55% HFCS.[81] It now seems that Coca Cola and Pepsi Cola will eventually allow total inter-

change between sucrose and 55% HFCS.[82] The second generation HFCS is already produced in greater quantities than the first generation syrups, since processors reduced the output of their first generation facilities and give preference to second generation plants. A key to substitution is the adoption of flexible pricing of HFCS, relative to the world market price of sucrose. (see Table 5.10)

Table 5.10 High Fructose Corn Syrup Prices, Relative to Sugar, Chicago-West Market, 1979-83

Calendar Year and Quarter	HFCS-42	Sucrose from Beet
1979	13.15	19.68
1980	23.64	38.29
1981	21.47	28.26
1982	14.30	27.62
1983		
I	11.70	28.00
II	17.64	28.87
III	-	-
IV	-	-

Source: USDA Economic Research Service. 1983.

Isoglucose outside the U.S.A.

EEC

Maize is very much a crop of prairies and steppe and does not grow as satisfactorily in more maritime climates. Much of the maize grown in EEC countries is unsuitable for wet milling so the considerable wet milling industry in Europe is based almost entirely on imported U.S. maize although this position is considered to be changing as European varieties continue to be improved. Most EEC countries are capable of growing sugar beet and wheat very efficiently, especially if given a cash incentive, hence the much publicised EEC "mountains". Clearly the economic incentive to adopt HFCS technology in the EEC is much less than in the U.S.A. Nevertheless EEC companies approached the new technology with as much enthusiasm as their transatlantic counterparts, though their plans were for much smaller plants. Since the U.K. joined the EEC there has been a partial restitution of the import levy on maize from starch production which applied also to HFCS. By 1977 the growth, or planned growth, of HFCS production in the EEC coupled with restitution payments with the consequent prospect of growing sugar surpluses so alarmed the beet sugar producers that they pressed, successfully, for these restitution payments to be removed for HFCS becase it was regarded as a direct competitor to sugar. The EEC reacted accordingly and imposed a levy on HFCS production, effectively raising the price. The levy was shown in 1979 to be illegal and it was greatly reduced but argument for the restitution of payments was lost and a quota system instituted similar to that applying to sugar production.(83) The present HFCS production capacity in the EEC is 265,000 tonnes but the total quota allocated in 1981 was for 186,462 tonnes, of which 146,245 tonnes are "A" quota and 40,217 "B" quota. (see Table 5.11).

Table 5.11 A and B quota production and producers, and estimated plant capacity in tonnes, in the EEC for 1981-85

Company and country of location	A quota	B quota	Quota total (A and B)	Actual production	Estimated plant capacity
De Zetmeelbedrijven (Netherlands)	7,426	1,749	9,175	7,000	20,000
Maizena (West Germany)	28,882	6,802	35,684	35,000	55,000
Roquette Freres (France)	15,887	4,135	20,022	20,000	35,000
SPAD (Italy)	5,865	1,257	7,243	7,572	10,000
FRAGD (Italy)	10,704	2,645	13,228	11,761	20,000
Amylum (Belgium)	56,667	15,583	72,250	75,000	80,000
Tunnel (UK)	21,696	5,787	27,483	28,000	45,000
Biamyl (Greece)	6,376	1,502	7,878	6,000	13,000
ZAAE (Greece)	4,146	976	5,122	5,000	10,000
EEC Total	157,649	40,436	198,085	195,333	288,000

Source : Towalski, Z., and Cohn, N.C. (1)

"A" quota production attracts no levy but "B" quota attracts a production levy, a payment to the EEC as a contribution to the Agricultural Fund in Brussels to offset the costs of exporting sugar should they be needed. Any HFCS produced above the quotas, must be exported at full EEC production rates, which include the tariff on importing maize. To date very little HFCS has been exported from the community. Worse it is now impossible for new producers to enter the market, therefore the likelihood of further development is slight, i.e. no second generation syrups. However with the entry of Spain into the EEC and their 55% isoglucose facilities this situation may need to change.

Although the technology used by EEC companies is similar to that developed by the U.S.A. companies and based on maize, Amylum in Belgium, the EEC's largest HFCS producer, uses wheat starch as the raw material. The sale of wheat gluten supports the price of starch in a similar way in that sales of oil, gluten meal, bran and steep water support the price of the maize starch.(84)

The consequences of the EEC's attitude to HFCS are interesting to record. British companies were particularly affected. Of the British wet milling companies Tunnel Refineries had acted most rapidly and were producing HFCS in time to receive a quota. They are the only U.K. producers of HFCS. CPC U.K. and Garton's Glucose Ltd. announced no plans for HFCS production but Albion Sugars Ltd., a subsidiary of the Dutch firm Koninkliijke Scholten Honig(KSH), planned and began constructing a new plant including a corn wet mill. With the announcement of the subsidy withdrawal and the introduction of a levy, KSH (who had a similar plant under construction in Holland) collapsed and their assets were acquired by the American Company Cargill. The

Albion plant was completed so as to be able to produce glucose syrup. This resulted in over capacity in the sweetener market which in turn resulted in the demise of Garton's, the least efficient cane sugar producer. Had the levy not been applied, however, and Albion had gone into full scale production it would probably have resulted in the closing of yet another cane sugar refinery, four of which have been closed since 1978, a direct consequence of the EEC policy supporting the domestic sugar beet industry.(85)

The future for HFCS in Europe

Since the EEC is founded on protectionist policies the failure of HFCS will be determined chiefly by political considerations. As has been mentioned above, there is no demonstrable need for HFCS within the EEC with its self sufficiency in sugar. The motive to substitute, based essentially on savings in raw material costs, has a different set of variables to contend with. As far as first generation syrups are concerned, HFCS will do nothing as an ingredient that cannot be done as well by sucrose or inverted sucrose syrups, but this argument no longer holds for the sweeter second generation syrups. The EEC community usually produces an excess of sucrose and in an ideal year for agriculture, as 1982 was, a very large "sugar mountain" can accumulate. Such mountains receive adverse publicity when they are accumulating, (little mention when they are sold), as occurred in the Spring of 1982. Sale of EEC surplus sugar however is on the open market and Third World producers have accused the EEC of dumping. Sugar stocks are desirable because their existence keeps down the world sugar price in times of shortage, but if they grow too large they can drive prices down and deprive cane sugar producing nations of much needed income. Nevertheless each time there is an

extreme world wide shortage of sugar, as occurred in 1974 and in 1980, there will be a tendency for HFCS to gain markets which through flexible pricing make it difficult for sugar to regain completely on restoration of sugar supplies. It still remains to be seen if the abnormally low world sugar prices in 1984-85 have enabled sugar to regain some of this market.(86)

Although the EEC countries are usually self reliant in sugar production they see a moral need to continue to buy raw cane sugar from countries which are signatories of the Lome convention. The Lomé convention is an agreement between the EEC and 46 African Caribbean and Pacific (ACP) countries that, in general, imports from ACP States will enter EEC countries free of customs duty and similar charges. There are no signs of any radical change in the present state of affairs at the time of writing. In practice only the U.K. imports significant quantities of raw sugar stocks which are refined in Tate & Lyle's refineries in London and Greenock.

Europe is also becoming self sufficient in wheat, although the hard North American wheats are preferred for bread and biscuits manufacture, especially in the U.K. due to their lower alpha amylase contents and are consequently still imported in large quantities from North America.

Other countries

(a) Japan

Japan is a net importer of both corn and sugar and has moved enthusiastically into HFCS. The prime reason for this is that sucrose is subject to import duty and taxes whereas HFCS is not. Nevertheless sucrose is likely to remain the major sweetener in Japan for some

time. It is common practice in Japan to blend sucrose solutions with HFCS to prevent the crystallisation of glucose whose transport can be a problem in cold countries.

There appears to be four main enzyme systems in use. The Denki/ Nagase system used by Showa Sangyo, Sanwa Denpun and Nihon Denpun; Sanmatsu/Mitsubishi technology by Sanmatsu Kogyo; CPC technology by Nihon Shokushin Kako, and Novo Technology by Kato Kagaku. (See Table 5.12.).[87]

There seems to be little information freely available about the types of enzyme used in Japanese plants but there is no reason to assume that any major differences exist between Japanese plants and those used elsewhere.

(b) Eastern Europe

Most East European countries are self sufficient in sugar consumption so have no pressing need for HFCS. However plants producing modest amounts of HFCS have been constructed in Hungary (currently 32,000 te expected to expand to 64,000 te) and Yugoslavia (less than 39,000 te), using Miles Laboratories technology.[88] It is interesting to note the roles that CPC and Miles have adopted in developing overseas manufacturing bases and acting as vehicles for the diffusion of this technology throughout the world. The U.S.S.R. is not self sufficient in sugar consumption or in corn for livestock feed. Furthermore it has a shortage of foreign exchange. Thus although there are occasional reports of plans for the construction of HFCS plants in the U.S.S.R. and intense interest in glucose isomerase in Russian (and other East European) academic laboratories, it is unlikely that HFCS will be produced

TABLE 5.12 1981/82 ESTIMATED ANNUAL HFCS PRODUCTION CAPACITIES AND ACTUAL PRODUCTION LEVELS IN JAPAN

metric tons wet including 25% moisture

Manufacturer	Location	Production Capacities		Production Levels*	
		HFCS-42%	HFCS-55%	HFCS-42%	HFCS-55%
Showa Sangyo	Ibaraki-ken	42,000	42,000	19,400	27,400
Gunei Kagaku	Gunma-ken	24,000	18,000	10,400	14,900
Gunei Kagaku	Hokkaido	18,000	18,000	3,600	10,400
Sanmatsu Kogyo	Ghiba-ken	96,000	78,000	57,000	53,000
Sanmatsu Kogyo	Fukuoka-ken	30,000	18,000	27,000	25,000
Aito	Aichi-ken	96,000	-	29,000	-
Nihon Corn Starch	Aichi-ken	96,000	108,000	39,600	38,200
Sanwa Denpun Kogyo	Nara-ken	72,000	72,999	44,800	48,600
Nihon Denpun Kogyo	Kagoshima-ken	36,000	42,000	23,300	23,300
Kato Kagaku	Aichi-ken	120,000	108,000	54,500	54,500
Nihon Shokuhin Kako	Shizuoka-ken	72,000	96,000	59,500	34,000
Others		103,000	60,000	24,800	47,100
Total		805,000	660,000	402,900	376,000

* Production levels for fiscal year 1981 are assumed equal to consumption.

Source: Vuilleumier, S., World Outlook for high fructose syrups. 1983 International Sweetener Colloquium

in large quantities there, but both the U.S.S.R. and Bulgaria may begin its production in the future.

5.8. Factors affecting the diffusion of HFS technology

5.8.1. Introduction

It is interesting now to consider the question why HFS have been so successful in substitution since a number of alternative sweeteners are currently available on the market. I shall discuss the following factors; sugar economics, alternative sweeteners, and the organoleptic properties of sugar and glucose and fructose.

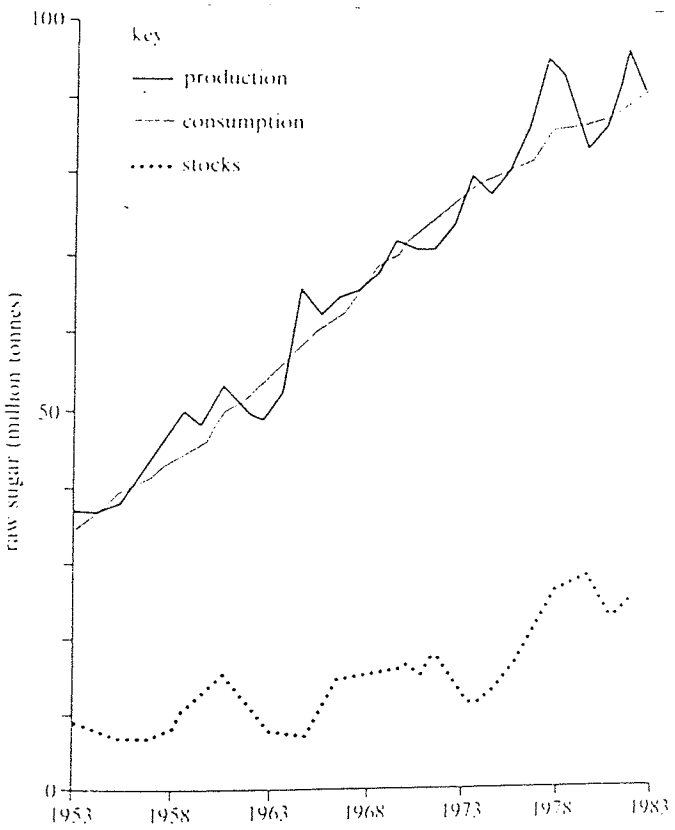


Fig.5.8. Worldwide production, consumption and stocks of sucrose from 1953 to 1982

Source : Towalski, Z., and Cohen, N.C. (1)

5.8.2. Sugar economics and the diffusion of high fructose syrups

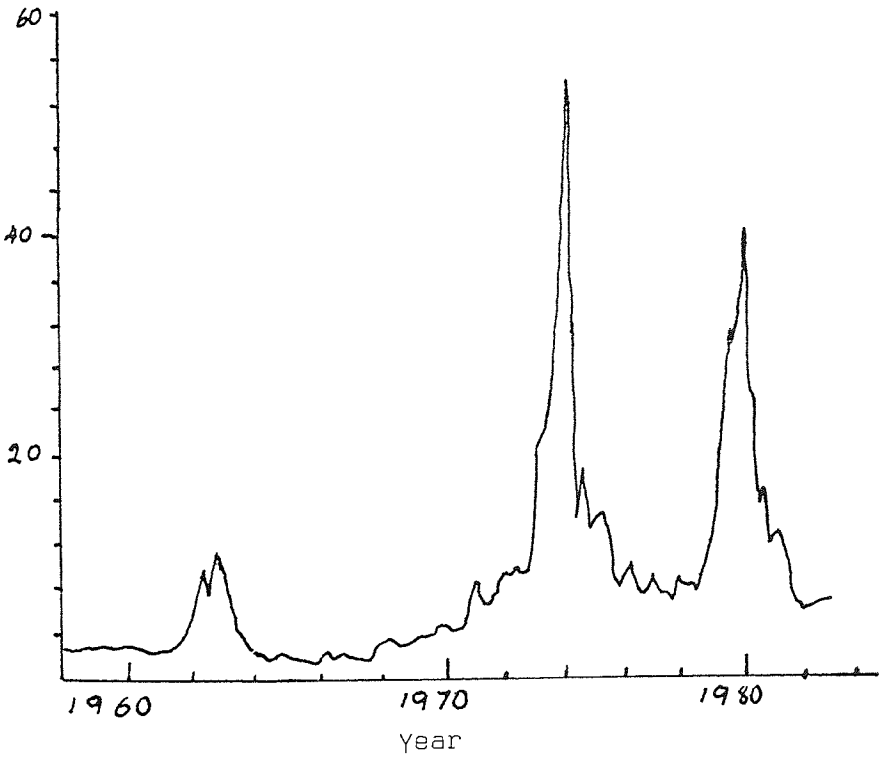
So far I have shown that the diffusion of high fructose syrups is related to the price of sugar. World wide consumption of sugar is still growing, in particular in Asia and Africa, and by 1985 the total world consumption of sucrose as a food should reach 100 million tonnes. (See Figure 5.8.) The demand for sugar is generally characterised as being non-elastic with a relatively stable consumption. The price of sugar is principally the effect of two factors :

1. sugar crop/refining production
2. consumption.

Prices fluctuate due to a mismatch between supply and demand which are moderated through the purchase and sale of buffer stocks. Poor harvests, caused by adverse weather conditions and pest damage occasionally affect the crops and reduce sugar production. Figure 5.8. shows that in the period from 1970 onwards on the graph there were two occasions where supply failed to keep pace with demand. It was in early 1970 that rising world consumption of sugar began to outstrip production, and world sugar stocks began to be reduced to low levels. In 1974 disappointing sugar beet harvests affected sugar supplies in the U.S. The Soviet Union, Japan, Canada and the Arab nations were also badly affected and began buying heavily on the world market. Sugar speculators working within exporting nations, sensing a bearish market held back on shipments, either to force prices up or to maintain supplies to meet domestic contracts. In addition in 1974 Congress failed to renew the Sugar Act, an Act that sent the domestic price

of sugar higher than the world price and which protected the U.S. grower. Failure to renew the Act caused some uncertainty as to how the future price of sugar would be affected by the absence of protection. Consequently the price of raw sugar in world markets rose by more than 300% as shown in Figure 5.9.

Figure 5.9. World Raw Sugar Price F.O.B. Stowed 1958 - 1984



Source : F.O. Licht's For historical series.

These sugar prices could not be maintained. A bumper harvest in 1975 caused sugar prices to fall. Once the fall started the downward trend was fed by speculators unloading their stocks forcing prices even lower. By 1976 the New York spot price fell

as low as 9-10 cents per lb. U.S. growers applied pressure on the President and Congress to restore price protection. A system of subsidy payments was proposed but ruled illegal. The Agricultural Act 1977 made provision for a price support scheme under the de la Garza amendment. However the Bergland plan was implemented which allowed the sugar beet producers to be paid a variable subsidy depending on the world sugar price and the amount of sugar they produced.

Poor harvests again occurred in the 1980's and sugar prices once again rose steeply, but by this time there was a bulk alternative source of supply for a sector of the market, and prices reached 40 cents per pound.

In western countries consumption of sucrose is slowly declining for a variety of reasons. Take the case of the U.S. for example; in the U.S. there has been a steady increase in the use of low calorie sweeteners since the 50's. This trend is the result of certain sectors of the population becoming concerned about the calorific intake of sugar in processed foods, as well as high calorie foods in general. High sugar diets have been implicated as a contributing agent in the development of dental caries and diabetes, and are implicated by circumstance with contributing to obesity and coronary heart disease. A reduction in the sugar content of diets appears to have fallen when prices rose sharply in the 1974-75 period. Despite this the main reason for the decline in consumption of sugar in the U.S. has been its substitution by HFCS.

It is predicted that by 1990, sucrose will still have 90%

of the world sweetener market, starch-based sweeteners 8% and other 2% on the basis of sweetness compared with sucrose.

5.8.3. Differences in the diffusion rates of sugar substitution

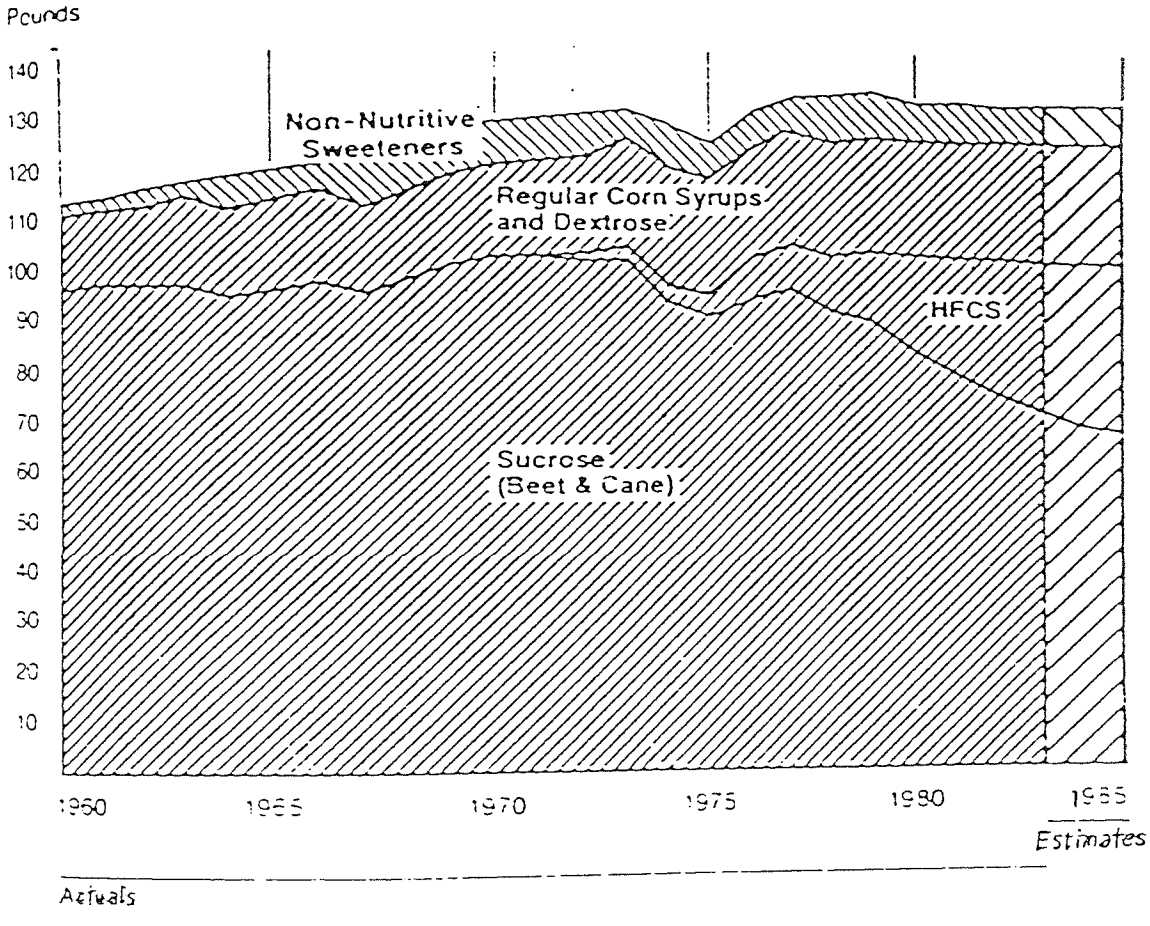
The trends in the rates of substitution of non-nutritional sweeteners and HFCS are markedly different (See Fig. 5.10). This suggests that if sweetness was the only quality of sugar, then the non-nutritive sweeteners would have had a greater effect on substitution than they have had to date.

This indeed is the case, for sucrose provided not only sweetness but also a range of other useful properties. Sucrose acts as a bulking agent, a cheap extender, it serves as diluent or carrier for other ingredients. It is readily soluble in water (Less soluble sugars such as lactose separate either directly by crystallisation in products such as ice cream, and give it a gritty texture or such as glucose, have a lower solubility at lower temperatures so are correspondingly less useful as sweeteners.) Its solubility is a key to its use as a preservative. At high concentrations sucrose acts as an osmotic dehydrater passively removing water from microbes and their spores. A more extensive list of such properties is presented in Table 5.13.

Saccharin because of its low price is widely used as an alternative sweetener despite its very definite bitter-metallic after taste. Yet it does not have a completely clear bill of health in toxicity tests having been implicated in rat trials as possibly causing bladder tumors in some second generation rats fed with massive doses in two general experiments.

Figure 5.10.

U.S. Nutritive and Non-Nutritive Sweetener
Per Capita Consumption
1960-1985



Source : Amstar Corporation

Table 5.13. The features that make sucrose an attractive commercial product

* It is a digestible and metabolisable form of carbohydrate (hence a food).
* It may be used as a bulking agent and carrier for other ingredients.
* It is high soluble and remains so in cold water.
* It has excellent storage stability when crystalline.
* It is cheap.
* It is a natural product and when refined is colourless and chemically pure.
* It is microbiologically pure when crystalline (less so when in liquid form).
* It provides good preservative properties.
* Through reactions with proteins and starch it helps to generate structure and texture in food.
* It can be used to form flavours and colours on heating (such as caramel).
* It provides beverages with mouth feel +
* It is less reactive chemically than many other sugars and may therefore be used at higher temperatures.

+ 'Mouth feel' is an elusive subjective property given by some substances when dissolved. It is most readily described by its absence from so-called low calorie soft drinks when compared to 'regular' soft drinks.

When the tests were rerun the evidence they provided remained inconclusive, yet saccharin appears to be not metabolised, and is rapidly excreted via the kidneys. Despite this there is still an element of concern over its use and so there is a race to introduce replacements for it. The Sweetener Cyclamate has failed toxicity tests[89] in the U.S. In Europe Aspartame and Acesulphan K have been approved as sweeteners.[90] Aspartame is close to sucrose in sweetness quality but unstable at the low pH's of many soft drinks and it has possible problems in that it may interfere with the production of certain hormones in the body as well as being a possible source of harm to phenylato nuina sufferers. Acesulphan is stable but has a "saccharin like" aftertaste. At least two more high intensity sweeteners with properties sweeter than any material currently available are under development such as Talin and trichloro galactose.[91] However at present no one high-intensity sweetener gives the "mouth feel" of sucrose nor does any of them have preservative or bulking properties although Aspertame is close. (See Table 5.14 for a comparative list of characteristics of the major types of alternative sweeteners).

In the acid conditions in carbonated drinks for example, the cola's pH's can be less than 3.0, so sucrose is completely hydrolysed "inverted". This has little effect on the sweetness because whilst glucose is less sweet than sucrose the fructose is sweeter. It follows therefore that an approximate 50-50 mixture of glucose and fructose produced from sources other than sugar cane or sugar beet could replace sucrose in soft drinks and in other liquid sugar formulations.

Table 5.14 *The characteristics of sucrose and other, alternative sweeteners*

Sweetener	Sweetness factor (times sucrose)	Nutritive (+) or non-nutritive (-)	Sweetness quality	Mouth feel (arbitrary scale)	Price £/tonne sucrose sweetness equivalent
Sucrose	1	+	Very good	+++	405
Glucose	0.7	+	Good	+++	435
Fructose	1.3	+	Good/fruity	+++	710
Saccharin	300	-	Sweet/metallic/ bitter/poor	+	15
Acesulpham-K	150	-	Sweet/chemical/ bitter/fair	+	150
Aspartame	180	-	Sweet/slightly lingering/good	+	740
Cyclamate	30	-	Clean, sweet/fair	+	50

To be acceptable to the soft drink manufacturers, many of whom are extremely fastidious as to the quality of their new materials, the new material must be no more expensive than sucrose and must match sucrose in performance. This should also be better than sucrose with respect to colour, mineral salt content, microbiological contamination, but since crystalline sucrose is over 99.9% pure these standards are difficult to reach, (in liquid form the purity standards for sugar are somewhat lower).

5.8.4. Future prospects for this technology.

Glucose is one of the basic carbohydrate building units found in nature occurring in structural materials such as cellulose and other polysaccharides including the major plant storage material, starch. Starch granules are made up of linear starch called amylose and branded starch called amylopectin. Starch can be hydrolysed by acid to produce glucose but this reaction is unsatisfactory because colours, and undesirable flavours are produced at around 50% hydrolysis of the starch. This process has been developed commercially after Kirchoff discovered this reaction in 1811. Syrups derived from starch are described by their dextrose equivalent (DE) which is a measure of the extent to which hydrolysis of starch has occurred expressed as a % of glucose equivalent of the dry solids. Thus pure glucose has a DE of 100, maltose a DE of 50. Commercial hydrolysis to produce high DE syrups containing monomeric glucose is carried out by enzymes. Bacterial alpha-amylase and gluco-amylase are added to slurries of starch containing at least 30% W/W solids. Alpha-amylase disrupts the starch granules and starch chains to produce shorter soluble saccharide chains.

Glucoamylase hydrolyses them to produce large amounts of glucose (dextrose) in the hydrolysate.

At this concentration the maximum DE which is attainable is 97 and the product contains around 6% of maltose and higher oligosaccharides. Pure glucose can be crystallised from such syrups under such conditions it forms crystals which include 1 molecule of water and is hence called glucose monohydrate. Neither 97 DE syrups nor glucose monohydrate crystals have found wide use as sweeteners because :

* glucose has 0.7 times the sweetness value of sucrose so greater quantities must be used to match the sweetness of sucrose
* glucose crystallises readily from high DE syrups, and makes shipment difficult
* glucose monohydrate is hygroscopic (able to absorb moisture from the air) becomes wet more easily than sucrose and is therefore difficult to store.

Fructose on the other hand is found in varying extents in honey, vegetables and all ripe fruits in conjunction with glucose and sucrose. Sweet fruits such as pears and grapes possess higher percentages of this sugar hence its name fruit sugar. Generally it is not found in structural polysaccharides so the only source other than sucrose is in the storage polysaccharide (inulin) of plants of the daisy family and of liliaceous plants such as Iris. The composite plants chicory and Jerusalem artichoke are receiving considerable attention as potential crops for alcohol production but they are at present not practical

commercial sources of pure fructose nor is there an industry capable of producing such fructose on a large scale. Thus at present the only way to produce an "invert" syrup from sources other than sucrose is by converting glucose to fructose. This situation is therefore likely to continue in the foreseeable future.

5.9. SUMMARY

From this case study we can make the following summary observations :

1. That the scientific breakthroughs necessary for the successful development of this technology arose from work that bore no direct or immediate relation to the needs of the industry.

2. That applied research in Japan in the early 20th Century was crucial to the development of the enzyme technology.

3. The importance of monitoring scientific developments within a company.

4. The important role that the company and its policies/ strategies play as an instrument in the development of an innovation in an oligopoly.

5. That a number of isomerases could be found by screening other microbes hence enzymes could be obtained that fitted a range of different processing requirements quite closely.

6. That the development of an immobilised enzyme was a direct result of the commercial pressures on processing companies to recover the enzyme, and that this led to the development of the immobilised enzyme reactor and resulted in a more efficient method of processing.

7. That the provision of an immobilised catalyst was an acceptable solution in cost reduction because of the high degree of purity of the substrate.

8. That in the search for new commercially viable processes, enzyme companies, which already had a working relationship with the end users (starch processing companies) were able to develop the technology and sell it as a job lot to the users, or negotiate joint ventures.

9. That the patterns of research followed by the competing companies were largely similar and were aimed at cost reduction of the enzyme; at increasing the yield of enzyme, and in improving its working life.

10. The diffusion of the technology was increased considerably by technology made available by an enzyme producer (Novo) which enabled those companies who neglected to carry out any R & D to enter the market and to effectively compete with the early innovators.

11. The various mechanisms by which companies were able to exploit the technology such as licensing the technology (negotiating national patent rights); (Clinton; A.E. Staley) entering into joint ventures with companies which had developed the technology (Cargil and Miles), buying in the technology (ADM from Novo) or buying out companies which had developed the technology (Heinz buying Hubringer).

12. The marked effect that market forces had on the diffusion of this technology, such as large volume sugar imports,

a significantly large industrial sugar utilising sector, high sugar prices, a cheap source of starch, an efficient distribution network and available investment capital.

13. The marked effect that political policies (national and international) which affect relative pricing have on process economies and hence the diffusion of the technology, e.g. U.S. price guarantees and EEC quotas.

14. That the scientific research linked to this technology undertaken by companies was initially stimulated by a perceived market opportunity i.e. syrup sweeteners cheaper than sugar.

15. That economically alternative sweeteners different in many respects from sugar, which had become established as the norm in sweeteners, and high fructose syrups matched inverted sugar syrups in all respects but one, they are cheaper to produce than sugar.

16. That the speed of adoption by other corn starch processors created a band wagon effect and the withdrawal of certain projects due to over capacity e.g. Penick & Ford.

17. The defensive innovation by sugar producers such as Amstar and Tate & Lyle investing in isoglucose technology.

18. That the diffusion does not fit the normal or Gompertz curves closely in this case, since it has a marked inflection in the middle due to the development of 55% glucose technology.

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CHAPTER 6 : CASE STUDY : WASHING POWDER ENZYMES

6.1. Introduction

This case study was selected because the SRC Enzyme Committee implied that this development was an example of recent innovative activity and growth. The retrospective analysis of the technology indicated that this technology had been around for years, certainly since 1913. I concluded therefore that certain factors had been operating on the diffusion of the technology which warranted further analysis.

I shall begin by discussing aspects of washing clothes and the reasons for the use of soaps and detergents.

Water alone is not efficient for cleaning clothes unless a great deal of time and energy is spent on the process (for example, by thrashing the clothes against rocks in a stream and rubbing the clothes). As well as using up time and energy, this damages the clothes. All this energy is required as water is not particularly good at penetrating woven surfaces. Clean water has a high 'surface tension' because of the properties of its molecules, so that it forms droplets on the surface of soiled clothes and doesn't easily penetrate the fabric. Soaps and detergents, however can reduce the energy needed for this. They do this by coating the fibre structure of the fabric with a surface active film, which attracts water and helps wet the surface of the clothes, helping the water to get throughout the clothes to do its job. Soaps reduce the surface tension of water but detergents reduce it even further. Surfactants also surround the dirt particles and force them up off the fabric and into the water and help to keep them

suspended in the water. Agitation by hand or machine helps by loosening the dirt and causing droplets in suspension to break up still further.

Soap is one of the oldest surfactants known and has been around for at least 100 years. The Celts made soap from animal fats and plant ashes and it later came to be made from tallow or vegetable oil heated with caustic alkalis. Salt was then added to separate the soap from the liquid. Production of soap was a local activity but the large scale production was started in 1823, by William Lever. The small soaperies such as Hudson's, Knight's and Pear's were bought out by the larger producer and formed the giant Lever Brothers. Likewise, a similar process of concentration in the U.S. produced the giant Procter & Gamble. In part, economic criteria led to a loss of consumer sovereignty and consumers are forced to accept whatever the two major manufacturers choose to sell. This loss to the consumer has resulted in limited choice of product type, with catering for 'minority' markets being uneconomical. Improvements in the technology were the result of chemical discoveries which led to the production of wetting agents that were superior to soap. The term 'detergent' is nowadays usually applied to synthetic soapless detergents which were first introduced in the 1930's. The majority of these are made from chemicals derived from petroleum and which has a mineral oil origin.

Detergents usually also contain 'builders' such as phosphates which help the washing action by softening the water, preventing the corrosion of metal parts in the washing machine and help to remove dirt by forming soluble complex ions which repel the fibre and help loosen the dirt from its surface. Other chemicals are added to either

boost or stabilise foam, sodium perborate which acts as a bleaching agent to remove stains by oxygenating the water, fluorescent materials which increase brightness and sodium carboxymethyl cellulose which help prevent the dirt from being redeposited back onto the garment by helping to keep it suspended in the water.

6.2. Enzymes in washing preparations

In chapter 2 I have discussed why enzymes are useful industrial catalysts and two factors that appeared to hasten their introduction during the 1960's were that they are non-polluting and biodegradable.

It is the enzymes ability to catalyse reactions at relatively low temperature ($10^{o}C$-$80^{o}C$) and normal atmospheric pressure that is proving an important aspect of this innovation in the 1970's and 1980's.

Enzyme utilising processes however have their own special problems, most enzymes operate in conditions of mild pH (6-8) and washing is usually carried out in strongly alkaline solutions (pH 9-12). In this case the complex protein structure of enzymes so vital for their function is also the cause of their vulnerability. Their protein structure makes enzymes susceptible to denaturation and inhibition even by slight alterations of their physical environment, switching off their catalytic action, or by triggering off other mild forms of chemical change. Enzymes are also susceptible to a range of poisons and therefore generally require high standards of purity from the substrate and clean operating conditions. Many enzymes require specific metal ions to activate the enzyme itself, some have a requirement for cofactors which need to be present as 'carriers' for the transfer of distinct chemical groups, before the catalytic reaction is to proceed to completion.

These requirements vary from enzyme to enzyme and need to be determined for each enzyme in turn, which explains why it is the more robust broader acting extracellular enzyme that at present have found most industrial uses, and in washing preparations in particular. Dirt may be grouped into four types: grease - resulting largely from the natural lubricants of the body, mixtures of hydrocarbons, fatty acids, esters and glycerol and industrial fats and oils; proteins - food, blood etc; grime - made up largely of solid particles, and polysaccharides such as mucus. They all prove difficult to remove from clothes only washed in water. These biological molecules are less frequently encountered in the home, but can be a major problem on protective wear used by some industries such as food processing. Enzymes could be used to digest any of these biological compounds such as grease, protein or carbohydrates and help to loosen or dissolve the staining materials. In a laboratory a wide range of enzymes could be used to produce satisfactory results but not all lend themselves to being used as pre-soaking agents.

The development of washing powder enzymes proved to be problematic because of the strongly alkaline properties of industrial soaps and detergents. Innovation of enzyme preparations as cleaning agents shows three distinct phases of development, each of which had quite a different commercial impact.

6.2.1. The first phase

The initial invention and innovation in the use of enzymes as washing aids occurred in Germany, in 1913. Otto Röhm took out a patent[1] for the purpose of protecting the use of pancreatic enzymes to break down fat and protein in a presoak on the basis of the assumption that the main part of

dirt in fabrics used by humans was composed of those two materials. Preliminary findings indicated that fabric could be cleaned in a shorter time, using less strength (these were the days of soap and washboard and the clothes boiler) and at a tempoerature far below the boiling point of water than when enzymes were not used. In addition in the main wash less harsh alkaline chemicals could be used, less soap was necessary and the fabric had a better appearance after washing. The Company Rohm and Haas of Darmstadt began to market this presoak washing powder as BURNUS.[2] It received moderate success and was sold for about 50 years in European countries until the 1960's. The product consisted mostly of a mixture of sodium carbonate (washing soda) and small amounts of pancreatin containing a mixture of enzymes including trypsin (a protease to digest protein) and emulsin (a lipase to digest fat). Unfortunately sodium carbonate is very alkaline and in solution has a pH of 12-14. At such a pH it adversely affected the stability and chemical activity of the enzymes. Nevertheless Rohm's idea of using enzymes to digest away stains rather than to boil or bleach them out was a good one, and was picked up in 1934 by a Dr. E. Jaag - working for the firm of Gebrueder Schnyder based at Biel in Switzerland.[3] The use of other enzymes was also proposed - lipase by Altenberg[4] and amylase was used by Balantine and Sons[5] in the US.

6.2.2 The second phase

A shortage of fat during World War II, led to a severe shortage of soap in Europe during this period. This triggered a search for alternatives and Zscharn[6] in 1940 suggested that enzymes could be used as an 'ersatz seife' or soap substitute. During the war enzymic presoaking agents gained increasing

importance in Europe brought about by a severe shortage of fats and soaps. In Switzerland, however, pancreatic glands a source of the enzyme trypsin were removed to produce insulin, by a process which destroyed the glands enzyme activity, and production of an enzyme based washing powder had to await the cessation of hostilities.

Jaag's investigations published in 1947[7] indicated that the use of enzymes as washing aids were of considerable value but needed to be used carefully when washing protein based fibres, such as silk and wool. After the war a pancreatic trypsin preparation with bile salts for fat emulsification was produced by the Swiss Ferment Co. at Basle and marketed as Bio 38.[8]

Jaag realised that if enzymes were to have any future they would have to be incorporated and used under normal wash conditions. However, after the War soap powders began to be replaced by much more efficient surfactants detergents. By 1959 Jaag succeeded in developing an improved product using a bacterial neutral proteinase which was more effective as a presoak agent. This presoak enzyme detergent was called Bio 40, and produced by Gebrueder Schnyder in co-operation with the Swiss Ferment Company, Ltd. of Basle. (Now part of NOVO).

6.2.3. The third phase

Independently of these developments Novo Industrie A/S of Copenhagen, Denmark began investigating a laundering problem, blood and mucus stained protective clothes from the local meat and fish processing industries. In 1958 they began a programme of research

which lasted for three years and resulted in the introduction of an alkaline bacterial protease for the purpose. Novo researchers developed a six stage washing protocol that involved an enzyme treatment of the garments at stage III. This method was technically successful but too complicated for the laundry to use and hence failed to be adopted.[9] Undeterred Novo researchers identified the reason for failure as the complex washing procedure and looked around for alternatives. Knowing that they had a better enzyme to offer than the neutral protease produced by the Swiss Ferment Co., they entered into a co-operative agreement with Gebrueder Schnyder who introduced Novo's alkaline protease, trade named 'Alcalase' in their Bio 40 detergent preparation and improved its efficiency.[10]

Gebrueder Schnyder however began to suffer from local competition from the large soapers. Consequently Novo and Gebrueder Schnyder entered into technical co-operation agreements with 2 small detergent producers Kortman and Schulte based in Rotterdam and Blumøller in Odense, Denmark. In 1963 Kortman and Schulte began to market a presoaking agent Biotex in Holland and in a short time captured 20% of the Dutch detergent market.[11] Since the detergent market had been more or less static since the introduction of optical brightness in the 1950's a loss of 20% to a newcomer was considered to be serious and the two big soapers Procter & Gamble and Unilever began to look at enzymes with renewed interest.

The main aim of business is to make a profit. One way of increasing the profit is to increase the share of the market of

the goods produced. So the detergent manufacturer has to consider ways of at least maintaining and preferably increasing the market share of each product line. With just two manufacturers producing between them over 90% of the washing powders available in the U.K. the competition between the two companies Unilever and Procter and Gamble is intense. Each brand of powder has a "brand image" which aims the product at a particular section of the community. Such brands and images, as well as an overall "company image" are crucial for sales.

6.3. The Production of washing powder enzymes

Most of the enzymes used by the detergent manufacturers are produced by one or other of the two major producers, MAXATASE is marketed by the Dutch Company Gist-Brocade and ALCALASE by the Danish Company Novo. Of the two, Novo is the major producer.

The production of extracellular enzymes is theoretically very simple, it relies on the culture of micro-organisms in a suitable medium. Enzymes produced by the micro-organisms are then recovered from the medium or the cells. To make this process commercially viable, with respect to price, quality and quantity and to satisfy stipulated standards of safety and hygiene.

The production of alkaline protease is based on the organism Bacillus lichenformis (misclassified as B.subtilis) in a process which was developed in the late 50's and early 60's. Large fermenters culture this organism at temperatures between 30-40C and this produces large quantities of protease. By holding the medium at pH7, self-destruction is prevented, as this inactivates the enzyme since its optimum pH lies

about pH9-10. In the presence of sequestering agents such as sodium tripolyphosphate and ethylenediamine tetra acetic acid the enzyme remains stable. It is also stable in the presence of mild oxidising agents such as sodium perborate. The wide ranging catalytic activity of this protease is illustrated by its ability to break down between 30-35% of the milk protein, casein.

Processing of the culture begins with the separation of the culture medium from the cells, by centrifugation. The liquid is de-watered by either vacuum evaporation or ultrafiltration or both. Remaining bacteria are filtered out. The enzyme is then separated out by salt or solvent precipitation. In the Novo process, granulation of the enzyme is with common salt which acts as a preservative, mixed with carboxymethyl cellulose which acts also as a binder and water to form a paste. The paste is then extruded and passed to the marumeriser for shaping into spheres. The dried spheres are then coated with a non-ionic wax material. This wax coat is a relatively biologically inert material which protects the biologically highly active enzyme, and is less liable to form a dust. Shrouding of the enzyme in this manner is designed to protect both the workforce and the consumer from contact with the enzyme through inhalation. Recent developments in the wax coating and enzyme particle producing technology have replaced the marumerisers, with processes that make spherical enzyme agglomerates to a higher degree of uniformity, further reducing the risk of producing enzyme-containing dusts. This development has therefore further reduced the risk to the workforce/consumer of contact with the enzyme through inhalation.

6.4. Initial Market Growth

Many different procedures are used to wash clothes. Novo's experience with complex procedures suggested that if enzymes were incorporated in washing powders in a one step method then market resistance to their introduction would be reduced. I shall therefore consider only the main two washing procedures, the one in use in the U.S. and the one in use in Europe. In the U.S. method, clothes are washed at about 50°C for 10 minutes and then a bleaching agent is added separately. In the European method the temperature of the hot wash is raised slowly to just below boiling and held for 30-40 minutes. The bleaching action takes place in the second part of the wash since the washing powder contains sodium perborate which releases its oxygen at temperatures above 50°C. See table 6.1. for a comparison between European and U.S. washing powder compositions.

By 1966 enzyme washing powders had assumed a 2-3% market share of the European laundry detergent market. In 1966 enzyme washing powders first appeared in test marketing exercises in the United States.[12] Growth in the market share of the estimated enzyme washing powder market reached 50% in Europe by 1969 and was almost 45% in the United States. See fig. 6.1.

Table 6.1. A comparison between European and U.S. Detergent washing powders formulas.

European Enzyme Detergent Composition

Constituent	Composition %
sodium LAS[a] (40%)	25.0
alkylphenol ethoxylate (11 EO)[b]	3.0
soap, high-titer (80%)	3.0
CMC[c] (59%)	1.6
sodium tripolyphosphate (STP)	38.0
sodium perborate tetrahydrate	25.0
sodium metasilicate	1.0
alcalase 1.5M	0.9
sodium sulfate	ca 2.5
Total	100.0%

United States Enzyme Detergent Formulas

Constituent	Composition %	
	Phosphate	Nonphosphate
sodium LAS[a]	10-20	10-22
tallow alcohol sulfate	0-10	
tallow ether sulfate	0-30	0-12
sodium tripolyphosphate	up to 34	
sodium carbonate		20
sodium silicate	up to 12	up to 20
sodium carboxymethyl cellulose(CMC)	0-0.5	0-1.0
fluorescent whiteners	0.1-0.5	0.1-0.5
alkaline protease	1.0	1.25
sodium sulfate and perfume	qs	qs

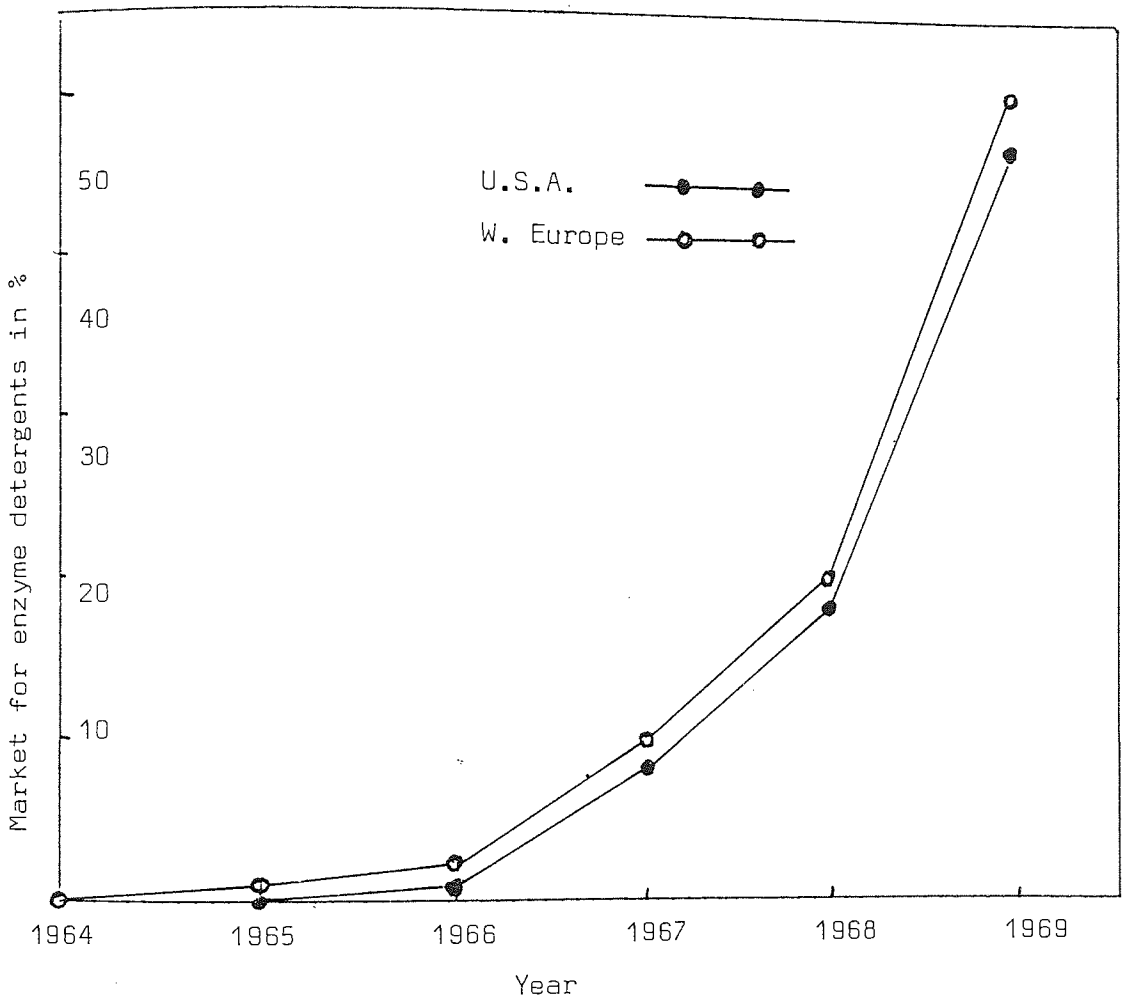
[a] Linear alkanesulfonate surfactant

[b] Average of 11 ethylene oxide units (see Surfactants).

[c] Carboxymethyl cellulose, sodium salt.

Source : Kirk Othmer 1980. (25)

Figure 6.1. The market entry rate of enzyme detergents in the United States and Western Europe on the basis of tonnage



Source : C.Dambmann et al. (3)

Growth in the market share was largely attributable to the demand for enzyme washing powders.

6.5. Diffusion of enzyme technology - the social dimension

In chapter 2, and in this chapter I have stated that enzymes are proteins and this can cause problems in their useability. Another source of difficulty arises in the way the human body interacts with enzymes present in the environment. Enzymes may have a direct effect on the body as specific chemical catalysts, indeed enzymes may be used to clean dirty wounds from dead cells and pus in topic dressings or to lyse blood clots.(13) Risks of damage by digestion are quite low compared to the allergic effects that may be triggered in certain people initiated by the enzyme protein being recognised as 'non self' and the setting in motion of an immune response towards it. The immune response complex chain of events during the initial sensitising period there is no response. Sensitisation is dose related and it may take a matter of hours or years or may never reach the level to trigger a clinical response. During the period of sensitisation antibodies are produced in response to the enzyme which acts as an allergen and these become attached to mast cells within the connective tissues of the body. Once the individual has been sensitised if there is further exposure to the same substance an allergy reaction will occur. Renewed exposure to the enzyme now causes the mast cell to explosively release mediators such as histamine and bradykinin chemicals which act on smooth muscles, mucus glands and blood vessels of the body. If a massive release of mediators take place anaphylaxis ensues which may result in a range of symptoms that include, skin rashes and eczema, swelling of the mucus

membranes of the nose and throat, vomiting, vascular collapse and bronchospasm. Hay fever-like symptoms carry with them severe discomfort but severe asthma attacks may prove to be fatal. Asthma is now recognised as an industrial disease if the patient has been exposed to the proteolytic enzymes used in detergent manufacture. People who suffer from allergies are called 'atopic'.[14]

Screening of 'atopic' individuals is recommended by the 1971 SDIA report according to the following procedures.
"Skin testing should be done by the prick method using an agreed enzyme reagent, grass pollen, aspergillus fumigatus, dermatophagoides culinae and a control". A questionnaire is completed, base line assessment of lung function carried out, chest X-rays performed as well as the skin testing using the prick method using an agreed enzyme reagent, some other known allergens and a control. "Those with significant findings as a result of any of the above procedures should not be recommended for work with enzyme powders." (SDIA report 1971).[15]

"The ventral surface of the forearm is used ... One drop of the skin test solution is placed on a marked ... area of the arm. The tip of a number 20 hypodermic needle (26 S.W.G.) is passed through the drop and inserted into the skin at an angle so that the skin may be lifted on the bevel. The needle is withdrawn and the excess solution is wiped off immediately... A positive reaction is indicated by a weal larger than the control and should be read at 15 min. A positive reading is either a weal of 1mm diameter or larger, or a weal greater than that which may be produced by the control".

Dr. Flindt,[16] a former Unilever doctor, described the symptoms of workers exposed to enzyme dusts in the early days as breathlessness,

usually acute onset and sometimes very severe. At its worst peak, patients were unable to get out of bed and thought they were dying (Lancet (1) p.1177, 1969). This clinical picture has been confirmed by other investigators in the United Kingdom, the United States of America, Switzerland and Italy.[17] Juniper summarised some of the points as follows:

> Alcalase is an allergen; inhalation could sensitise the individual; subsequent inhalation could affect nose and lungs; effects could last a few minutes or up to 24 hours; removal of patient from Alcalase could effect a cure whereas failure to do so could mean increasingly severe episodes: repeated episodes could lead to incomplete recovery and even long term impairment of health.

During this initial period of incorporating enzymes into powders, as more became known about the health hazards of enzymes, more precautions were taken such as exhaust ventilation, filter masks, specially designed overalls and health checks.[18] (New Scientist Vol.44 No.671, 1969). The safety precautions were those recommended in the Soap and Detergent Industry Association (SDIA) report of 1971[19] in which great emphasis is laid on the screening of "atopic" workers. Such workers are not recruited for employment. Thus those people who are likely to develop a sensitivity to enzymes are screened out. The SDIA report states that perhaps 30% of the population "may have atopic characteristics" but even non-atopics develop sensitivity, the ratio believed to be about 2:1 atopic to non-atopic.

The detergent industry spent a great deal of money in protecting the workforce from the hazards. The USDAW branch secretary at one site said "In the past we had occasion to eliminate silica from Vim. It had been used for years before it was identified with silicosis... as far as I'm concerned Lever Brothers did everything in their power on safety. It was a difference of medical opinion. I'm concerned about those I represent and the evidence. NSDs were made without any noticeable problems but when the 'bios' were introduced, the same workers were then affected".(19) In 1975 Lever Brothers opted out of the enzyme detergent market for commercial (not medical) reasons. The price was too high. "It's regrettable that we didn't make progress nationally. I hope I'm wrong, but people in Proctor & Gamble might develop chest pains they haven't had before. The NSD department here is a lot happier now".(M. Boxer, Private communication)

The decision to pull out of enzymes was also possibly prompted by the public's disenchantment with the product, owing to the unfavourable publicity attributed to enzyme dusts and continued reports of allergic reactions. Radient in particular was receiving a lot of adverse publicity. Such effects on the sale of microbial enzymes world wide was dramatic as the market share for enzyme detergents fell to pre 1966 levels. (See Fig 3.27 Chapter 3). The withdrawal of enzymes by Lever Brothers in 1975 was done relatively quietly after considerable discussion with the union at the factory involved. The union (USDAW) were concerned that there was a medical problem as incidents convinced them that enzymes were the cause of some workers becoming "distressed with asthmatic attacks". Initially, the enzymes were regarded just as a new ingredient but after a while, when the dust hazard was highlighted the enzymes were encapsulated (shrouded) by fixing the enzyme on to

granular sodium bipolyphosphate and rendering the resulting granules substantially dust free by spraying non-ionic material in the Novo process the enzyme was also crisped and screened to remove lumps, which made them heavier and more likely to fall to the floor instead of blowing around.

Eight years have passed since Lever Brothers withdrew enzymes from powders. During these 8 years the other manufacturers have continued to include them. The enzymes themselves have been modified, now being encapsulated in wax which melts in the wash to release the enzyme. There has been a substantial improvement in enzyme handling and plant designed to protect the work force. During this time, consumer washing habits have also changed that go beyond machine ownership. There is now much more emphasis on economy; so instead of washing 'whites' at high temperature, they are switching to the low termperature 'coloureds' cycle to save electricity. A mood that Proctor and Gamble exploit to the full with their 'Ariel' advertisements on television encouraging the housewife to economise by showing a temperature of 40°C in use and a 'save it' logo. There is a more flexible attitude to washing, abandoning the old washday concept and housewives shopping habits have altered with less frequent shopping and a preparedness to buy larger sizes. Because of product matching and the commercial competition between Lever Brothers and Proctor & Gamble and the success of major launches Ariel Automatic and Bold 3 in 1981 and 1982 respectively, Levers were obliged to introduce a product suitable for a low temperature wash.

Lever Brothers' answer was to reintroduce enzymes into one of their powders, but this time into Persil Automatic, rather than Radiant

or Omo as before. Persil Automatic enjoyed at that time 42.8% of the market for low suds powders, with the nearest rival being Ariel Automatic (Proctor & Gamble) with 17.4%. The only other low suds powder marketed by Levers was Surf Automatic with 9.6% of the market share. However, the "Persil" name has always had a "gentle and caring" image and this product image (so we are told by advertising executives) is what influences the consumer. Evidence that all was not well with enzyme washing powders was accumulating from the trades unions, where certain groups of the workforce were not atopically screened and whose members came into contact with enzyme powders. Such was the case in the departments dealing with "money off coupons" cut from the packets of powder. Certain sorters experienced a variety of allergy reactions whilst handling those coupons from powders containing enzymes.

Other evidence of difficulties began to trickle in from groups such as the National Eczema Society, whose members reported that clothes washed in enzyme-containing detergents were associated with skin problems. Until the introduction of enzymes into Persil Automatic, it had been the one powder recommended by the National Eczema Society for those who experienced discomfort from other powders. It was the powder with the fewest complaints of skin problems as a result of its use. Following the introduction of enzymes, this advantageous situation was lost. A variety of articles appeared in the popular press and popular scientific journals.[22] It was recognised that with Lever Brothers' decision to change Persil Automatic into an enzyme powder, it effectively reduced consumer choice of low lather, enzyme-free detergents and now excluded a section of the population that suffer from eczema from using its favourite detergent formulation.[23]

Evidence of causality is always difficult to obtain without experimentation. Unilever carried out exhaustive trials before launching the new product and independent research by two leading dermatologists. The former revealed no problems but the latter showed that indeed some people do suffer adverse effects when using a New System Persil Automatic, or any other enzyme-containing washing powder. It is interesting to note that Proctor and Gamble say publically that the tests they have carried out have proved negative[24]. Perhaps the tests they ran were not sensitive enough to detect these effects. Both companies state that the majority of those using enzyme-containing washing powders do not suffer from any side effects. However, on 22nd June this year, Lever Brothers stated publically that they would reintroduce the old formula Persil Automatic within two months.

The soap and detergent market has long been regarded as having lost consumer sovereignty through being dominated by a few large companies where sale of brand name products was skilfully manipulated through brand image advertising. Competition in the market restricted to a choice between rival brands. The opinions as to consumer need were ascertained by market research and marketing functions. The recent activities show how tenuous and fragile are the brand images which are so carefully and so costly created. This policy reversal by Lever Brothers, which also included the re-launch of their other low suds powder Surf Automatic as a low temperature powder WITHOUT enzymes, is a modest victory for the consumer and it took a product of biotechnology to bring it about.

The lesson for other products must be clear, innovators that develop new products or processes that will interact with people, must consider the SOCIAL DIMENSION of the market as well as the economist's criteria of price, demand and supply.

5.6. SUMMARY : CASE STUDY OF ENZYME INNOVATION PROCESS FROM SOAPING INTO LAUNDERING AND DRY CLEANING (See 4.10 above)

1. That the basic scientific work which led to the initial innovation of enzyme technology was carried out in the 19th century.

2. That the initial development was by a scientist entrepreneur, Rohm.

3. That the product initially and for a long period of time thereafter enjoyed limited market success.

4. That the opportunity to improve the product by improving the enzyme was a significant motivation to encourage further scientific research. (Jaag and Balantine)

5. That soap shortages during World War II created a market demand that could not be met because of problems in supplying the appropriate enzyme.

6. That bacterial enzymes were gradually and eventually identified as more suitable than pancreatic enzymes.

7. That the Novo company initially identified an industrial laundry problem and developed an enzyme-based solution to it. In the first instance this proved unsuccessful but subsequent scientific reanalysis of the reasons for failure led to the identification of causes for failure, their technological solution and led ultimately to commercial success.

8. That at that point Novo and Swiss Ferment examined other socio-economic developments and commercial opportunities identifying a new or established market for their product.

9. That initial marketing success was dependent upon the improved benefits of an enzyme presoak product and caused the large soapers notably, Unilever and Procter and Gamble to re-evaluate the use of enzymes in washing preparations.

10. That rapid industrial diffusion of the new enzyme technology followed, since most of the development problems had been worked out by Novo.

11. The subsequent consumer problems with allergic reaction caused the enzyme washing powder product to lose favour with the general public, resulting in a loss of sales and hence a declining market for these preparations.

12. That resulting in scientific and technological improvements in shrouding the enzyme the contact problem for most individuals was overcome. Contemporaneous innovations in the use of synthetic materials to make clothes and improved washing machine design led to changes in washing and laundering habits. This change was first exploited by Procter & Gamble and led to the reintroduction of enzyme washing powders by Lever Bros.

13. Thus the development of washing powder enzymes has depended very much on matching the product to user requirements. The role of the enzyme developing, refining and producing company in meeting end user needs was crucial to the diffusion of this technology.

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CHAPTER 7 - CASE STUDY : RESTRICTION ENDONUCLEASES - THE GENETIC ENGINEER'S SCISSORS

7.1. Introduction

Restriction nucleases are enzymes that cut double stranded DNA. (The cut is also called a nick). They have been found in many prokaryotes where they role is surmised as effecting degradation of foreign (i.e. non-self) DNA, such as that injected into a cell by an infecting virus or that takes place during conjugation. These enzymes can be separated into 3 groups according to the position of the site of DNA cleavage relative to the position of the DNA sequence recognised by the enzyme, relative molecular weights and cofactor requirements.

Class 1 restriction endonucleases cut double stranded DNA at positions outside of the recognition sequence and produce randomly cut fragments of genetic material. They are the largest of the restriction enzymes, with a molecular weight of about 300,000 - 400,000, are composed of 3 non-identical subunits, require magnesium ions 3-adenosyl methionine and ATP cofactors, and perform two different functions, DNA methylation and DNA cleavage; during their reaction the ATP is used up.[1]

Class II restriction endonucleases cut the DNA strand within the recognition sequences. Most of the Class I endonucleases isolated so far appear to recognise pallindromic sequences of base pairs of 4 to 6 units long (sequences that are identical whatever way they are read). (See Fig.7.1.). Class II endonucleases are smaller than Class I or Class III restriction enzymes, have molecular weights less than 80,000, require magnesium ions only for nuclease activity and S-adenosyl methionine for modification (see Section 7.2.)[2]

Type III endonucleases were originally grouped with Class I endonucleases but are now considered to be part of a third and distinct group. These enzymes which are of intermediate complexity comprise 2 subunits which have a combined weight of about 250,000. They require magnesium ions, they do not have an absolute requirement for S-adenosyl methionine but appear to be stimulated by it and do not hydrolyse ATP during the reaction but require it for the reaction to proceed.[3]

Strictly speaking 'restriction nucleases' are only those enzymes which have been shown to be part of the restriction modification system. This has been shown for only a handful of enzymes used for genetic engineering, consequently a pragmatic definition has come into existence in which any sequence specific endonuclease is called a restriction endonuclease.[14]

The abbreviated name for each restriction endonuclease enzyme is derived from the parent organism, thus Eco from Escherictia Coli, or Hpa from Haemophilus parainfluenzae, and Sal from Streptomyces albus and so on. If necessary the strain or serotype are identified next hence Hind from Haemophilus influenzae strain d and Hinc from Haemophilus influenzae strain c. Finally a number is added in order of the chronology isolation. Taq I the first endonuclease isolated from Thermus aquaticus in 1977 and Taq II the second nuclease isolated from Thermus aquaticus.

It is the type II restriction endonucleases which cleave DNA at or very close to the recognition sequence that has proved so invaluable in genetic engineering research.[4]

7.2. The discovery of restriction endonucleases

The first evidence for the occurrence of restriction nucleases was obtained in the 1950's by Luria and Human[6] whilst looking at the interaction between T-even phages (viral parasites of bacteria) and the bacterium itself. They found that the growth of phages originally grown on one bacterial strain lost their ability to infect that strain, once they had been used to infect another strain. Analysis of this phenomenon indicated that it was due to some mechanism operating within the cell itself rather than the phage, and so they suggested that 'host modification' was responsible for this change.

Bertani and Weigle[7] investigated the growth of Coli phages lambda, λ, and P2 (viruses of E.coli) and observed that these phages appeared to have widely differing efficiencies of infection on several closely related strains of bacteria. Thus the efficiency of infection increased dramatically when phages with initially low infection efficiency were replated on the same bacterial strain. Since the growth of the phages was restricted the mechanism used by the E.coli was called a 'restriction system'. They concluded that this modification was not a heredity genetic adaptation since it could be lost by subsequent propagation of the phage in another bacterial strain. It was subsequently shown that these two systems were different and that the system described by Luria and Human was unique to the T-even phages, whereas the system described by Bertani and Weigle was more widespread. The modification of T-even phages involves the glycosylation of hydroxymethylcytosine residues.[8]

Some years later Arber[9] and colleagues working in Geneva investigated this phenomenon further using phage lambda and two strains of Escherichia Coli, K & C. They showed that phage grown on

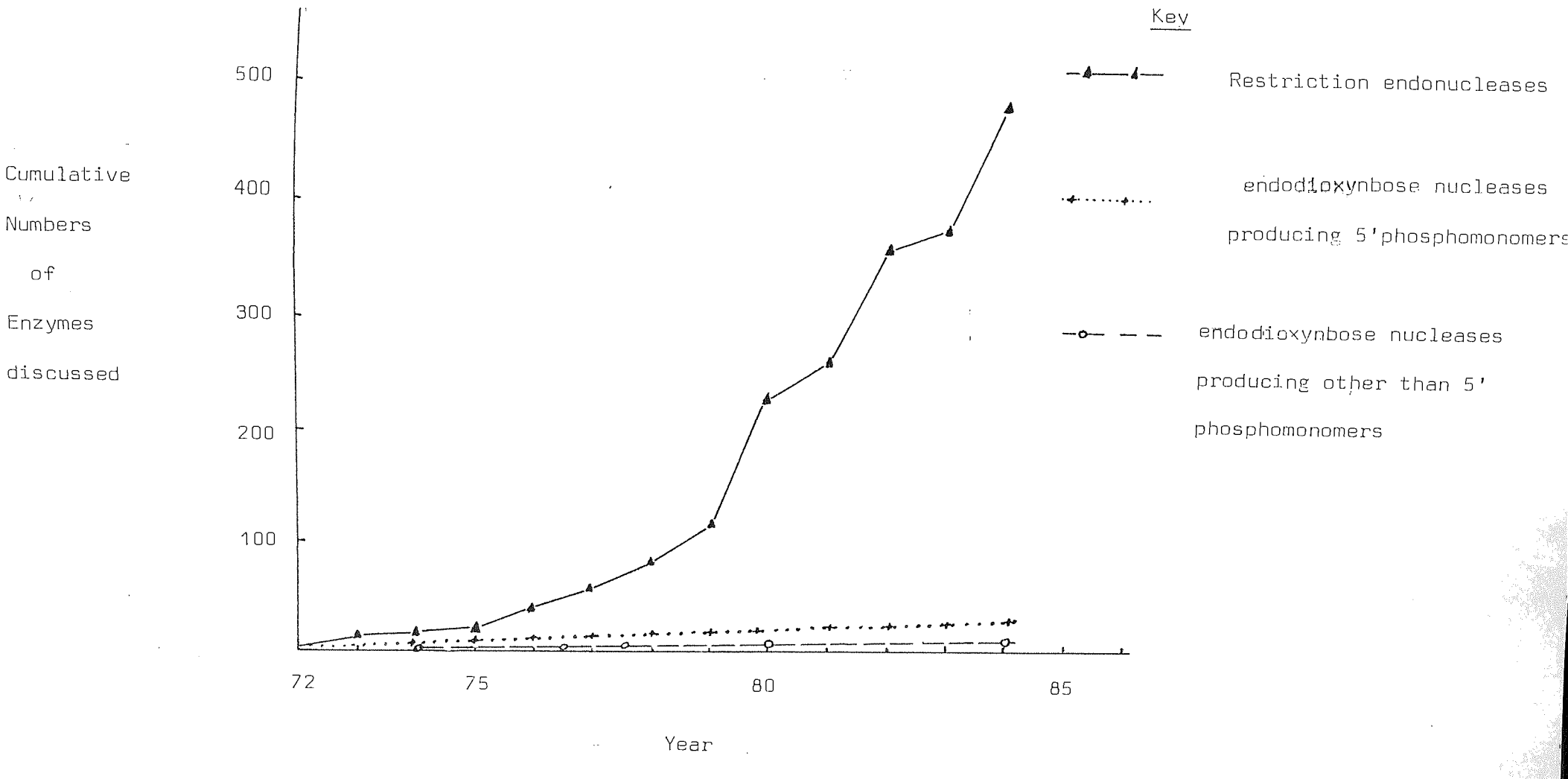
the K strain was capable of infecting C strain but phage from C strain on reinfecting strain K produced only one thousandth of the expected progeny. However, if these K phages were used to infect more of the K strain organisms then the number of offspring returned to normal. They concluded that phages grown on C strain had to be specifically modified to be grown on the K strain and that modification was a non-inherited phenomenon. The observations that DNA labelled with radio isotopes when grown in the C strain was broken down into smaller fragments with lower molecular weights, suggested that in addition to modification, this process described involved a nucleolytic degradation or restriction.

In 1968 Arber[10] and colleagues showed that modification of the phage DNA had also occurred. In the K modified Lambda phages the adenosine of the phage DNA became methylated to form 6-methyl adenosine in certain positions which made it resistant to attack by K the E.coli strains restriction system.

On the basis of these results they postulated the existence of two systems within a host cell. A restriction system that cuts the DNA at specific sites into small fragments and a modification system which recognises the same sequence but methylates them in such a way that they cannot be cleaved by the endonucleases of the first system. From these observations it was also possible to hypothesise on the function of this system, i.e. that since it is designed to cope with invading DNA it has evolved in bacteria as a response to viral attack.

Prior to these experiments, nucleases were considered to be non-specific in their mode of action, although the TI RNase was found to

Fig. 7.1. A comparison of the rates of discovery of three different endodioxynbose nucleases



act adjacent to guanine residues. The recognition of the restriction modification phenomenon led to a concerted effort by several laboratories to focus on the enzymes involved in the DNA restriction phenomenon. All these initiatives were the result of work undertaken by research workers engaged in the molecular biology branch of pure science.

7.3. Isolation of restriction endonucleases

In 1969 Meselson[11] and co-workers and Arber and co-workers reported almost simultaneously the isolation of a protein from E.coli strains K[12] and strain B[13] which had the ability to cleave phage DNA *in vitro*. These nucleases however belong to the Class I of enzymes and did not produce specific fragments. These Class I enzymes had a large molecular weight 400,000. They needed ATP and Mg^{2+} as cofactors and also S-adenosyl methionine for activity. The presence of just one methyl group on one strand of the double stranded DNA was sufficient to stop the enzymes action as a restriction endonuclease and direct the enzyme to methylate the second strand. None of these early E.coli restriction enzymes were of use as research tools, because although they recognised specific sites on the DNA, they cut it at what were in effect random locations, making it very difficult to piece together the nucleotide sequence of the nucleic acid.

Two years later in 1970 however, Hamilton Smith[14] and his co-workers[15] based at Johns Hopkins University in the U.S. followed up the chance observation that a cell extract from the bacterium *Haemophilus infleunzae* rapidly broke down P22 phage DNA, but had no effect on the Haemophilus DNA itself. Kelly and Smith found that highly purified extracts of the enzyme Hind III produced the breaks

in T7 DNA occurred at symetrical sequence where the hydrolysis occurred at the phosphodieter bonds, indicated by the arrows

5'...Gp Tp Py↓...pPu pA pC...3'

3'...Cp Ap Pup...Pyp Tp Gp...5'
↑

i.e. the enzyme cut the 6 base palindromic sequence of the double DNA strand between the Purine and Pyrimidine bases as indicated by the arrows. This was the first indication of Type II restriction nuclease activity. The number of restriction endonucleases today (1985) stands at 475[16] from 103 different species of microbes (some 363 strains). The rate of discovery of these enzymes is shown in Figure 7.1.

The genetic and biochemical characterisation of these enzymes is poor and are isolated as site specific endonucleases without it being genetically determined if they are involved in DNA restriction or modification.[17] The majority of these restriction endonuclease preparations are relatively crude preparations and little or nothing is known about the properties of the enzymes, save their unique affinity for the recognition sequence which can be in the range of 4 to 7 base pairs.

All enzymes are arranged at 37°C and are routinely used at this temperature even though it may not be the optimum temperature which gives the maximum rate of DNA cleavage. Some are assayed in units which digests 1.0µg of lambda (λ)DNA in 15 or 60 minutes at 37° in a total reaction mixture of 0.05ml.[18] Most restriction enzymes are stable and lose approximately 10% of their activity over a 12-18 month period if stored at -20°C in the appropriate storage buffer.[19]

The genetics of the restriction modification system have been analysed in Escherichia coli and in Salmonella typhimurium. In these microbes, type I restriction systems produce 3 different polypeptide units so the model predicts three genes. Type II systems are simpler with only 2 genes, one coding for the restriction endonuclease the other for the modification methylase. Whilst Type III systems probably have 2 genes.[20]

It has been shown that methylation of either adenosine or cytosine within a recognition site can inhibit the action of some Type II enzymes. However several enzymes work on a methylated DNA e.g. Dpn I form Diplococus pneumoniae, Bom HI from Bacillus amyloliquifaciens Bbr SI from Bacillus brevis, BsuRI from Bacillus subtilis strain R, BsuM from Bacillus subtilis Marbura 168, BsuFI from Bacillus subtilis FI strain and so on.[21]

Some enzymes recognise the same sequences such as XmaI and SmaI and are called isoschisomers although the cuts they produce in the DNA may be different. Although isolation sequences vary between different laboratories the procedure relies on the removal of nucleic acids by an agarose gel separation followed by DEAE and phosphocellulose chromatography. After these processes the enzyme is sufficiently pure for use in restriction experiments. Additional purification may be necessary for analysis and sequencing of the restriction sites and may involve chromatography using ω-aminoalkyl Sepharose.

7.4. Factors contributing to the rapid growth in the discovery of 'endonucleases'

It is unusual to find such a large number of enzymes, so poorly characterised.

The reasons for this are complex. Type II restriction endonucleases have been widely used for mapping studies as well as for cloning experiments.

The first restriction map using restriction enzyme was made by Nathans and Danno at John Hoskins University.[22] They used the Hind II enzyme to cut the Simian Virus 40 circular DNA. Separation of the fragments on agarose gel produced well defined bands consistently in different experiments. Nathans produced a restriction map from 11 fragments indicating the presence of 11 sites attacked by the restriction enzyme.

The availability of reproducible restriction fragments made it possible to sequence them by the use of the RNA polymerase technique developed by Sanger. Weissman and Fiers had by 1976 sequenced over 50% of the 5200 base pairs of the DNA of SV40. In 1975 Sanger derived a rapid sequencing technique of 100-500 base pair fragments using the plus and minus method,[23] based on the elongation of DNA chains with DNA polymerase. In 1977 a method of DNA sequencing based on the chemical degradation of DNA chains was developed by Maxam and Gilbert.[24]

Sanger later developed a second method for sequencing DNA and again used enzyme techniques to do so.[25] These techniques led to the rapid sequencing of the phage G4.

In 1971-72 Lobban and Kaiser[26] demonstrated that DNA ligase would rejoin 2 DNA fragments, that had previously been cut by a restriction endonuclease permanently. This realisation led to the development by Boyer and Cohen[27] of a practical method of inserting DNA into the small pSC202 plasmid. Thus these two groups of experiments opened the way for the analysis of DNA structure at the molecular level. Thus restriction endonuclease were needed as research tools, and providing they performed the task of being the genetic engineer's 'scissors', little else was required of them to function. As research or genetic engineering tools, these enzymes are used in very small amounts, they are still expensive to produce and consequently very little characterisation work has been carried out on them.

The rapid expansion in the discovery of these enzymes is attributable to two other factors. The research carried out by the restriction enzyme group of Richard J. Roberts at Cold Spring Harbour Laboratory, U.S.A., and the important service that this group has performed in identification, isolation, and the characterisation of new restriction endonucleases,[28] and their role in the dissemination of information (hence the frequent reference to unpublished work about these enzymes in review listings and the catalogues of restriction endonuclease suppliers). The other factor has been the setting up of a number of specialist firms that purify and sell these enzymes.

Although the better known commercial suppliers of biochemicals such as Miles Laboratories (the first commercial restriction enzyme producer) and P.L. Biochemicals are important suppliers the production of restriction endonucleases is dominated by a number of small companies. New England Laboratories based in Beverly, Massachusetts was founded in

1974 by Donald G.Comb - when he was still at Harvard Medical School, and is wholly owned by Comb and his wife.[29] Comb is keeping his company small, in 1980 it was only 22 strong. In 1975 the company made a world first by bringing a rDNA product on the market - a DNA ligase from an Escherictia coli strain from a gene cloned by Lehman of Stanford.[30] By 1980 the company featured three other rDNA-made enzymes. Comb and his company have had a major impact on the production of restriction enzymes and in 1980 began to diversify into rDNA technology working on a cure for malaria.[31]

The other small company is Bethesda Research Laboratories (BRL) which was founded in January 1976 by Stephen Turner, a former Becton Dickinson employee.[32] As the market for restriction endonucleases began to grow so Turner decided to start his own company. Using $30,000 of his own he hired a technician and rented 7000 square feet in Rockville in Maryland and began to visit nearby companies of NIH and John Hopkins University selling restriction enzymes from an ice bucket.[33] By 1976 he sold $100,000 worth of enzymes and hired two more technicians. The company began to expand rapidly and employed 35 Ph.D's and achieved sales in 1981 of $2.5M. Turner diversified as fast as possible into other fields, using rDNA projects. Turner considered that by making new techniques available as soon as they are developed he could shrink the lead times. Turner owns about 85% of BRL.

An analysis of the restriction enzyme market proves to be difficult. New England Laboratories considers that on the basis of citation in the literature it controls 75% of the market - a figure that is reflected in the relative sizes of the company's turnover for 1980.[34]

Between 30 and 40 restriction endonucleases were available in 1979 from companies such as Bethesda Research Laboratories, New England BioLabs, Miles Laboratories, Boehringher Mannheim and Worthington Laboratories[35] (By 1980 New England BioLabs alone was offering 52 endonucleases).[36]

By 1981 65 restriction endonucleases were available from a number of commercial sources.[37] Recently other biochemicals producers have begun to distribute restriction endonucleases e.g. Amersham (1984)[38] and others to produce and distribute these enzymes in the U.K., Cambridge Biotechnology Laboratories.[39]

The reasons for the expansion of production has been the increase in the number of corporate and university laboratories engaged in rDNA work. By 1980 27 U.S. and U.K. companies alone were reporting to have rDNA projects under way, and at least 38 research institutes and research laboratories[40] were claiming to be working in this area. The reasons for this upsurge of rDNA work during the 80's will be discussed in the next section.

7.5. Restriction nucleases and genetic engineering

The experiments that showed that it was possible to recombine fragments DNA from different sources and cleaved by the same restriction enzyme was carried out in Stanford University by Janet Mertz and Ron Davis in 1972.[41] The use of the restriction enzyme EcoRI in conjunction with DNA ligase made it possible to carry out genetic recombination in vitro.

Janet Mertz whilst still a student in Paul Berg's laboratory

talked about Paul Berg's plans to clone a tumour causing virus, Simian Virus 40 (SV40) into the genome of the generally benign gut bactenium Escherecia coli.[42] This talk brought about some reaction from the scientific community in particular Robert Pollack working on SV40 mediated transformations of mouse cells. He had some doubts about the safety of SV40 itself, and considerable doubts about the wisdom of carrying out such a genetic transfer into a bacterium that could act as a vector and initiate Simian Virus induced cancers in humans. Pollack telephoned Berg who saw no reason to panic but made the decision to refrain from cloning tumour virus genomes. During this period the problem of inserting DNA into a bacterium made cloning still a difficult procedure to achieve. This however was about to change for in 1973 DNA fragments were inserted into an E.coli plasmid pSC101 by Herbert Boyer and Stanley Cohen producing a new hybrid plasmid.[43] Plasmids have the advantage that they are discrete pieces of DNA that can be taken up by a bacterium and multiply with it as it divides. This refinement of rDNA technology increased the incidence of rDNA research so much so that in the Gordon Research Conference, safety issues were discussed.[44] The question of whether to proceed with these experiments was not the issue, the scientists overwhelmingly felt they needed to proceed with their work. The scientists were concerned with investigative procedures.

However the delegates voted by a narrow margin, to send a letter to the National Academy of Sciences and the National Institute of Medicine requesting that a committee or study group be instigated to investigate potential hazards of rDNA technology to workers and the public and to alert the scientific community to these dangers. This letter by Maxine Singer and Dieter Soll was reprinted in Science Vol.

181, 1973, p.1114. The NAS appointed a committee who in July 1974 asked through an open letter by Paul Berg and printed in Science and Nature, for a moratorium and called for a meeting to discuss potential hazards. The letter also requested that the Director of the National Institutes of Health consider the formation of an advisory committee to develop methods to evaluate potential hazards and frame guidelines for research workers and research institutes. In response to this call the Recombinant DNA Advisory Committee of the NIH was formed in the U.S. in October 1974.

The international conference called for by the NAS committee was held at the Asilomar Conference Centre near Monterey, California, in February 1975. The conference discussed the scientific issues and largely ignored the ethical and moral issues. A new unanimous consensus was reached about the need to restrict rDNA work to genetically weakened strains of micro-organisms and to biological containment i.e. that work should continue with appropriate safeguards involving physical containment as well.[45]

In the U.K. the suggestions in the Berg letters were upheld by the Medical Research Council,[46] and the official investigations of hazards on behalf of the British Government were undertaken by the Ashby Committee whose report (Command 5880 1085) was delayed to coincide with the added official publication of the Godber Committee's findings (Command 6054) based on investigating safety in research laboratories. These reports were followed up quickly by the Williams Report (Command 6660 1976) which led to the setting up by the Government of the Genetic Manipulation Advisory Group (GMAG).[47] The Williams committee also proposed the establishing of containment categories with emphasis on the physical

containment of the genetically engineered microbes. Yet another official body, the Health and Safety Executive, proposed very broad definitions of experiments which needed to be controlled.

In the U.S. after Asilomar the rDNA debate began to attract political comment from non-scientist members of the public. In the U.K. GMAG drew its membership from representatives which included scientists, public organisations, trade unions, industrialists and civil servants, and in that it was a departure from the usual self-regulation of science by scientists. This radical mix was a means by which various community interests could be seen to be served in the event. In December 1976 GMAG met and the Health and Safety Executive definitions of safeguards were aligned so as to follow the Williams committee's categories.

In reality the workings of the committee were fraught with problems, the industrialists refusing to discuss their experiments in open, with the trade unions or other representatives being present.

Pressure from academics to be allowed to proceed with rDNA experiments and from industrialists anxious to exploit the new technology gradually ensured a reduction in the initial framework of state controls and led to the committee's eventual reorganisation.

With the emphasis on regulating rDNA technology by developing weakened strains of bacteria, safe plasmids and a heavy emphasis on physical containment, rDNA technology was allowed to develop. The underpinning enzyme technology so necessary for rDNA work was effectively allowed to proceed unrestricted, the option of controlling rDNA work by controlling Class II restriction endonuclease was never considered.

Thus the political debate about rDNA affected the growth of rDNA technology which combined with the specialist nature of the market and uncertainty over its size and potential created a commercial environment which a scientist entrepreneur or science related entrepreneurs could exploit. In this case 2 small companies were able to supply a growing market that the more highly diversified larger companies were slow or loathe to enter. It was only once the market had begun to develop and with the relaxing of state regulations that the larger specialist scientific organisations began to make inroads into this market and offer restriction enzymes as part of their product range e.g. Amersham 1984.

Despite their strategic value as the tools without which genetic engineering would be impossible, the making of restriction and ligation enzymes was largely left to private enterprise, a classis case in mind is that of D.Coombs who acted as an entrepreneur in this respect and moved into this area, forming New England BioLabs (a collaborative laboratory) and proceeded to recruit newly qualified Ph.D. scientists who had as part of their doctoral or post doctoral programmes isolated new restriction enzymes, and who now made these enzymes available for others working in genetic engineering research. However the prime mover in this field was undoubtedly the research carried out by Richard Roberts at Cold Spring Harbor and university workers from a number of universities.

7.6. Summary

1. Type II restriction endonucleases are being discovered at a faster rate than other endonucleases.

2. The impetus for their growth is their importance as research tools and tools for industrial application of rDNA technology.

3. The majority of research and development in these enzymes is attributable to R.J.Roberts of Cold Spring Harbour Laboratory U.S.A. but other universities and institutes have subsequently also played important roles in the development of this area.

4. That the distribution and dissemination of information about these enzymes coupled with the availability of bacterial strains processed and developed by Roberts has led to the rapid diffusion of these enzymes in the west.

5. The role played in the production and development of restriction enzymes by companies such as Miles, New England Labs and Bethesda Laboratories has been helpful to the development of this technology.

6. The commercial incentive to obtain new nick-producing enzymes has created a commercial environment and market in which companies such as New England BioLabs and Bethesda Laboratories could flourish.

7. The scientific and commercial development of these enzymes appears to have been largely unaffected by the rDNA debate and State regulation.

8. That the coupling of scientific knowledge to a research technique and ultimately to a production technique has led to an increase in scientific activity in the search for new Type II restriction endonucleases.

9. That the non-functional aspects of restriction endonucleases have attracted less scientific attention, either at the level of the microbes genetics or the working characterists of the enzyme.

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CHAPTER 8 : CASE STUDY : IMMOBILISED ENZYMES

8.1. Introduction

I have selected immobilised enzymes as a case study because my survey of the literature, my analysis of patents and the scientometric analysis of a case journal, all indicate that this is an important area of scientific research, invention and innovation.

I shall therefore begin by defining my use of the term immobilised enzyme.

The term immobilised enzyme was introduced in 1971 at the 1st Enzyme Engineering Conference in 1971, and superseded various other terms such as 'water insoluble enzyme', 'trapped enzyme', 'fixed enzyme' and 'matrix supported enzyme'.[1] I have adopted a narrow definition of immobilised enzyme and consequently this discussion does not include immobilised cells. Immobilisation of enzymes is considered to be an important step in extending the application of enzymatic processes to industrial uses.

Immobilised enzymes will always be more costly to produce than soluble enzymes, but their introduction, and use, is justified for the following reasons :

(i) An improvement in the enzymes stability

(ii) Modification of the enzymes operating condition enabling it to withstand use in harsher environments, such as extremes of pH, elevated temperatures or strong solvents.

(iii) Increase in the quality of production due to the reduction of enzyme traces in the product, ability to control the reaction by removal of enzyme from the substrate at the optimum conversion of the product; as well as preventing the formation of undesired side reactions, thus saving on raw materials and reducing the need for elaborate down stream purification procedures.

(iv) Reducing the cost of the enzyme by making the catalyst reusable.

(v) Raising the efficiency of processing by making possible continuous operation.

(vi) Creation of new processes and procedures hitherto not possible for enzymic processing, such as the use of immobilised enzymes in a continuous process which are inhibited by the product.

A summary of factors to be considered for the industrial application of immobilised enzymes or cells is provided below (See Table 8.1.).

Table 8.1. Factors to be considered for Industrial Application of Immobilised Enzymes including Immobilised Cells

Factors		Soluble enzyme Batch system	Immobilised enzymes Batch system	Immobilised enzymes Column system
Enzyme	Cost: High	-	suitable	suitable
	Cost: Low	suitable	-	-
	Stability & reuse	low impossible	moderate-high possible	high possible
	Control of by product	difficult	difficult	easy
Enzyme reaction	Rate: High	-	-	suitable
	Rate: Low	suitable	suitable	-
Product	Purity	low	high	high
	Yield	low	high	high
Equipment	Initial cost	low	moderate	high
	Automation Applicability	difficult high	difficult high	easy moderate
Running cost (Labour cost)		high	moderate	low
Economic merit scale		low	low	high

Source - L-Amino Acid Production by I.Chabata et al. in Immobilised Enzyme Technology. (Research ' Application) Edited by H.H.Weetall and S.Suzuki. Plenum Press N.Y. & London 1975, p.25

For many industrial processes, the immobilisation of cells provides an often preferred alternative to the use of immobilised enzymes, hence the term immobilised biocatalyst is often preferred. However I have restricted this discussion to purified and immobilised enzymes per se.

8.2. Immobilisation Methods for Enzymes

Enzymes can be immobilised by a number of methods:

1. They can be physically retained in place by the use of the following:-[2]

   a. Enclosure within ultrafiltration membranes.

   b. Enclosure of the enzymes in microcapsules.

   c. Entrapping the enzymes in a matrix e.g. a gel, or fibre.

2. They can be adsorbed to a carrier by weak van der Waals forces or absorbed within a process matrix and retained by surface tension effects.

3. Use can be made also of the ionic forces between enzyme and carrier to immobilise the enzyme. Ionic adsorbants are generally anionic in character. Ionic bonds are chemical bonds that can be readily broken by altering the pH of a solution, and affecting the overall change on a protein molecule.

4. Activation of the surface of a support by a transition metal compound, which allows direct coupling of the enzyme to it by the formation of chelates.

5. Use can also be made of covalent linkages, energy requiring chemical bonds, either between the enzymes by use of intermediate chemicals to provide spacer arms or by bonding the enzyme directly onto a carrier. For covalent immobilisation of an enzyme any one of the following chemical groups can be used :

   (i) the amino group

   (ii) the carboxyl group

(iii) the sulphydral groups of cysteine

(iv) the imadozole groups of histidine

(v) the phenolic or hydroxyl groups of serine or threonine.

6. By cross linking the enzyme to other enzymes or to a carrier.

The advantages and disadvantages of the various systems and examples of their industrial application are shown in Table 8.2. below.

For a summary of the characteristics of immobilised enzymes and summaries of criteria for their choice see Table 8.3. below.

The enzymes used predominantly by industry are the hydrolases, isomerases or oxidoreductases. Of these the isomerases require no cofactors, the hydrolases require ubiquitous water as a cofactor to bring about their reaction, oxidoreductases require oxygen, a peroxide or commercially available cofactor such as NAD.

The cost of preparing cofactors and problems of their regeneration has been a major obstacle in the development of this branch of enzyme technology. Purification and co-immobilisation of cofactor and apo-enzyme or the "holoenzyme complex" is unsuccessful technically because suboptimal compromise conditions are required by such a process and this makes the operation of such catalysts inefficient.(3) Various ingenious solutions to this problem have been attempted. A process has been developed by workers at the Gesellschaft für Bio-technologische Forschung mbH, Braunschweig, which has been commercialised by Deguss AG (F.G.R) so making L-Alanine in an ultra filtration reactor. The enzyme involved is a dehydrogenase. The NADH cofactor is immobilised on a soluble polymer and is regenerated continuously by

Table 8.2. The Advantages and disadvantages of alternative immobilisation methods and some examples of their industrial application

Immobilised Systems	Advantages	Disadvantages	Industrial Applications	
			Enzyme	Cells
1. Enclosure in an Ultra-filtrations Membrane or microcapsules.			Analine dehydrogenase Degussa.	
	a) High retention b) Initial activity maintained c) Ease of regeneration	a) Stabilisation a problem b) Substrate of high purity required c) High pressure required to drive substrate through ultrafiltration reactor. Microcapsules have problems of mixing of substrate and diffusion of products.		
2. Entrapment in a Matrix				
	a) Easy to prepare b) Easy to handle c) Initial activity maintained	a) Some inactivation of enzyme on immobilisation b) Leaching a problem c) Regeneration requires swelling of matrix, difficult to perform		E.coli (aspartase) 1975 E.coli (fumerase) 1974
3. Adsorption or absorption				
	a) Easy to prepare b) Initial inactivity usually high c) Good hydraulic properties d) Regeneration of enzyme possible	a) Low yield of immobilised enzyme a problem b) Diffusion of substrate and products often reduced c) Leakage a problem d) Porous supports costly if used.		
3. Adsorption onto a changed support.				
	a) Easy to prepare b) High activities maintained c) A shift in optimum d) Stabilisation of enzyme often achieved	a) Hydraulic properties limit use to low pressure drop reactors b) Leaching of enzyme from support c) pH control critical		Aspartase Ammonia lyase, Glucose isomerase Penicillin acylase

Table 8.2. continued

	Advantages	Disadvantages	Industrial Applications: Enzyme	Industrial Applications: Cells
5. Chelation	a) Easy to prepare b) Stabilisation of enzyme achieved c) Good hydraulic properties	a) Initial activities seldom difficult to attain b) Leakage of enzyme from support working with c) Low pH a problem		
6. Covalent Binding onto a carrier	a) Good retention of activity in use b) Good hydraulic properties c) Stabilisation of enzyme sometimes achieved e.g. temp.	a) Initial activities seldom achieved b) Leakage of activating chemicals a possibility (toxicity a problem) c) Immobilisation and re-generation costly operations		Glucose isomerase Miles technology. Penicillin amylase
7. Cross Linking with/out Bonding onto carrier	a) Good retention of activity in use b) Stabilisation of enzyme often achieved c) Improvement in desired properties possible	a) Initial activities often lowered b) Most supports costly, require reuse, re-use difficult.	Glucose isomerase glucoamylase	
8. Cross Linking with/out Bonding onto carrier	Similar to carrier bonding.			

Source: Towalski, Z., Enzyme Technology Patents as quantitative indicators of activity, 1982.

Table 8.3. Summary of the various commercially important characteristics of various Immobilised Systems

Characteristics	Physical			Carrier Binding Method				
	Ultrafiltrations Membrane	Matrix	Microencapsulation	Physical Adsorption	Ionic Binding	Chelation	Covalent Carrier Binding	Cross Linking with/out Binding onto carrier
1. Ease of preparation	xxx	xx	x	xxx	xxx	xxx	x	x
2. Activity	xxx	xxx	xxx	x	xxx	xxx	xx Some inactivation of enzyme by chemical process	xx The enzyme tends to be buried by the cross linking agent
3. Substrate specificity	same	same	same	same	same	same	modified	modified
4. Retention (Binding force)	high	high	high	low	moderate	moderate	high	high
5. Regeneration of carrier	possible	possible in some	possible	possible	possible	possible	rare	impossible
6. Cost of Immobilisation	low	low	low	low	moderate	moderate	high	high
7. Stability				low	moderate	moderate		high

KEY : x slight
xx medium
xxx high

Source : Towalski, Z., Enzyme Technology Patents as quantitative indicators of activity, 1982.

a formate dehydrogenase that is supplied with formate ions.[4]

Immobilisation of whole microbial cells containing the enzymes and cofactors overcomes this problem. This technique is an attractive industrial alternative since it does away with the need for cofactor regeneration and reduces costs, by obviating the need for enzyme purification, cofactor isolation and system development. Enzymes within a cell are already stabilised to work within the intracellular environment and their high activity and half life is consequently prolonged, making them attractive for continuous operations.

As immobilisation of cells offers considerable savings in the initial processing of enzymes costs, it also makes small product runs a commercial possibility.

The cost of the immobilised enzyme may be so high that only reuse enables it to be economic, hence the desire to find methods of prolonging the life of the catalyst. I shall now discuss the development of immobilised enzyme systems and then proceed to discuss the industrial applications of immobilised enzymes listed in Table 8.2.above.

8.3. Development of immobilised enzyme science and technology

Although the majority of enzymes made by a cell are immobilised or bound to some supporting membrane, the isolation and rebinding of enzymes is not a recent phenomenon. The first conscious attempt to immobilise an enzyme was made by Michaelis and Ehrenreich in 1908, who immobilised the enzyme invertase on charcoal.[5] Although the scientific literature describes the first scientific recording of an immobilised enzyme to the work of Nelson and Griffin[6] in 1916 who

absorbed the enzyme invertase onto animal charcoal and reported that the invertase retained most of its activity. Nelson and his co-workers Hitchcock[7] also showed in 1921 that alumina (kaolinite) acted as a suitable binding material. Since then other enzymes have been immobilised by adsorption onto a variety of inert supports by what are commonly referred to as weak van der Waal forces.

The first attempts to apply this knowledge was made by immunologists who were concerned with effecting a separation of antigens for the isolation of specific antibodies.[8] However the unpredictable behaviour of these systems, and the difficulty of obtaining efficient separation of the antibody fractions, led the early investigators to realise that stronger methods of retention were needed than just the adsorption of the protein to a carrier. They began to look at chemical methods of attachment. Thus the initial attempt to covalently link proteins onto insoluble supports were consequently carried out by immunologists.[9]

The first industrial application of an immobilised enzyme system was carried out by the Tate and Lyle Company during the 1940's.[10] Animal charcoal (bone char) was used to decolourise golden syrup produced by the acidic hydrolysis of sucrose. In a modified process these columns (10ft diameter x 20ft deep each containing 40 tons of bone char) were charged with invertase obtained by the plasmolysis of yeast. 6 columns decolourised and inverted 32,000 gallons of syrup during a 6 day production cycle. Although the process made significant cost advantages over normal batch inversion a continuous operation proved difficult to achieve. Problems were also experienced with reproducible immobilisation of the enzyme to the carrier, to microbial contamination of the filter bed and inadequate process control.

After World War II the first experiments on covalent binding of proteins were carried out. Early workers, mostly biochemists, biophysicists and chemists began to realise that immobilised enzymes could be of use in research on the organisation of cellular metabolism by providing models of the way enzymes behaved in living systems. Others felt that immobilised enzymes could be useful as insoluble chemical reagents and possibly be of use as insoluble industrial catalysts.

Binding was restricted however to the then commercially available supports, derivative of celluloses and styrene polymers. In parallel similar approaches were tried for the immobilisation of enzymes[11] The amount of bound protein was low due to the hydrophobic nature of the carriers which also facilitated the leaching of the enzyme for the 'carrier'.

These reactions were improved by increasing the hydrophilicity of the carriers,[12] whilst concurrently copolymers of methacyril acid and methacrylic acid, 3 - fluoro - 4, 6 - dinitroanilide were also developed.[13]

The upsurge of interest in enzyme immobilisation during the mid-1960's followed several different lines of investigation. Some work was directed at the chemical and mechanical nature of the support material, some at the development of milder techniques for immobilising the enzyme reducing the need to use harsh chemicals, which improved the enzymes stability but reduced its activity. Others sought to develop alternative techniques to the covalent binding of the enzyme to a carrier, thus new data began to emerge. During the late 1960's it became apparent that enzyme activity and stability were functions of the support material, the nature of immobilisation, and of the micro environment in which

the enzyme found itself working. Ability to influence these three factors proved to be crucial to the development of immobilised enzymes as industrial catalysts. Thus enzyme technology became a major field of research activity of biochemists and chemists. This led to more coherent attempts to design supports with predetermined characteristics such as mechanical stability, limited hydrophobicity and the functional group which would be linked to the protein. These included derivatives of cellulose e.g. Diethyl amino ethyl cellulose[14], cyanogen bromide activated Sephadex and Sepharose[15], derivatized acrylic polymers and copolymers,[16] derivatized porous glass[17] and derivatised nylons.[18]

The mainstream of academic research focused on covalent linkage, but non covalent fixation of enzymes also received some attention.

During the1960's most of the fundamental principles and practices that underpinned the use and development of immobilised enzymes had been carried out. In 1964 Goldstein et al[19] showed that the pH activity of profiles of polyanionic derivatives of 2 hydrolases enzymes were displaced to a more alkaline pH and that polycationic derivatives were displaced towards more acidic values, and that the observed lowering or increase in value of the apparent Michaelis constant could be accounted for by the partitioning of the substrate and repulsive or attractive interactions with the polyelectrolytic support. Goldstein et al[20] could relate the observed shifts in pH activity curves and the Michaelis constant to the electrostatic potential in the domain of a charged enzyme particle. These observations had been anticipated by other workers[21] but their findings did not enter the scientific literature because they were directed at soil chemists interested in clays.

The role of the enzymes micro-environment highlighted the limitations of the main thrust of scientific thinking that was divided at reconstituting metabolic pathways of cells in vitro and beginning the extraction and reconstruction of enzyme complexes, and immobilised multienzyme systems.[22]

In 1965 Goldman et al.[23] found that enzyme membranes set up pH differences which at the time were attributed to local accumulation of hydrogen ions as a result of the hydrolysis of later substrates within the porous membrane. Later[24] they showed that substrate and product concentration gradients were established within an enzyme membrane due to diffusional limitations on the translocation of the substrate and product. These studies introduced the concept of a micro-environment generated by enzyme activity in a system where access to the enzyme is limited. Similar findings came to light from parallel studies carried out on enzyme reactors.

The studies on the various engineering aspects of immobilised enzymes catalysis such as mass transfer kinetics led to early work on enzyme columns[25] and led to a high degree of sophistication in enzyme reactor design.

By 1969 pilot plant operations involving two immobilised enzyme systems were in operation. One involving the DEAE, cellulose immobilised glucose isomerase of the Clinton discussed in Chapter 7, the other the immobilised penicillin acylase being studied by Lilly at the Biochemical Engineering Dept. of University College,

Table 8.4. Salient Design Paramaters in relation to the immobilised enzyme

Parameters	System properties	Goal
Enzyme Kinetics Cost	Reaction rate : enzyme loading	Cost per pound product
Carrier Cost Particle size and shape Surface area and pore size Composition Surface treatment	Coupling efficiency: immobilised enzyme life: additional processing cost	Cost per pound product
Enzyme immobilisation Technique Temperature Concentration Time pH	Equipment cost: operating cost; heat transfer; pressure drop (columns)	Cost per pound product
Reactor Type Dimension	Product quality	Cost per pound product
Operating Conditions Temperature Feed rate Feed composition pH Pressure		Cost per pound product

Source : Pitcher, W.M., Immobilised enzymes, antigens, antibodies and peptides, Preparation and characterisation, Weetall H.W., Ed. Naral Dekker, 1975.

London.[26] The level of scientific activity in this field has grown rapidly. By 1970 some 300 papers had entered the literature concerning aspects of enzyme immobilisation. Five years later this number had risen to over 1,000 and continues to grow.[27]

The most active researchers and major proponents of this technology were members of the academic community both in the U.K. and abroad in the U.S. During the period 1971-75 the National Science Foundation (NSF) organised under its Research Applied to National Needs (RANN) programme, research into enzyme science that cost $8.6M and funded 93 separate projects in this field.[28] Despite the copious publications, comparisons between the different types of immobilisation did not take place and critical analysis of results using commercial criteria such as those listed in Table 8.4 are absent. However the degree of industrial participation in immobilised enzyme research became more noticeable in the U.S. during the late 70's as criteria for the commercial evaluation of immobilised enzyme systems began to emerge.[29]

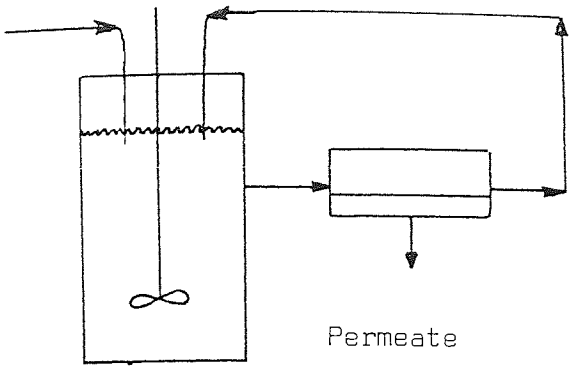
8.4. Commercial criteria influencing the use of immobilised enzymes as industrial catalysts.

The development of immobilised enzyme processes is a costly undertaking and may be between $250,000 and $500,000.[30] A wide range of design parameters have to be taken into consideration which relate to the immobilised enzyme and the design of the reactor. A list of salient design parameters is presented in table 8.4. above.

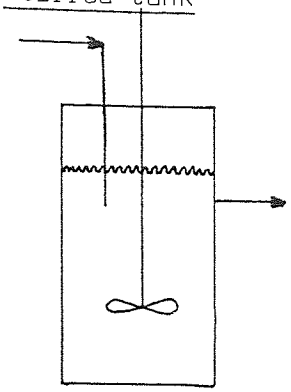
It is possible to use immobilised enzymes in a number of different reactor configurations. I shall mention just four in passing: continuous flow stirred tank reactors, combined continuous flow stirred

Figure 8.1. Some enzyme reactor configurations

Stirred tank with an ultrafiltration unit

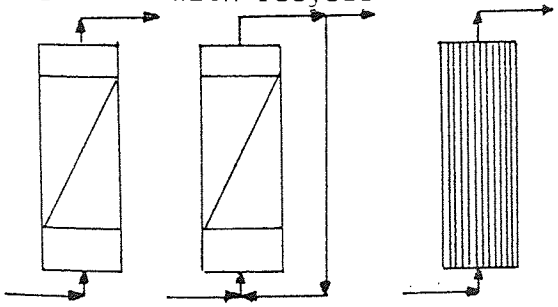


Stirred tank



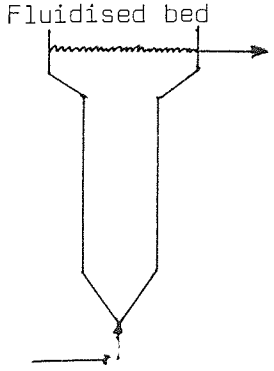
1. Combined continuous-flow stirred-tank reactor (CSTR) ultrafiltration system for free or soluble immobilised enzymes.
   Colloidal or insoluble substrates can be processed
   Poor stability of the enzyme for long-term operation
   Enzyme loss by adsorption onto the membrane
   Concentration polarization of the enzyme at the membrane surface.

Packed bed | Packed bed with recycle | Tubular



2. Continuous-flow stirred-tank reactors
   Easy to control pH when necessary
   Colloidal or insoluble substrates can be processed
   Easy replacement of catalyst
   Higher yields for substrates undergoing substrate inhibition.

Fluidised bed



3. Plug-flow reactors
   Higher conversion efficiency (compared to CSTR)
   High yields for substrates undergoing product inhibition

4. Fluidized-bed reactors
   Better heat and mass transfer characteristics
   Freedom from plugging
   Insoluble substrates can be used
   Low pressure drop
   Large power requirement for fluidizing the bed
   Uncertainties in reactor scale-up

tank reactors with ultrafiltration systems, plug flow reactors and fluidised bed reactors. These are illustrated in fig.8.1 and characteristics of each reactor are listed.

Factors influenceing thechoice of reactor type have been summarised by Veith et al and are listed in table 8.5. below.

Table 8.5. Factors influencing the choice of reactor type.

1. Form of immobilised enzyme.
2. Nature of substrate.
3. Operational requirements, e.g. pH control
4. Reaction kinetics.
5. Carrier load capacity.
6. Catalytic surface to reactor volume ratio.
7. Mass transfer characteristic therefore microdiffusional, macrodiffusional efficiences.
8. Ease of replacement, regeneration.
9. Ease of fabrication.
10. Reactor costs.

Table 8.6. Commercial Immobilised Enzyme Reactors

Enzyme	Immobilising Method	Reactor type	Operating Mode	Company	Starting Date
Aminoacylase	Adsorbed	Packed bed	Continuous	Tanabe Seiyaku	1969
Glucose Isomerase	Adsorbed	Packed bed	Continuous	Clinton	1972
	Covalent	Stirred tank	Batch	Novo	1974
	"	Packed bed	Continuous	Novo	1975
Lactase	Entrapped	Stirred tank	Batch	Snam Progetti	1977
Penicillin amidase	Adsorbed	Stirred tank	Batch	Squibb	1966
	Covalent	Stirred tank	Batch	Astra	1973
	Covalent	Stirred tank	Batch	Beecham	1974
	Entrapped	Packed bed	Continuous	Snam Progetti	1975
Alanine dehydrogenase	Enclosure	Ultrafiltration membrane	Continous	Degussa	1985

8.5. Industrial applications

8.5.1. Introduction

Despite the high-level of research activity in university departments and institute laboratories and the progress that was being made, the number of industrial applications of immobilised enzymes was low. (See Table 8.6. above).

All of the developments were initiated by enzyme companies or were developed by enzyme producers. In terms of volume and value only two enzymes are produced in quantities larger than 1 tonne per annum - immobilised glucose isomerase and immobilised penicillin amiclase. In the following section I shall discuss the development of these and several other enzymes of some industrial importance, and identify the factors which contributed to their successful use as industrial catalysts.

8.5.2. Immobilised glucose isomerase

The immobilisation of glucose isomerase was a major development in the catalytic conversion of glucose to fructose syrup. The need to immobilise the glucose isomerase obtained from the Streptomyces microbes was a direct result of technological factors. They all had cost implications for the laboratories and companies concerned in that they were concerned with increasing the efficiency of the process and reduction of capital and operating costs of the technology.[31]

To begin with glucose isomerase is an intracellular enzyme and had to be extracted from the Streptomyces microbes hence it was costly to produce. Takasaki developed a method of

inactivating the activity of other enzyme catalysts present in the cells by heat treating the cells.[32] This procedure obviated the need for expensive purification of the catalyst since it was now possible to use the cells directly. Isomerisation was achieved by adding the enzyme to a solution of glucose syrup and holding the reaction at about 40°C for several hours. Since the equilibrium for the isomerisation of glucose to fructose shifts to produce more fructose with an increase in temperature, use of a high reaction temperature would produce a high product yield and the reaction would proceed much faster.[33] However at elevated temperatures, sugars such as glucose and fructose react with the enzyme proteins to form brown discolourations by the caramel or Maillard reaction, this inactivates the enzyme rather quickly. Using batch processing techniques the highest degree of isomerisation that could be achieved that was consistent with the production of a glucose fructose clear syrup was rather low.[34] Additional costs were also incurred during the batch process since the cellular enzymes had to be removed from the syrup after completion of the process. The fine powder like suspension of enzyme made removal of the enzyme difficult and also added to production costs.

These problems were solved by immobilising the isomerase using dimethyl amino ethyl cellulose.[35] This material absorbed the enzyme by ionic forces; the isomerisation reaction was initially carried out as a batch process but now the enzyme was easily separated from the fructose syrup product by filtration. Immobilisation reduced enzyme costs, to such an extent that it made batch processing feasible on a commercial scale, and led to the

In all cases the mechanical properties of the immobilised enzyme influenced the design of the reactor, and the design of the process.

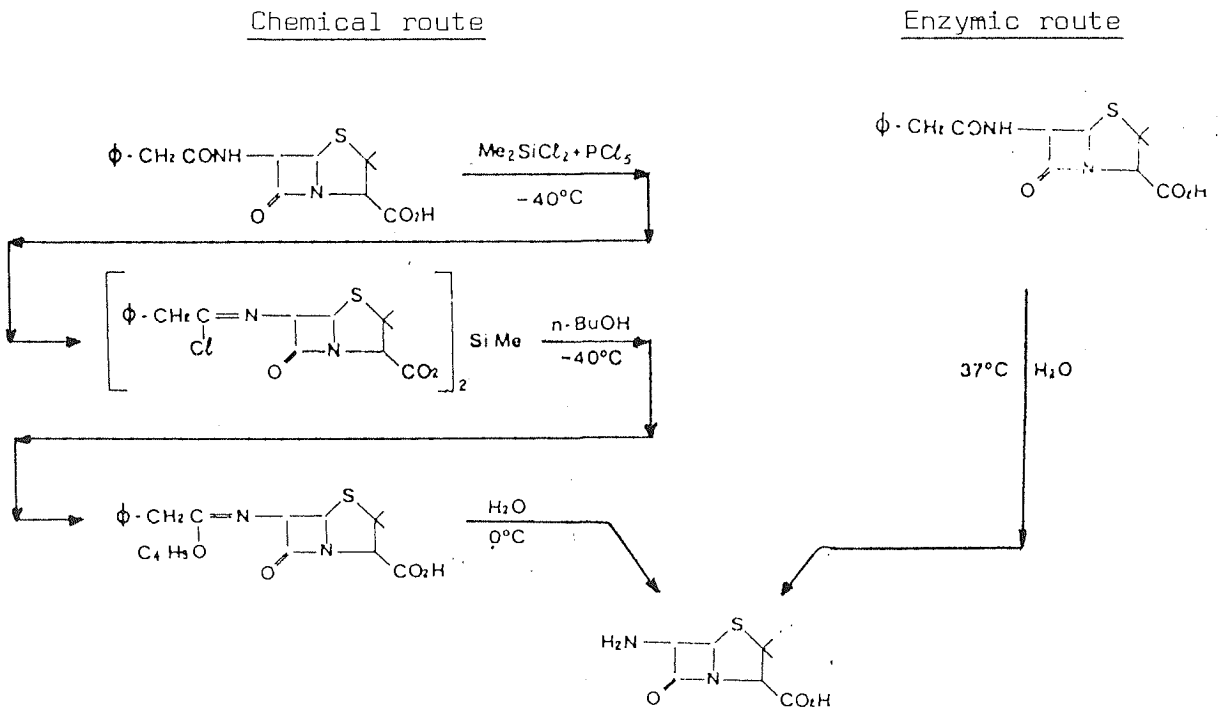
The relative adoption of Novo's immobilised enzyme catalysts by the industry can be attributed to the coincidence of a number of factors :

1. Having the necessary technology available at a time when the directors of the starch processing Corporations could forsee a rapid return on their investment.
2. Having an immobilised enzyme that had overcome most of the technical objections to the process, and which incurred reduced capital and running costs by making the additional outlay on ion exchange columns unnecessary.
3. An escalation of sugar prices during 1974 which made substitution of mid invert sugar by high fructose syrup commercially attractive and created a market for the innovation. (See Chapter 5 above)
4. A good immobilised enzyme product developed to pilot scale, sound technical backup and design engineering.

8.5.3. Immobilised penicillin amidase

The development and immobilisation of the enzyme penicillin amidase for the production of semisynthetic penicillins was initiated in the U.K. by Beechams.

Fig. 8.2. Chemical and enzymic routes for the production of 6-amino-penicillanic acid from benzyl penicillin.



Source : P.Dunnill. Immobilised cell and enzyme technology. Phil.Trans.P.Soc.London. B290, [409] 133, 1980.

production of Isomerose(R) 30 syrups (15% fructose) by Clinton in 1967.(36)

The availability of an immobilised isoglucose catalyst made possible the next development in this technology i.e. continuous processing of fructose syrups by contacting glucose syrups with the immobilised enzyme in an enzyme reactor.

Despite the undoubted technical success of the Clinton's technology, their immobilised enzyme catalyst was not adopted by the industry as a whole. Instead two enzyme producers, Gist-Brocades B.V. and Novo Industri A/S supplied the catalyst for the glucose isomerase plants developed by rival starch processors. In both cases their success is attributable to the development of glucose isomerase enzymes that did not require Cobalt ions as co-factors, which the Streptomyces glucose isomerases needed, and which gave this system a potential cost advantage.

Secondly, the method of immobilisation adopted by Novo Industri A/S was simply to cross link, heat treated cell homogenates of Bacillus coagulans with gluteraldehyde, an easy process that produced flocs of immobilised catalyst with good mechanical properties, able to withstand the pressure within industrial bed reactors. Gist-Brocades N.V. glucose isomerase obtained from Actinoplanes missourensis was also immobilised simply by mixing cell homogenates with gelatine and cross linking the mixture with gluteraldehyde. The immobilisation technology also opened the way for non-starch processes to enter the market, in particular chemical companies such as I.C.I. and Monsanto as well as others like Reynolds Tobacco and Corning Glass.

The opportunity to produce semisynthetic penicillins was created by a market need. After World War II, penicillin had become established as a major broad spectrum antibiotic, but during the 1960's there was considerable concern within the health care sector that strains of microbes were beginning to evolve that were increasingly tolerant to high doses of the antibiotic. Considerable interest was shown by the industry in the chemical modificants of these antibiotics but the processes were difficult to develop. A penicillin amidase absorbed onto bentonite has been reported as being used for the production of 6 amino-penicillionic acid by Squibb.[37] Around 1969 both Beecham and Bayer developed an enzyme route using E.coli penicillin amidase which has a preference to hydrolyse benzyl penicillin under alkaline conditions. The advantages of the enzyme route over the chemical route lies in its marked simplicity.[38] (See Fig.8.2.)

Immobilisation of the enzyme in this case was a key factor in maintaining the necessary purity of the product. Little has been published about the method of immobilisation used but it probably involved a DEAE-cellulose.[39]

In the 1970's this method of immobilisation was abandoned and the producers of semisynthetic penicillins changed to more rigid supports and a more highly bound enzyme. Beecham (UK) chose a polymer methacryl gluteraldehyde support, Bayer (Germany) cyanogen bromide activated dextran and Astra Lakemedal (Sweden) a cyanogen bromide activated Sephadex.[40] The need to retain the enzyme by covalently coupling it to the carrier proved to be important in each case. Typically much of the process details are covered by trade secrecy.

It is generally accepted that the 6 Amino Penicillin Acid (6APA) is produced by these methods by the major pharmaceutical companies engaged in the production of semisynthetic penicillins i.e. Bayer, Gist-Brocades, Beecham, Biochemie, Pfizer and Bristol-Meyers.[41]

Both immobilised glucose isomerase and immobilised penicillin amidase remain as the only two immobilised enzyme catalysts that are produced in quantities of over one tonne per annum.

8.5.4. Immobilised glucoamylase

Glucoamylase is the most widely used enzyme in the U.S. starch industry.[42] The development of an immobilised enzyme would enable savings to be made by replacing the large tanks used to carry out the saccharification of starch into a high D.E. syrup, by the installation of relatively small fixed bed reactors.

Early studies of immobilisation of the enzyme were carried out by Wilson and Lilly (1969)[43] using a triazine method of attachment of the enzyme to a cellulose carrier. O'Neil,[44] discussed in a later report, the pros and cons of both Continuously Stirred Tank Reactor and Packed Bed Reactors for saccharification. The immobilised enzyme used in this case had a low coupling efficiency of only 20-30%, a reduced pH optimum but a five fold increase in its Michaeles constant. Smiley[45] immobilised the enzyme on DEAE cellulose in 1970 and obtained a coupling efficiency to 50%, however the enzyme had a much narrower pH optimum and reduced thermal stability, and was deabsorbed by salt solutions in excess of 0.1M per passing through the reactor.

The current problem concerns the efficiency of conversion. Saccharification using the batch method takes 49-110 hours to complete but produces a syrup with a glucose content of about 95-96%. The highest figures that have been achieved by the immobilised catalyst is 94%. This is significantly lower and of considerable importance to a glucose syrup producer whose business is to convert starch to glucose or other sugars. Adoption of the immobilised glucoamylase technology is however influenced by other factors especially the following:

1. the relative low cost of soluble enzyme
2. the fact that soluble enzyme is simple to use
3. the fact that technology using the soluble enzyme is well tried and tested
4. the lower thermal stability of the soluble enzyme
5. and the fact that the industry has already made its investment in the large saccharification tanks.

Consequently although the potential market for immobilised glucoamylase is very large its introduction does not have the opportunity to produce immediate returns to the investors that glucose isomerase technology had.

Two commercial immobilised glucoamylase preparations are available on the market made by Tate and Lyle (UK) and Novo Industri (Denmark). Both enzymes were developed in response to the anticipated cost reduction that would accompany the use of smaller saccharification tanks by the starch processing industry. Even with eventual development of a technologically superior enzyme catalyst, the adoption of immobilised glucoamylase as a method for

saccharification is likely to continue to grow slowly since it lacks the opportunity for the commercial user to obtain a rapid return on capital which is insufficient to warrant the scrapping of the existing technology.

8.5.5. Immobilised lactase

The reasons for developing an immobilised lactase is to hydryolyse lactose the milk sugar dimer into its monomer glucose and galactose. There are two potential uses for the product:[46]

(i) to make milk and milk products acceptable as a whole food to a larger sector of adult population who are unable to digest cows milk because they lack the necessary enzymes. This would open up a new market for milk products for people from African or Asian extraction or origins.

(ii) to improve the utilisation of whey, a major waste product of the cheese manufacturing process.

The process characteristics of the two processes are remarkably different. A lactose from yeast (Kluyveromyces lactis)[47] is preferred for the processing of milk, where a lactase obtained from the fungus Aspergillus niger is preferred for processing the more acid whey.[48] Unfortunately whole milk is a complex emulsion and contains substances that inactivate the immobilised yeast lactase. Although pilot plants have been developed in several countries by Snam Progetti (Italy); Valeo (Finland);

Corning Glass Works (France and Britain) on the whole, although the technology has proved to be successful, neither of these processes has been more generally adopted.[49]

The only process to be scaled up as a production plant uses the technology developed by Progetti.[50] Their process produces about 10 tonnes of lactase treated milk per day at the Centrale del Latte in Milan.[51] The process has to be run at around 4°C to avoid contamination problems. Despite the technical success of these processes the market for lactase treated milk continues to remain small. The output of the Milan plant satisfies local needs for milk products and is used locally by the ice cream industry since it removes the poorly soluble lactose and therefore eliminates the gritty texture of ice cream. It is also used in products targeted at people who cannot digest lactose.[52]

The application of immobilised lactase to the utilisation of whey has been less successful because whey can be utilised in other ways e.g. as a feed directly, or as a substitute for the production of ethanol.[53] The lactose can also be hydrolysed into glucose and galactose by a chemical process which compete with the enzymic route. For example, acid hydrolysis of lactose to glucose and galactose is very cheap but increases the salt content of the product and reduces it's use as a sweetener in foods: hydrolysis of whey by ion exchange resins, however, is efficient and less troubled by contamination and inactivation than the immobilised lactase method. Even though whey is a waste product its initial high salt content and competition from

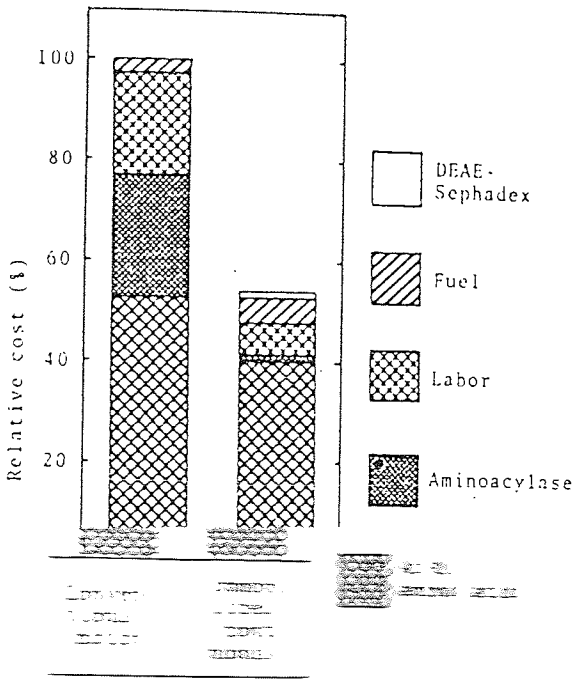
high fructose syrups makes the production of a high quality sweetener based on glucose and galactose unattractive.

8.5.6. Immobilised aminoacylase

Many chemical compounds exist in nature as isomers, chemically identical molecules that are mirror images of one another, and commonly referred to as D and L forms. Most biological systems however use only the L form. Thus L amino acids are used as the building blocks of proteins. Amino acids however can be synthesised by chemical or biological means, depending on economic criteria such as feedstock prices and the complexity of processing.[54] Methionine is one such amino acid which is obtained by a chemical reaction and is therefore made in the D and L forms.[55] In recent years the market for methione has been growing. By far the largest market for methione is its use as a supplement for soya protein stuffs. Although ruminants such beef cattle can utilise both the D and L forms of the amino acid (presumably by the action of their gut flora on the feed), L-methionine is needed to supplement the feeds of pigs and poultry and for therapeutic uses in humans.[56]

It is extremely difficult to separate the D and L isomers by chemical means (Pasteur managed it by picking out crystals of the isomers by hand), yet enzymes can be used to effect the separation efficiently. In a process developed by I.Chibata and Tanabe Seiyaku K.K. (Japan) acetylated D and L methionine is separated by the highly specific action of the enzyme amino-acid acylase on the L-isomer, which produces L-methione and an unaltered acyl-D-methione

Fig.8.3. Comparison of relative cost for industrial production of L-amino acids.



aminoacid. This mixture is then separated by the difference in this solubility of the two compounds. In a batch process, the enzyme amino acylase must be isolated from the L-methione, D-acyl-methionine mixture by adjusting the pH or heat treatment of the reaction's products. The Tanabe Process uses a DEAE sephadex immobilised enzyme. The selection of DEAE sephadex immobilised enzyme was made on the basis of its highest initial activity of any immobilisation system, its high-temp stability, high Michaelis constant as well as long half life. An advantage that an anionicly immobilised catalyst has over other systems of immobilisation, is that it can be periodically regenerated, (by flushing the system with fresh amino acylase).

The adoption of an immobilised catalyst made sense at the time, the process was being developed because of the lower capital and production costs of the continuous system of production. (See Fig.8.3. above).

Tanabe appears to have researched a number of immobilised enzyme catalysts before deciding on the one to use. In most of these cases the inhouse research and development was carried out by enzyme user organisations or enzyme companies. Yet despite their technical success they enjoy only limited commercial success for technical and commercial reasons. It is also apparent that it was only companies already working in a business where enzyme catalysts could be usefully applied and which were involved in supplying enzymes for a particular market who were successful in the development of industrial immobilised enzyme catalysts. Only they were in a position to be able to appreciate and evaluate the research

generated as a consequence of the RANN and SRC Enzyme Initiatives, and to apply it to a commercial situation. See for example a summary of research carried out by the company Tanabe Seiyaku during its research programme concerned with the production of amino acid acylase.[57]

8.6. Summary

1. That several different fields of scientific and technological research have contributed to getting immobilised enzyme research under way.

2. That the technology was tried unsuccessfully during the 1940's and failed because of the weak form of immobilising agent used and lacked biochemical engineering knowhow.

3. That the main development in academia concentrated on covalent coupling, whilst industrial processes favoured milder and cheaper forms of coupling.

4. The lack of an adequate dialogue between the scientists on the one hand and industrialists on the other allowed undirected research to proceed which continued to produce results of dubious commercial value.

5. That commercial criteria helped to focus research on to particular problems that enabled further developments to occur in the science.

6. The importance of commercial design criteria in the development of immobilised enzymes and enzyme reactors.

7. The economic importance of price advantage as a factor influencing diffusion of an immobilised enzyme technology.

8. The importance of real market size as opposed to potential market size in determining the uptake of the technology.

9. The ability of non enzyme companies to enter this area of technology by developing the carriers and immobilising the enzyme to it.

10. The ability of pharmaceutical companies to exploit their fermentation knowhow to develop their own enzyme and immobilise it for inhouse research and development use.

11. The importance of R & D departments within these various organisations to capitalise on and couple with developments that were going on in academic immobilised enzyme research.

12. The danger of linking one centre of excellence with concentrated research facilities to one company, which effectively prevented the diffusion and development of penicillin amidase technology in the U.K.

13. The reluctance of any of the bulk U.K. enzyme producers to become involved in this technology.

14. The importance of knowledge of the specialist market needs which helped the enzyme companies to maintain their dominant position in the industry.

15. The limited political stimulus to scientific and technological developments in the shape of the restricted concern within the health care sector (in the public sector in U.K.) about increasing microbial immunity to penicillin.

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CHAPTER 9 - MODELING INNOVATION PROCESSES

9.1. Introduction

As discussed in Chapter 1, section 2 above, a considerable amount of research has been undertaken and aimed at improving understanding of the innovation process. The studies referred to have covered a range of topics, from descriptions of cases of innovation, to economic factors of production to studies directed towards answering what constitutes a successful innovation in a variety of industries. Having then analysed enzyme innovations at a number of different levels in Chapters 2 to 8 above in order to understand the relationship between science, technology, and the firm we are now in a position to model the innovation process for the purpose of science policy analysis. But before beginning to build a new model of this process I shall critically examine a range of extant models in the literature, which appear to relate to the broad subject area of innovation.

As knowledge and understanding of the innovation process has grown so authors have put forward a number of models of the process. Such models provide us with plans of the key elements of the innovation process and the way they interact. The differences between them are attributable to the analytical framework used by the investigator or investigating group. Despite their differences some of the very simple models have had a disproportionate influence in the way science is perceived by scientists, by industrialists and policymakers alike.

In the main there have been two approaches to modelling innovation. One approach examines the innovative process in terms of organisational or functional aspects of a firm, or industry or industrial

society describing a time determined process as a sequence of events associated loosely or more strongly with one another, each making increasing or decreasing contributions to the process. The other approach concentrates on the mechanisms by which innovations are adopted in society. It being argued that one instance of an invention does not have an impact on the economy or society if it does not become widely adopted. The following section reviews a number of models which have been put forward at various times and are examples of these different approaches. In each case the models vary in the degree of their complexity.

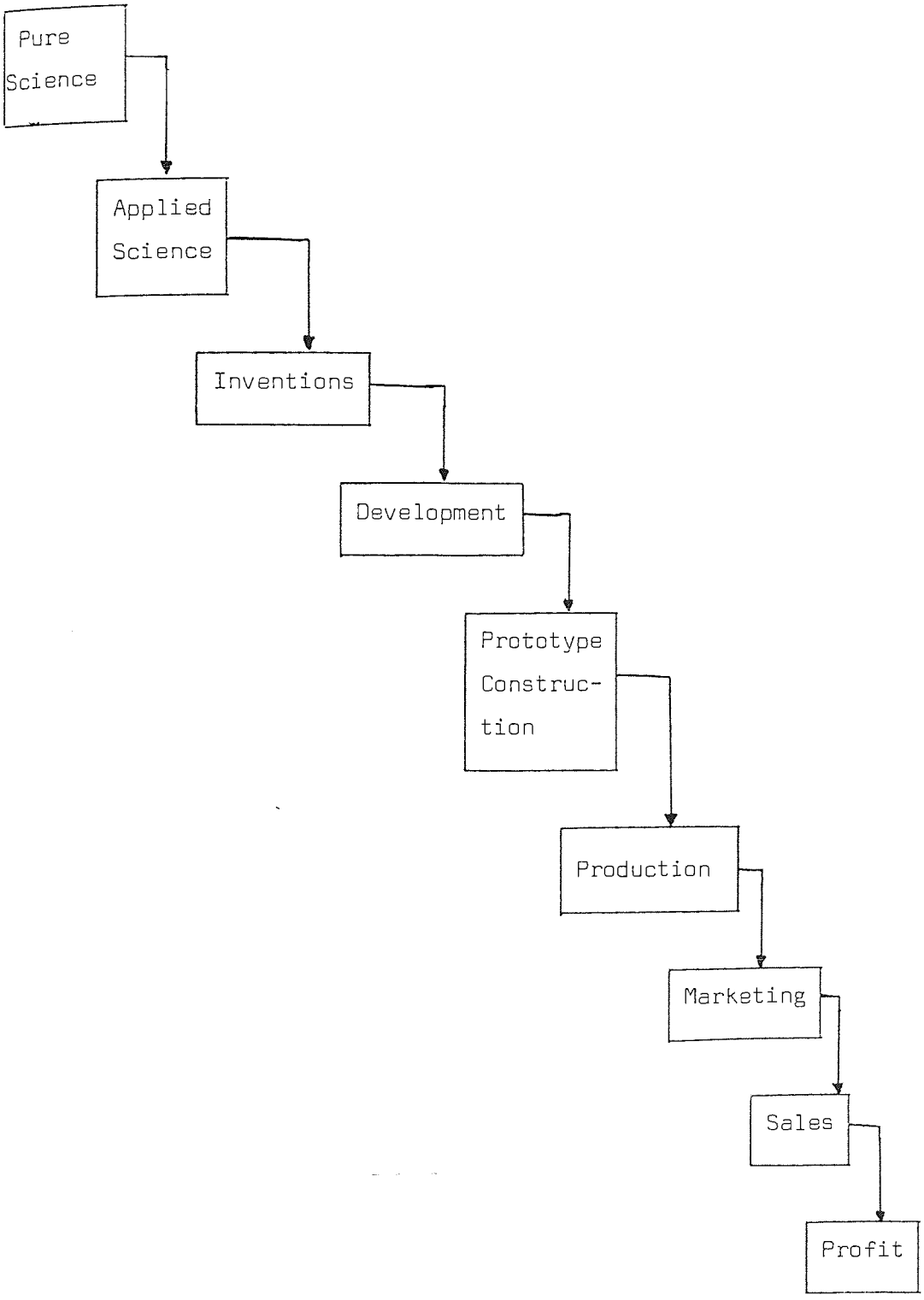
9.2. The organisation or function based models of innovation

9.2.1. Blackett's Science push model

As I have discussed in Chapter 1 World War II had a profound effect on the funding of science and did much to foster the conventional view that scientists brought about many important discoveries and innovations and industry applied them (see Price and Bass,)[1] This being the case it is not surprising therefore that innovation was seen by contemporaries of that period as an orderly process starting with the discovery of new knowledge, and moving through various stages of development to emerge as something is commercially viable. Such a linear process, linking outputs of science with profit, has been put forward by P.M.S. Blackett, a physics Nobel Laureate, and past president of the Royal Society.

In its simplified schematic form, successful technological innovation can be envisaged as a system of sequential steps beginning with pure science and ending with profit. (see Fig.9.1.)

Figure 9.1. Blackett's linear model of innovation



Blackett used this model to develop a case for the state funding of pure research stating that "Clearly the first steps cost money and only the later stages make money."(2)

Criticisms have been levelled at such models of innovations by various authors. Jevons[3] criticizes all linear models of innovation since they advocate a unidirectional flow of knowledge from research which apart from its cultural value relies predominantly on an element of serendipity when producing new ideas which industry can apply. Another difficulty of this model is that technology is seen to draw heavily on science and as Langrish[4] points out and as I discuss in chapter 4 above such a perception of the relationship between science and technology is historically inaccurate. It denies the existence of a relationship where technology leads and science follows. Langrish[5] also states that such a model is implicitly held by those who measure time lags between scientific discoveries and their applications, and by those who make statements such as "foreign industry is better at applying the fruits of domestic science efforts". Yet despite such criticisms these views of science research are commonly held by scientists today. The idea that science pushes is used by them to defend public funded unidirected research.

9.2.2 The Discovery push model

Langrish et al.[6] as a result of a study of innovations within a number of successful companies identified 4 models of innovation :

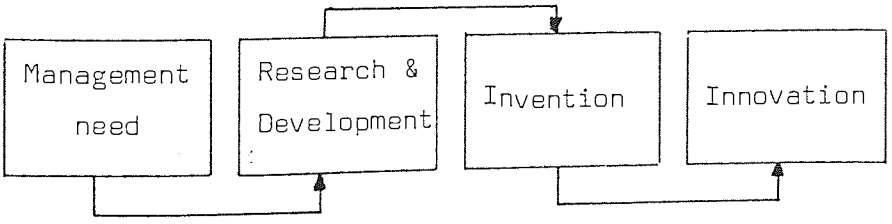
1. Science push } Discovery push
2. Technology push }
3. Market demand
4. Cost reduction induced (Management need)

The science push model has already been discussed. In the technology push case it is difficult to attribute a scientific contribution to a discovery that leads to an innovation. The push appears to come from a technological breakthrough e.g. the Pilkington float glass process. In this model I have grouped both Science and Technology together as instances of discovery push. Such models brings into question the contribution that pure science alone makes to the innovative process. Market demand or investment pull models which I shall discuss in the section 9.2.4.

9.2.3. Management Need Model

Langrish[7] identified the existence of innovations initiated as cost reduction exercises, where new and cheaper processes are introduced stimulated by internal pressures within a company to become more efficient. Such innovations appear to arise in response to management needs, see Fig.9.2., to make the process more efficient and Langrish used the example of a changeover from batch to continuous processing as one such example.

Figure 9.2. Management need model

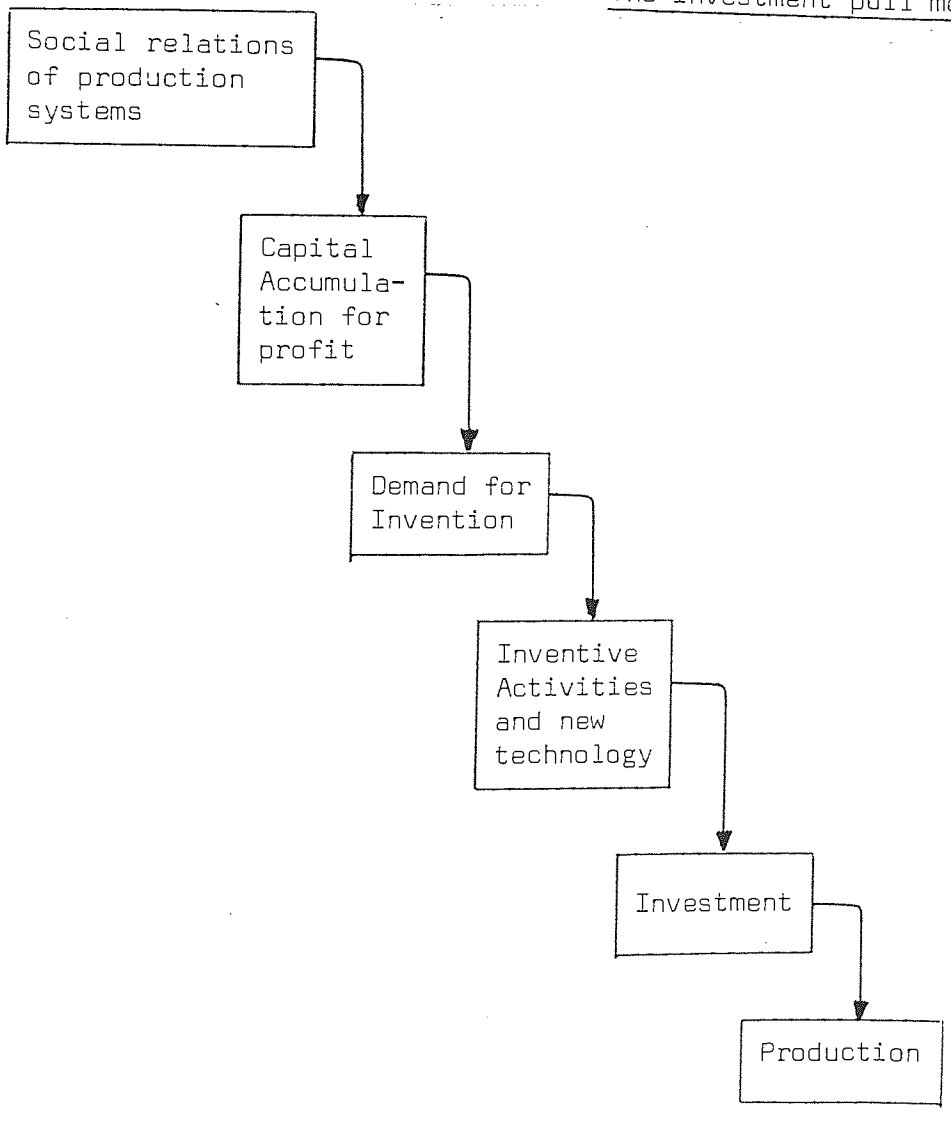


Langrish et al search for the major causes of innovation, give differing results, since different causes are attributable to the different factors in operation and different circumstances.

9.2.4. The investment pull models

Another variation on this theme is the model put forward by B.Hessen[8] who identifies a drive for new business investment as being a major factor influencing innovation. This, in turn creates a demand for invention which science (pure or applied) is stimulated to investigate, explain and lead to innovation. The following depiction is an adapted and augmented version of Speisers model. (see ref. below).

Figure 9.3. The investment pull model



In this model pure science is a weak influence on innovation (see Fig.9.3.) This model is supported by other studies e.g. Speiser,[9] who stresses that in both the case of the telegraph and the steam engine,[10] the science i.e. the telegraph equation and thermodynamics followed at a later date after the innovation. The role that science has to play in these cases reverts from being causal to being largely an effect.[11]

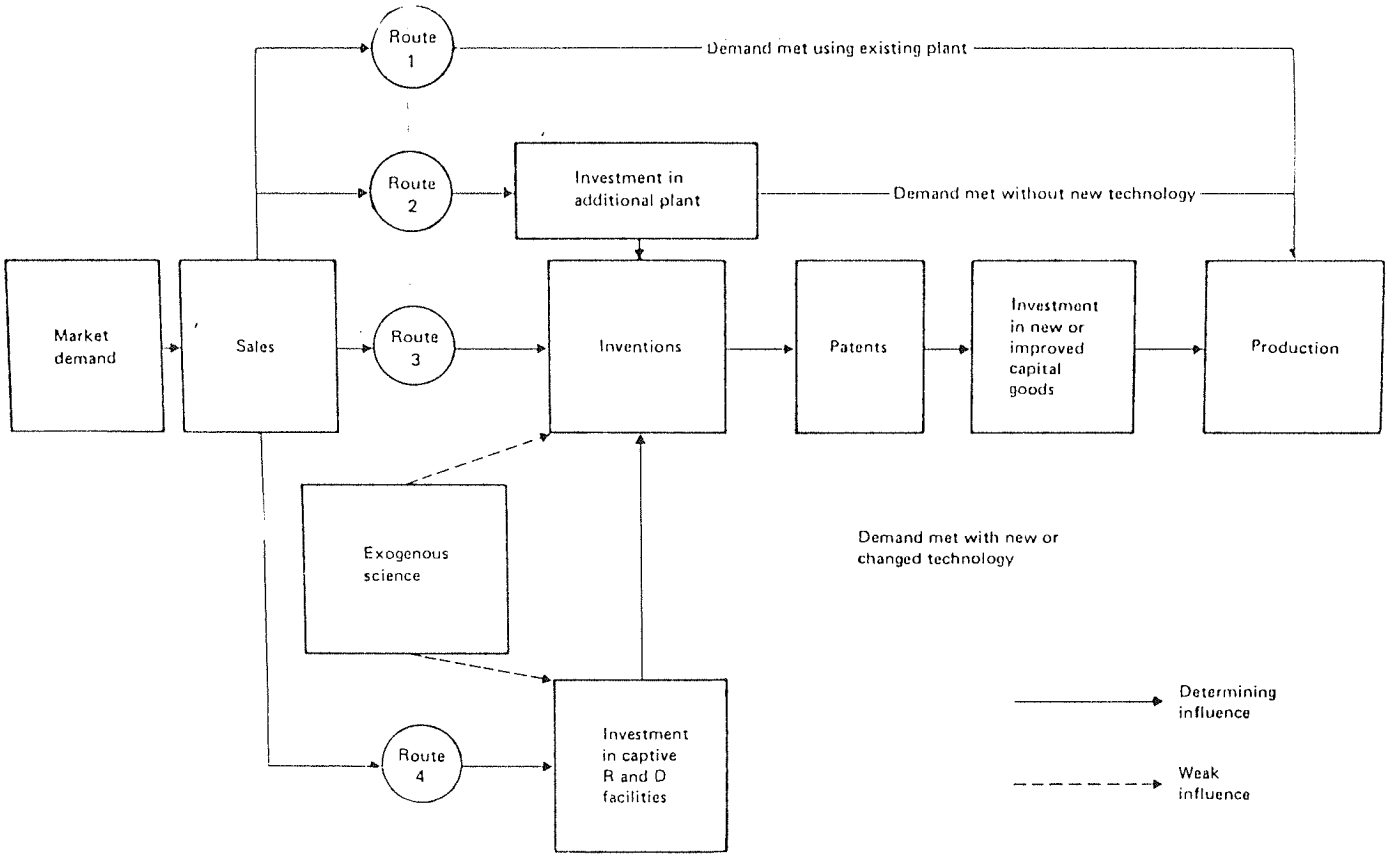
9.2.5. Schmookler's demand led invention model

The next two models have been developed by economists as discussed in Chapter 1. (Section 2) above.

Schmookler produced his model as a result of many years of careful and painstaking analysis of patents data.[12] Schmookler saw science as an exogenous influence in the production of inventions (See Fig.9.4. below). He carefully distinguished between inventions and innovations, in that patent counts are measures of inventive activity and not innovation. Time series analysis showed that investment precedes patenting activity in several cases. Schmookler explained this observation by the fact that inventions are pursued for economic gain, and the amount of investment in turn varies with expected sales. The expected sales are in turn determined by the state of current sales and the size of the market.[13]

Schmookler's model emphasises the fact that basic science may be an important independent influence but he does not see it's production as being demand led. Schmookler also points out that the existence of a demand does not necessarily result in an

Fig. 9.4. Schematic representation of Schmookler's model of demand led invention.



Source : Freeman C. (17)

invention, since it may be accommodated by utilising spare capacity, or by the purchase of additional production capacity. His model also caters for demand stimulated freelance inventions or company stimulated inventions produced by internal or captive research and development (R & D) facilities. This view of the relationship between science and innovation is understandable in terms of Schmookler's perspective on the process and his choice of measure of inventive activity.

It is perhaps worth remembering at this stage that despite its quantitative base, that this snapshot of the process may be distorted in favour of the larger companies in certain industrial sectors. Also Schmookler's analysis was intended to apply only to major innovations, which involve significant shifts to an entirely new production function.

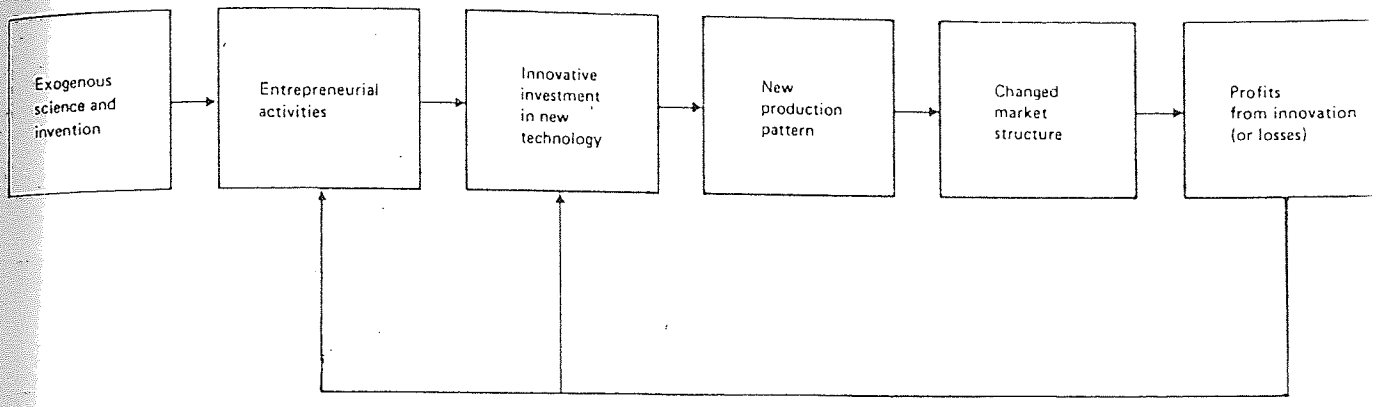
9.2.6. Entrepreneurial innovation : Schumpeter's first model

Another view of demand led innovation has been proposed by Schumpeter.[14] Schumpeter focused his attention on the first production of a new product process or system into a commercial or social activity of a country. This definition of innovation overcomes the problems of Schmookler's approach which counts inventive activity and does not take into account that inventions may not lead to any innovations. Inventions are thus exogenous to Schumpeter's system and he draws sharp boundaries between inventions, innovation and imitation. Within it the role of the entrepreneur is decisive, in that it is through the mediation of an individual that an invention is translated into an innovation.

is translated into an innovation. (see Fig.9.5.). In Schumpeter's model it is innovation counts and innovation frequency that are the true measures of the efficiency of combined scientific and R & D functions.

His observations of business cycles in the economy led to observations of the cyclic nature of innovative frequency, and the discontinuous nature of the innovation process. Schumpeter explains this by showing that great innovations trigger a series of smaller ones and which appear to develop in the wake of the important innovations. Schumpeter's model also postulates a change in the primacy of time between demand pull and science push as an industrial innovation grows to maturity. Exogenous science and technology tend to be important in the early stages of the innovation while demand becomes more important as the innovation becomes established in an industry. However the entrepreneur is the key individual concerned with matching the innovation to market needs. Philips[15] points out that there are two Schumpeterian models, the earlier model reflects the state of development of the nineteenth century enterprises, where entrepreneurial activity is possible because the market for the innovative product or process is still developing.

Figure 9.5. Schematic representation of Schumpeter's model of entrepreneurial innovation.



Source : Freeman, C. (17)

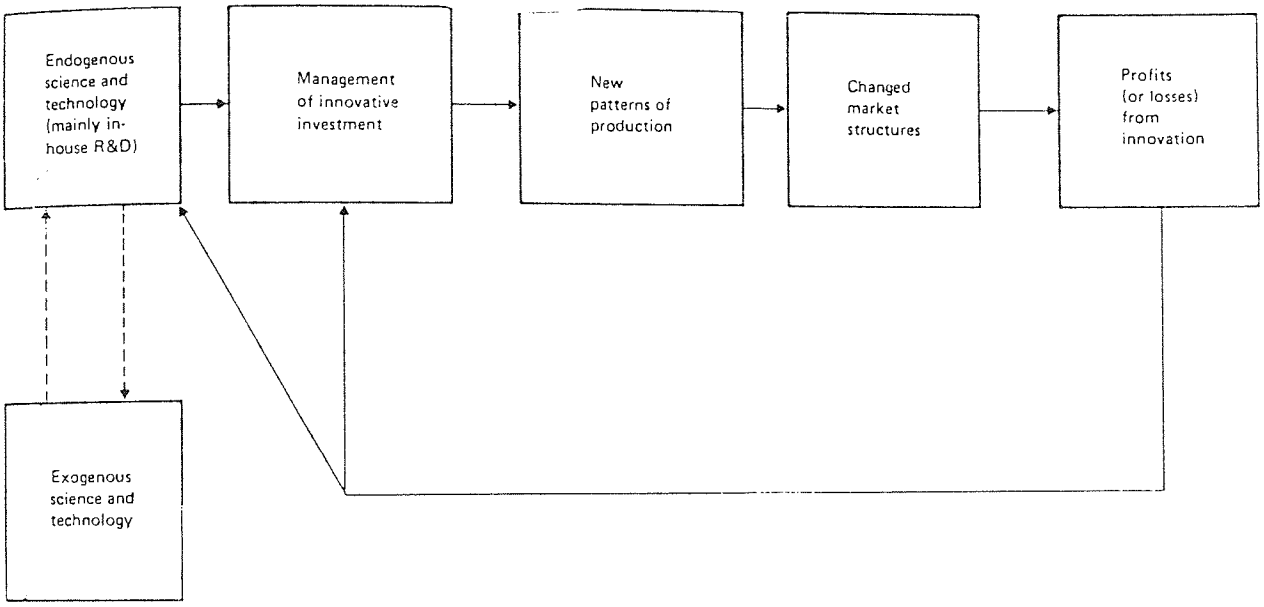
9.2.7. Large firm managed innovation: Schumpeter's Second Model.

Schumpeter's[16] later model reflects the change in the nature of business that occurred during the interwar years with the rapid growth of large corporations, and their ability to manage their own Research and Development functions.

The later Schumpeter model reinforces the view that inventive activity is increasingly controlled by large firms, and ignores the contributions made by smaller organisations. (See Fig.9.6.) However inventive activity itself is never examined as a continuing activity, inventions appear as an isolated phenomenon. The later model reflects the growth in the closer coupling together of science, technology, innovative

investment and the market, by larger organisations.

Figure 9.6. Schematic representation of Schumpeter's model of large-firm managed innovation (Second Model)



Source : Freeman, C. (17)

This view of association is supported by economists such as Galbraith.(18) Both Schmookler's different models and Schumpeter's models stress the importance of a series of stages than an idea must go through before it becomes a commercial reality. Attempts to relate inventive activity, or the feasibility of research or its contribution, becomes progressively more difficult outside the framework of the company and consequently more company orientated characterisation of innovation is needed.(19)

The research findings of Schumpeter and Schmookler indicate that large corporations account for most of the patent activity in most industrial sectors. Such information indicates the importance of looking at patent activity of large firms and corporations when examining the pattern of innovation in industry. In their models, the world of science is perceived to have an external influence.[20] Organisational models however reach the limit of their usefulness when organisational changes become a necessary response as part of the dynamics of the innovative process. Schumpeter is thus forced to discuss two models to explain the different types of innovation at work within a growing economy. Organisational models describe the innovative process as a series of still images, changing with time, and cast shadows of doubt as to whether they in fact are accurate descriptions of the real world. Neither do they consider the element of risk involved in innovation. A selection environment influencing the path of the innovation and which feeds back onto the R & D carried out by the firm is an important conceptual development.

9.2.8. Rothwell & Robertson's Innovation Model

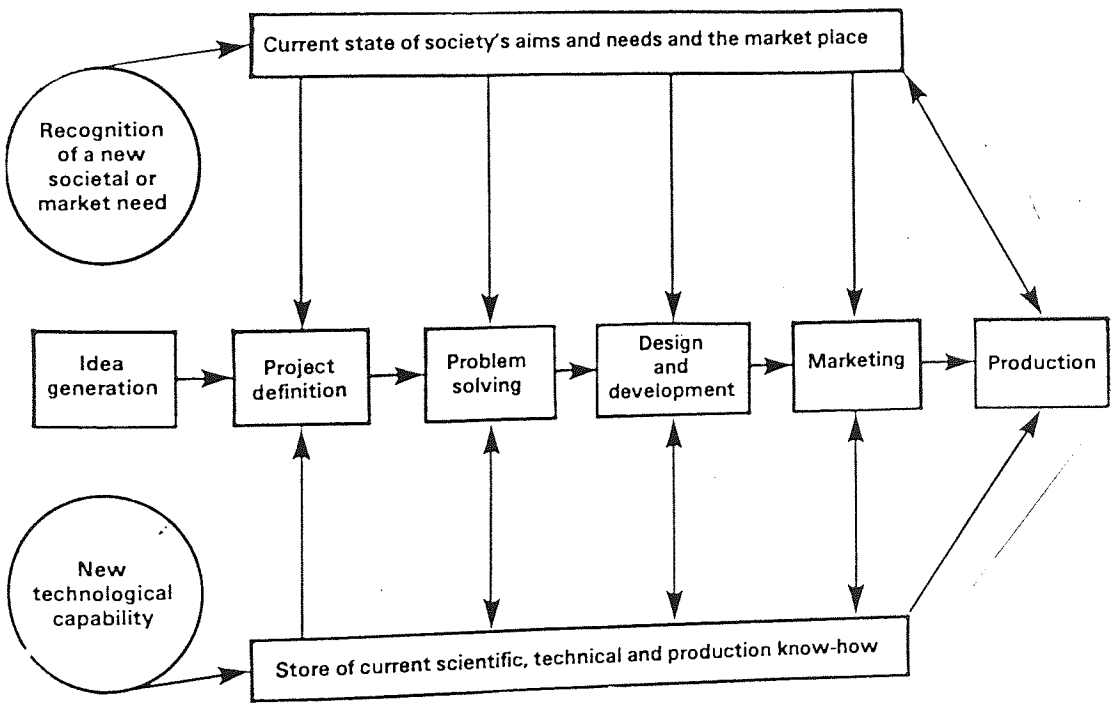
A variation on the organisational approach in which society's aims and needs and the market place influence the process was prepared by Rothwell and Robertson.[21] They examined the role of communications within the context of the innovative process. (See Fig.9.7.) This is a functional model of the innovative process in which the stages are interactive and interdependent, so that the overall pattern of the innovative process can be thought of as a complex of communication paths linking the

various stages with each other. They state that efficient communications are established and maintained both within the organisation and outside of it during the course of the innovation. Thus their model is both functionalist and interactive with respect to both internal and external factors.

Although essentially complete in one respect it is naive in its conceptual representation of the real world.

With time, models of the innovative process have increased in complexity, and since large corporations have a major effect on the economy so the models have gradually become more large corporation orientated.

Figure 9.7. Model for the innovation process.



Source : R.Rothwell, A.B.Robertson.[21] 1973.

The extent to which these models of innovation represent the real world, depends on the state of development of an industrial society. The society must be highly differentiated and the innovative process is institutionalised involving a knowledge producing sector and a market place. However such a model only reflects the innovative process found in large science and technology orientated companies as found in the chemicals, pharmaceuticals, electronics, computers or telecommunications sectors.

The economic importance of large companies and their potential for innovation corroborates such a model. Nevertheless this model is less representative of low technology companies such as leather producers; industries where company sizes have remained small; the new high technology start up, or specialist organisations who fulfil service roles supporting innovation. Thus such a model ignores the complexity of the innovation process, and distorts the picture of the real world.

9.2.9. Management models of innovation

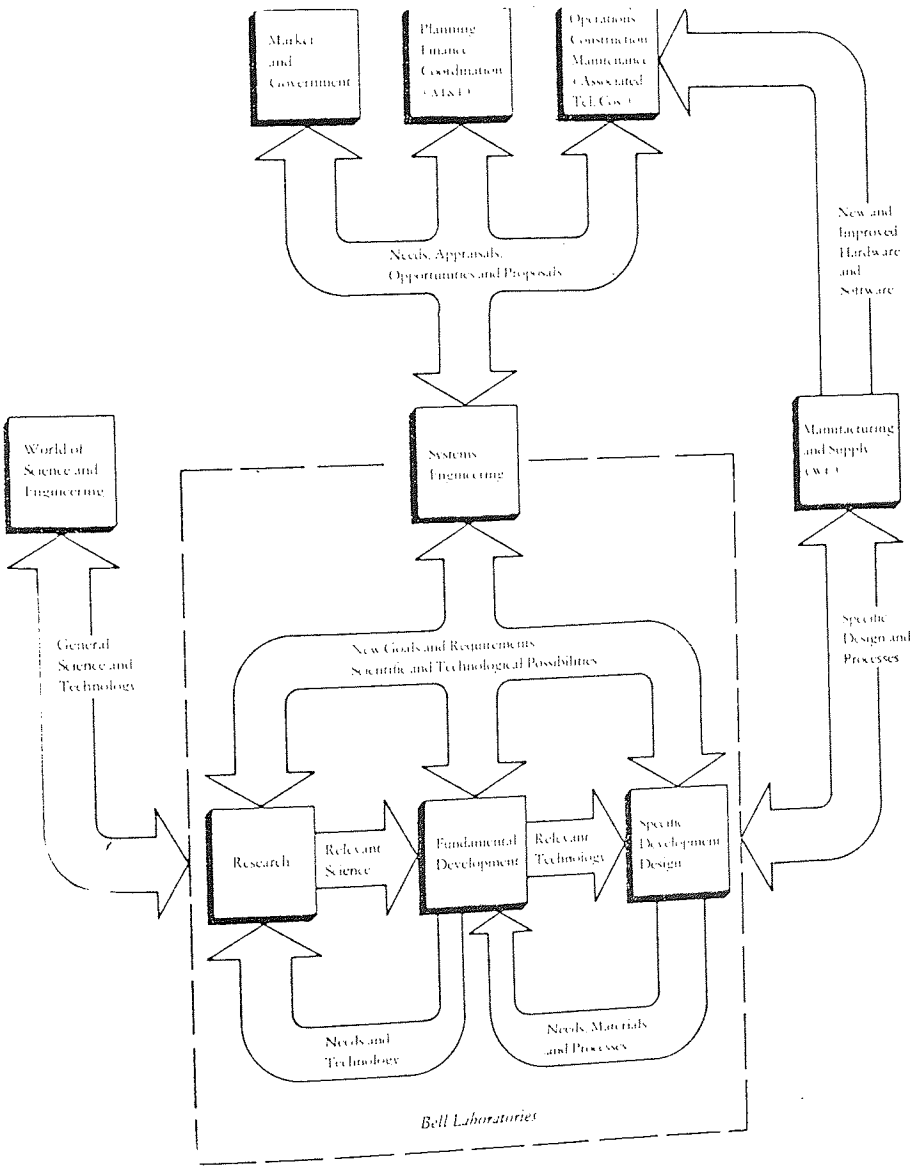
An example of a model of innovation put forward by a large research orientated organisation represents a management perspective of the process. The innovative process is perceived as being essentially similar in almost every industry. The synergistic interactive model proposed by Bode[22] lists elements of technological innovation processes which has proved to be both efficient and very productive at Bell Labs., Western Electric and A.T.& T. The classification is based on a concept of the innovation process conceived by J.H.Morton, a vice president of Bell Laboratories, and reflects a large R & D based company's version of this process. It shows the type of interactions that occur within those organisations. This model emphasises the flow of "intangible engineering information and its understanding" which Bode refers to as 'technical integration'. It cannot be evaluated in purely economic terms such as can the flow of steel or money.

Bode in his treatise emphasises that such a model works well for Bell Laboratories and it is likely that it applies universally. Further such a model emphasises the interaction between and the organisation structures and other factors that affect the innovation.

The model also depicts the relationships between the company and its operating environment. Viewed from a company perspective science is again seen as an external interactive influence on innovation.

Despite Bode's comment that this model could have wider applications it is biased towards an Electronics/Communications Corporation and reflects a highly innovative industry. It is consequently unlikely to apply to less innovative companies in other sectors, for example those concerned with the processing of food, or to smaller companies within the electronics/communications sector.

Figure 9.8. A Management model of innovation



Source : Synergy. Technical integration and Technological innovation in the Bell System. H.W.Bode - Privately Printed, Bell Labs, Murray Hill, New Jersey, 19th Edition.

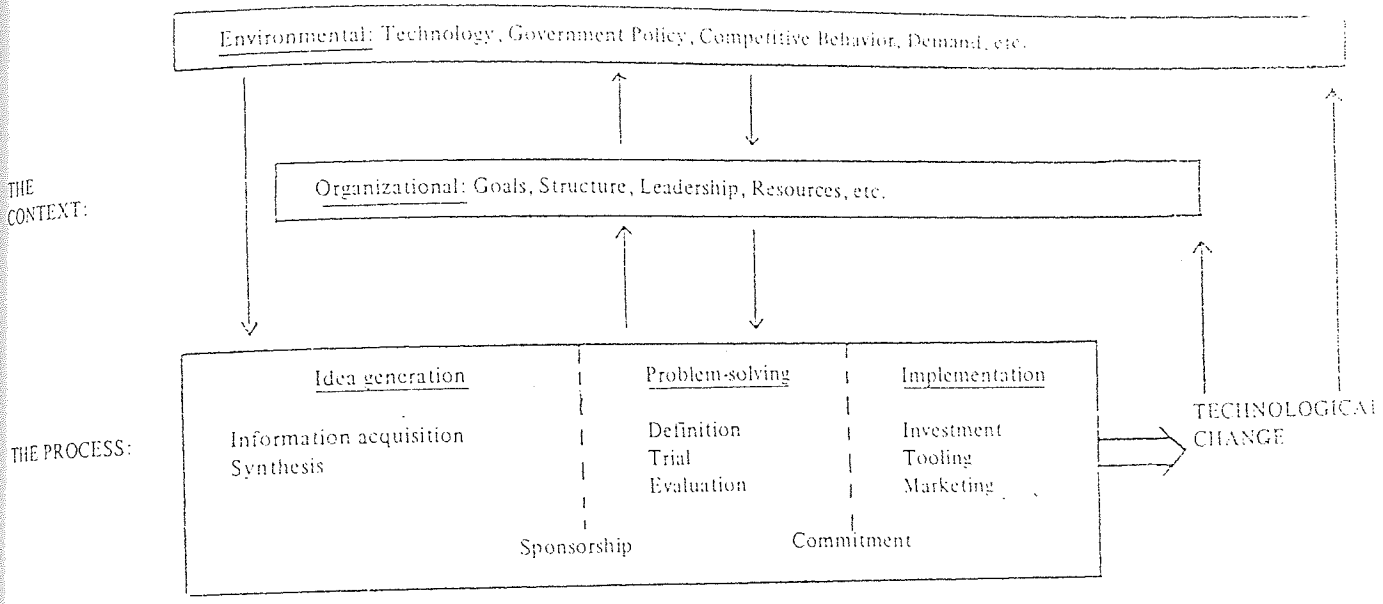
So far I have looked at specific models that have focused on different stages of the innovative process or have situated the innovative process within an organisational framework.

9.2.10. Extended models of innovation

Sociologists such as Cotgrove and Box [23] have also examined the process by which innovations enter society and have distinguished between fundamental, applied research and development and production phases of the process, and have stressed the seemingly linear but interactive appearance of the process. Others, have been less anxious to abstract it from the context in which it occurs.

Rosenbloom for example, attempts only to distinguish the innovative process from the context in which it occurs.[24] He sees technological change as a social process made up of three phases of tasks, the first concerned with idea generation, concentrating on information acquisition and synthesis, the second being the problem solving phase concerned with problem definition, trials and evaluation, and finally the third concerned with implementation of new knowledge gained. (See Fig.9.9). The initial context in which this process operates he defines in terms of organisations, and the organisations' environment. Although each organisation has its own goals structure, leadership and resources are organised to promote its interaction with the innovative process, it interacts with its wider environment, i.e., of Government Policy, market characteristics such as demand and competition, and the availability of the technology. Baker et al.[25] agree with Rosenbloom's findings and state that in addition the literature provides for an extended model including information flow of technological and market need capabilities.

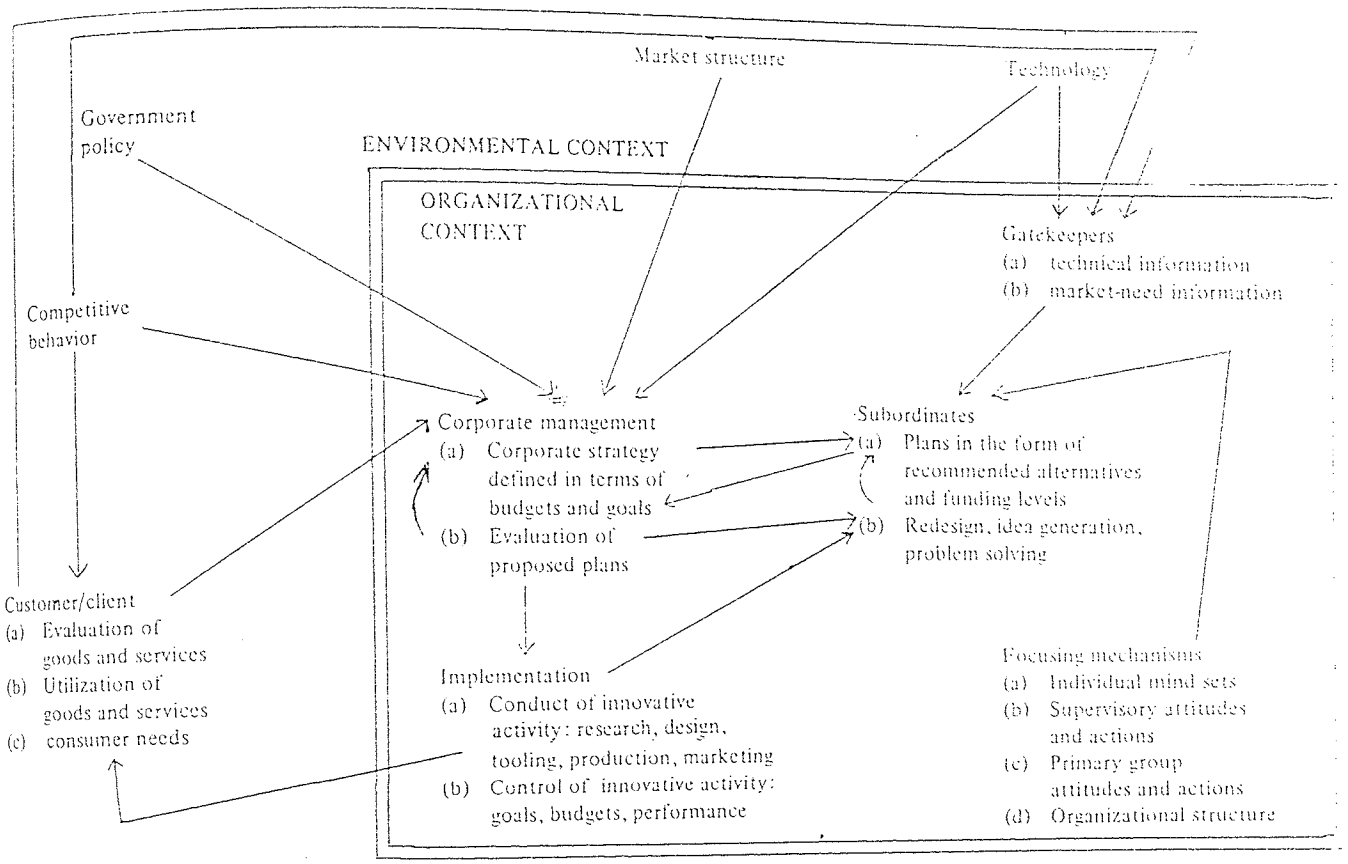
Figure 9.9 Rosenbloom's conceptual framework



Source : Baker, N.R. and Sweeney, D.J.[26]

Baker and Sweeney[26] have extended Rosenbloom's model (See Fig.9.10) of a corporate strategy that cuts across both external and internal boundaries to include other factors and interrelationships identified in the literature. More explicitly the implementation phase of organised innovation, the mechanism of focusing on the process, gatekeepers and exogenous input in the form of government policy, market structure, compatible behaviour, customer and clients needs.

Figure 9.10 An expanded conceptual framework: ——→ information, material flow; organisational boundary.



Source : Baker, N.R., and Sweeney, D.J. [26]

Hitherto all the models described have been static and time dependent since they concentrated on the organisation aspects or social organisation of innovation. We now examine more diachronic models of the innovation process allowing for temporal factors and change.

9.2.11. Representations of the Innovative Process

Tisdell[27] combined research findings into a composite model, (see Fig.9.11). It begins with an invention, which he defined as the time when the technical possibility of a new process or product is worked out and is proven. At this stage a patent application may be lodged. Innovation is the first commercial use of the invention. Development work has to be done in order that mass production or use of the invention can be made. Entrepreneurial ability and marketing acumen are necessary to ensure the commercial success of the innovation.

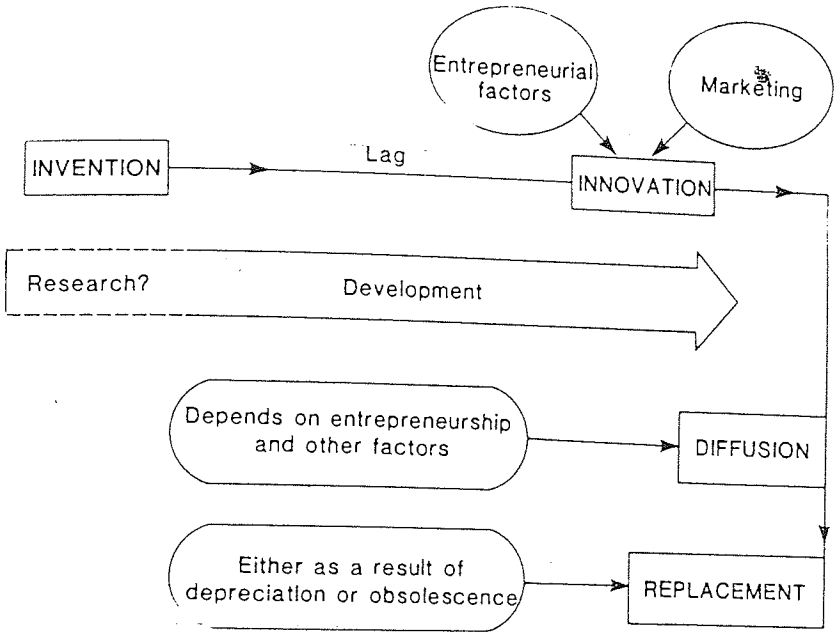
Once a successful innovation has occurred its spread depends upon many factors affecting diffusion. In the event of diffusion early adopters may have to replace equipment or that the innovation may be superseded so that they may need to replace it.

However this model describes the idealised version of the forces that mould product or process innovations and it does little to identify the role of science, pure or applied.

9.2.12. A Dynamic Model of Process and Product Innovation

Utterback and Abernathy[28] proposed a dynamic model of the innovative process describing major innovations which reflects the change in rate of innovative activity over time. Their model incorporates some of the organisational aspects of the various models already discussed. It incorporates the firms environment which they see as a determinant of the innovative process

Figure 9.11. Stages in the development and use of new technology.



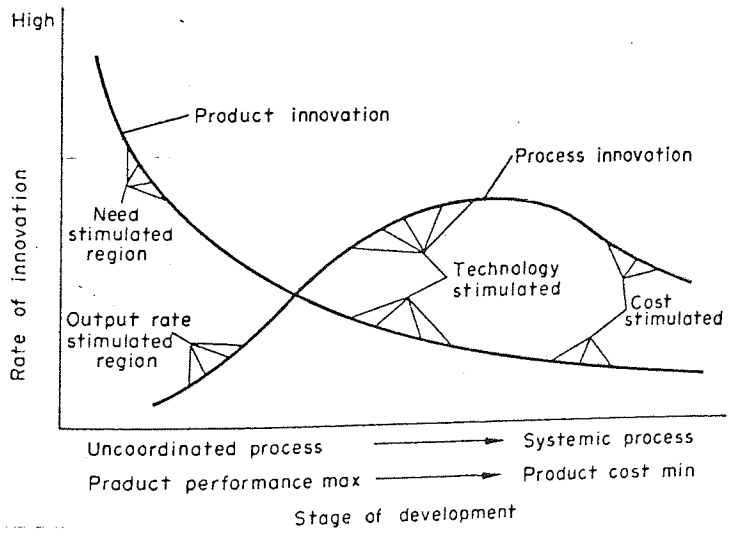
Source : Tisdell, C.A.,[27]

directing the strategy that the firm adopts to compete with other firms or to grow with the increasing maturity of an industry. Their model distinguishes between process innovations and product innovations by the change in the rate of innovative activity over time. They define a process innovation as anything that is used to make a product or service that is sold as a good. During the early life of a process innovation frequent improvements are made which enable it to expand into a growing market. Usually early process are

characterised by manual operations, that are unstandardised, the system is unco-ordinated and inefficient of necessity since it has to have a lot of flexibility: as the innovation matures, i.e. it has sufficient sales volume and stable product designs, production needs become more predictable, price competition becomes more intense, hence production becomes more streamlined, more formal in operation controls and segmental developments.

As the process becomes well integrated selective improvements become more difficult so that minor changes become costly, because changes now may require alterations to other elements of the process and may in turn affect the products overall design.

Figure 9.12. A Dynamic Model of Process and Product Innovation (28)



Source : Omega, Vol.3, No.6. p.645.

Product innovations on the other hand are associated with a new technology or combination of technologies introduced commercially to meet a user of market need. In the initial phase the competitive performance of the product is of paramount importance. (Innovations are often made by identifying relevant product requirements, i.e. are needs stimulated). This phase has the most uncertainty associated with it hence greatest innovative activity.

As experience of the product is gained by both producers and users, market uncertainty is reduced. Some product designers begin to dominate, as uncertainty in the market is reduced which enables product designs to make use of more costly and more advanced technology as a source offurther product innovation. Competition leads to measured product variation and also stimulates process innovations. Finally as product competition becomes cost-centred so product variety becomes reduced and products become standardised.

Utterbach and Abernathy's model is essentially descriptive but takes into account the natural changes in the loci of process and product innovation with time.

Nelson and Winter[29] develop the idea of natural trajectories of technical change in order to describe the pattern of invention and innovation. They refer to an internal momentum, the direction and course the innovations follow and put forward the idea that innovations have their own technological trajectories. Once again very interesting ideas and observations which are exceedingly difficult to model and quantify.

9.3. Diffusion models

9.3.1. Introduction

A logical extension of more extended models of innovation would be to consider the way innovations are adopted by an industry. Such studies have led to the development of 'Diffusion Models' of innovations which cocentrate on characterising and defining the process by which innovations are adopted, and on the factors that determine various aspects of the process.

Mansfield,[30] whose contribution has dominated this area of research, draws distinctions between the following :

(i) imitation or interfirm diffusions, or the spread of the innovation within individual firms within any industry

(ii) intra-firm diffusion or the spread of innovations within individual firms

(iii) overall diffusion or the spread of innovation throughout industry as a whole.

In this thesis, however, I will concentrate on interfirm diffusions since the subject of any thesis concerns the spread of ideas within enzyme science, enzyme technology and the enzyme industry.

The study of diffusion is not exclusive to economics as an academic discipline, but includes the spread of new drugs, the study of the diffusion of new drugs by medical sociologists, new teaching methods, by educationalists and even the spread of cast iron cooking pots by industrial archeologists.

9.3.2. The mathematical theory of epidemics and the logistic curve

All these disciplines in turn draw heavily on the classical mathematical theory developed to account for the spread of diseases. Thus the approach adopted by many workers, notably Mansfield above, involved a two stage analysis of the problem - the first involving curve fitting and the second, estimating slope parameters, etc. which are explained in terms of the characteristics of the industries and innovations concerned.

Mansfield[30] has shown that the use of the logistic curve fits the diffusion data surprisingly well, that the coefficients describing the profitability of installing an innovation relative to that of alternative investment, and the size of the investment outlay needed to install the innovation as a proportion of the average total assets of firms in the industry have the expected signs. However these results must be judged only on the basis of a small sample size of 12 observations used by Mansfield.

Griliches[31] new classical study of the spread of hybrid corn in 31 different states between 1932 and 1956, has a much larger sample size. Again he found that a logistic curve fitted the diffusion data very well, and proceeded to analyse factors affecting this phenomenon. These results have been interpreted by Rosenberg[32] as supporting Mansfield's findings on the importance of the profitability as perceived by firms when installing this innovation relative to the profitability of making an alternative investment.

9.3.3. The stock adjustment models

Use of stock adjustment models in the economic analysis of investment behaviour is becoming more common. Studies by Chow[33] and Stoneman[34] have shown that growth in product usages in any time period is proportional to the extent to which the actual stock at the beginning of the period falls short of the equilibrium stock value.

The Gompertz curve which is a skewed S-shaped growth curve with a point of inflexion value of 0.37 as opposed to that of the logistic curves values of 0.5 appears to fit the diffusion data better. However, this approach does not differentiate between interfirm and intrafirm diffusion since the basis for this curve is the measure of the diffusion by the level of stock, and hence does not differentiate between new users or existing users opting to increase the level of their existing stock.

9.3.4. The vintage model

This model has been put forward by Salter.[35] He assumes that new capital investment in new technology is embodied in new capital equipment so that gross investment is a measure of the process of diffusion. He also states that in this model old equipment is only replaced or scrapped when its operating costs exceed the rents it earns, and that new equipment is installed if its total costs are covered by the revenues it earns. Thus the appearance of a new innovation will make it more profitable to immediately replace some of the existing technology. Some

of the more efficient old equipment will still remain, however, because its operating costs are lower than the total costs of the new innovation.

Resistance to diffusion is therefore accounted for by the rate of depreciation of plant. In the U.K. most companies operate a 5 year pay off period for plant, which represents a measure of the fraction in the adjustment process. The age of the equipment and also the capital outlay for new equipment will determine in part the rate of adoption of innovations.

Other studies have identified still other elements that influence diffusion processes. Rogers[36] points out that adopter categories shift over time from one category to another and that laggards are most likely to drop out of the system.

Rogers and Shoemaker[37] proposed a model of the innovation decision process which has 4 elements :

1. Knowledge of an innovation's existence
2. Persuasion where the individual forms an opinion about that innovation
3. Decision where the individual begins the process of adoption or rejects
4. Confirmation where the individual evaluates his decisions and reassesses his earlier decision in the light of conflicting information reaching him about the innovation.

The model contains 3 major divisions (1) antecedents, (2) processes and (3) consequences.

On the question of needs or awareness, Rogers and Shoemaker point out that innovation can lead to needs and vice versa (p.105). In addition they distinguish between the knowledge of the innovation. However the long range competence of individuals to judge future innovations without principles of knowledge is facilitated by principles of knowhow.

Other characteristics such as the compatibility of the innovation with existing technology have had to be considered. Complexity of the innovation as perceived by the adopter is also important, as is the ability of the innovation to be tried in isolation with minimum disruption to the existing system of production.

9.4. Overview Discussion of Models

9.4.1. Introduction

The purpose of modeling is to represent the real or future system in order to predict how a system will behave under widely varying inputs. The subject of this thesis concerns the coupling of knowledge between science technology and industry and in this attempts to examine and predict the outcome of various policy strategies in the growth of enzyme technology in the U.K. It may be also possible to abstract these findings and discuss the policy for biotechnology in general.

Making a choice between the various models proves difficult since it is possible to associate with every model examples of enzyme innovations that will fit the model in question. For example from the HFCS case study (see Chapter 5 above) it is

possible to relate the pattern of innovation to that of Schumpeter's Model II (discussed above), and the HFCS innovation of Clinton Corn Processing Corporation. From the case of washing powder enzymes (see Chapter 6 above) to interpret the activities by Novo as fitting within Rosenbloom's conceptual framework model discussed above. In fact the simpler models appear to be in effect special cases of what appears to be a much more complex matrix of interactions. In the past the debate on the nature of innovation has focused on certain dialectics and much effort has gone into resolving these issues. Having put together a unique body of data about the nature of enzyme innovation, this thesis presents an opportunity to discuss some of these issues. I shall restrict this discussion to the following :

1. Which factors are more important for innovation, scientific discoveries or market demand (the science push versus the market pull controversy)?
2. Are large firms better at innovating than small firms?
3. Is the role of science exogenous or endogenous?
4. How crucial is the role of the entrepreneur?
5. How important is it to have an R & D department within a firm?
6. How important is the existence of an institutional framework for innovation?
7. How important is it to have a compatible product?
8. How important is it to have compatible technology?

There are other questions but again time constraints prevent me from examining other issues further.

9.4.2. The relationship between science and the market

This strictly throws some light on this debate. It is perhaps worth stressing that in Chapter 4 above (see Conclusions) that to proceed innovation does not need science, in fact it was the problems met by the need to increase production that appears more likely to have helped stimulate the development of science.

Once the technology has begun then developments in science make it possible to develop related technologies more quickly by providing a theoretical and experimental framework. However new technologies will not develop until there is a sufficiently large market to warrant their creation e.g. the development of pectinase enzymes discussed in Chapter 4 above.

Science provides a useful international public platform for the rapid diffusion of new knowledge, e.g. the role of R.J. Roberts and Cold Spring Harbour Laboratories mentioned in Chapter 7 (Conclusions) as point number 3. If the market demand is high then the technologically useful aspects of science will be exploited in preference to other scientific endeavours. Science thus becomes distorted by new discoveries. The underlying basic science associated with these discoveries consequently needs time to catch up, e.g. the case of restriction nuclease enzymes discussed in Chapter 5 above.

It is worth pointing out, however, that the level of market demand for the product may exist in advance of the technological

expertise and technical competence or scientific level of understanding, e.g. the case of immobilised invertase, discussed in Chapter 8 above. Failure to bring a successful innovation to fruition in this case is due to the lack of scientific understanding.

Mansfield's observation that it is the relative price advantage which appears to determine the rate of diffusion of the technology also holds true for, e.g. the diffusion of immobilised glucose isomerase enzymes and of immobilised amyloglucosidase enzymes discussed in Chapters 5 and 8 above.

There is evidence that the lack of knowledge of a market and customer needs can effectively be a major barrier to innovation, e.g. see Chapter 8 above.

Science however presents an opportunity for large firms to cross product sector barriers, e.g. the immobilisation of enzymes created an opportunity for chemical companies to enter production of chemical supports and of the immobilised enzyme itself, e.g. Corning Glass Works (see Chapter 5 above).

To conclude therefore it appears inappropriate to talk of science push or demand pull components of innovation independently of one another since both elements must be present for innovations to take place. It would be more appropriate to talk of a coupling between these and other important components when analysing the innovation process.

9.4.3. Firm size, invention and innovation

Freeman[38] discusses reliable and consistent evidence in the O.E.C.D. area which shows that forty of the largest firms accounted for more than half of all industrial R & D, and that small firms that carry out R & D are likely to fall into one of three categories, i.e.

1. firms which have just begun to develop or exploit a new invention,
2. high specialised firms trading on their particular expertise and
3. firms struggling to survive in industries in which new product competition makes R & D necessary.

Schmookler[39] has presented convincing patent data statistics to show that small firms have a higher propensity to patent hence to invent. Jewkes et al.[40] note however, that the ability to invent does not necessarily always lead to innovation and from their study show that well over half of the innovations made were developed by large firms.

In the enzyme industry during 1970-79 this study draws attention to the importance of large firms which act as major sources of enzyme invention. (see Chapter 3 above), especially tables showing company sizes.

This study also shows that small firms have an important function as innovators, where they are responsible in developing new markets with a high risk value, or markets with a small size.

It seems from our study that small firms are important vehicles for the rapid translation of new scientific knowledge into the market. But here the entrepreneur's role is vital in that it involves the coupling of scientific and technical knowhow with market knowledge and business sense. This coupling of scientific knowledge with market and business sense can be embodied in one individual e.g. the entrepreneur Takamine, or in a partnership, e.g. Rohm and Haas as discussed in Chapter 4 above. In certain small specialist markets, patent protection does not appear to be an important factor in facilitating innovation e.g. the case of restriction endonucleases, their production does not appear to be covered by patents, technical knowhow, processing skill and production and distribution for a discerning market are sufficient barriers to keep other competitors out. (see Chapter 7 above).

9.4.4. Is the role of science endogenous or exogenous to innovation?

This question of endogenous/exogenous science can be discussed in terms of Schumpeter's first and second models of innovation. In his first model Schumpeter places science as endogenous to the process of innovation, reflecting its important position as initiator of commercially useful knowledge. Schumpeter's second model reflects the rise of large firms with inhouse research and development facilities, which then pick and choose appropriate pieces of scientific information as and when they are needed. This is because in larger organisations the contribution that pure scientific research makes appears often to be of secondary importance. Inhouse research is targeted on projects specific to the

companies needs or to meeting the needs of new products. Any broad theoretical knowledge or recent scientific discoveries can be picked up by applied research workers whose task is to screen various lines of investigation, e.g. that of work in the public sector and that of applied research by rival companies that may be relevant to the solution of the company's immediate problem, e.g. HFCS production and the role of Clinton Corn Processing Corporation, or the role of Novo in providing the enzyme and design engineering support. (see Chapter 5 above). For smaller new start up companies science is endogenous as indicated in Section 9.4.3. above.

9.4.5. How crucial is the role of the entrepreneur?

The need for individuals with entrepreneurial ability is seen to be at the centre of economic growth and development. However with the increase in firm size the role of the entrepreneur has been incorporated into the company organisation and is associated with its marketing and research and development functions. Historically, the great entrepreneurs are associated with the industrial revolution before firms began to grow in size. From this study it is also interesting to point out that the incidence of more recent entrepreneurs coincides with a small but growing market need and a new opportunity to satisfy that need through recent developments in science, e.g. as in the case of the market demand for restriction endonuclease and the sources of their supply. (see Chapter 7 above).

This study shows also that entrepreneurs still arise from

two diverse backgrounds, they either have a knowledge of the market or have a scientific background and are able to make the product in demand and very rarely are the two combined in one person.

9.4.6. How important is it to have an R & D department within a firm?

Although most enzyme producing firms have an R & D department, it is interesting to note that the largest producer of high fructose syrup merely concentrates on market dominance through high volume production. The absence of an R & D department within this organisation did not prevent the company buying in the technology once it had been proven. Clearly such a strategy initially prevents the firm from exploiting small to medium specialised product markets. The presence of an R & D department, however, enabled firms like Clinton, (see Chapter 5 above), Rohm and Haas, (see Chapter 4 above), Novo (see Chapter 5 above) and Gist-Brocades (see Chapter 5 above) to develop a new product and to supply the market ahead of the competition.

9.4.7. How important is it to have an institutional framework for innovation?

In Chapter 1 above I stated that one of the criticisms levelled against the U.K. concerns the inability to capitalise on the good ideas emanating from science. There is very little evidence provided by this thesis for the usefulness of government-sponsored agencies in initiating innovations, or for their role in enhancing or bringing about science-based inventions into innovations. (see Chapter 3 above for the role of the N.R.D.C.)

In Chapter 4 however, it is interesting to note the opposite effect government action has on innovations in times of war. The difference appears to be in the way that government spending provides a unique market open to home industries and that of its allies. Which brings into question the rationale of attempting to bring about a coupling of science based research via an "honest broker" system such as the N.R.D.C., the N.E.B. or B.T.G. when couplings between the science and the market have not been forged in the past. Furthermore, there is little evidence from our study of an effect of legislative action either stimulating or inhibiting innovation although the information collected does not necessarily lend itself to an analysis of this problem.

9.4.8. The importance of a compatible product?

This study highlights the fact that successful major innovations are improved versions of a previous compatible product. (see for example glucose isomerase discussed in Chapter 5 above, or the early hydrolase enzymes discussed in Chapter 4 above). Several other observations are worth noting with regard to compatibility. The importance of taking into consideration social as well as political factors when assessing markets, e.g. the consumer backlash and the problems it caused for the diffusion of washing powder enzyme technology. Other aspects of the diffusion of enzyme technology also become apparent. Once established as an enzyme producer, many new companies then proceeded to diversify their production base into other enzymic and non-enzymic products. However, larger firms tend to diversify only if markets are sufficiently larger for compatible products

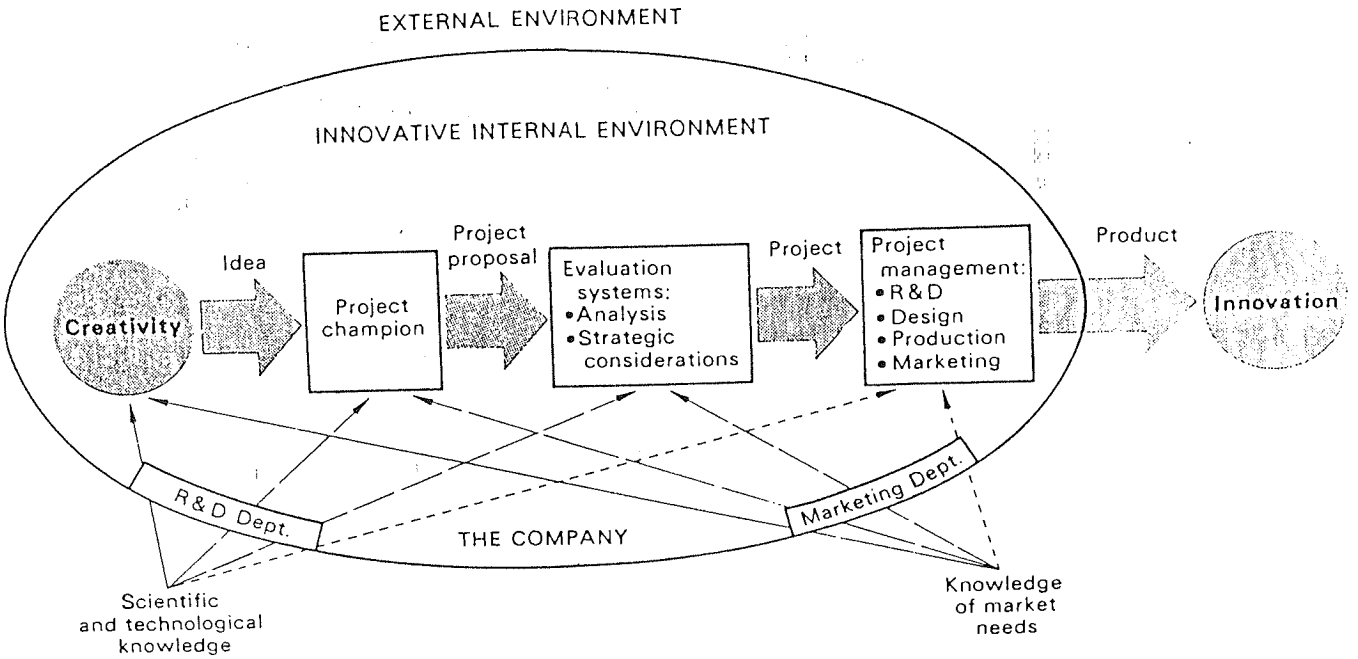
and therefore invest in the innovation. Innovations based on a knowledge of enzymes such as glucose isomerase, glucose oxidase, microbial rennets and microbial proteases were all developed by large companies only when they identified a sufficiently large market need and only then did they proceed to develop it by carrying out an inhouse programme of research, drawing on any developments in pure science as and when needed.

.5. A proposed model of innovation for policy analysis

Souder and Rubenstein[41] state that the decision to innovate is a complex one and involves an interaction between several groups of factors.

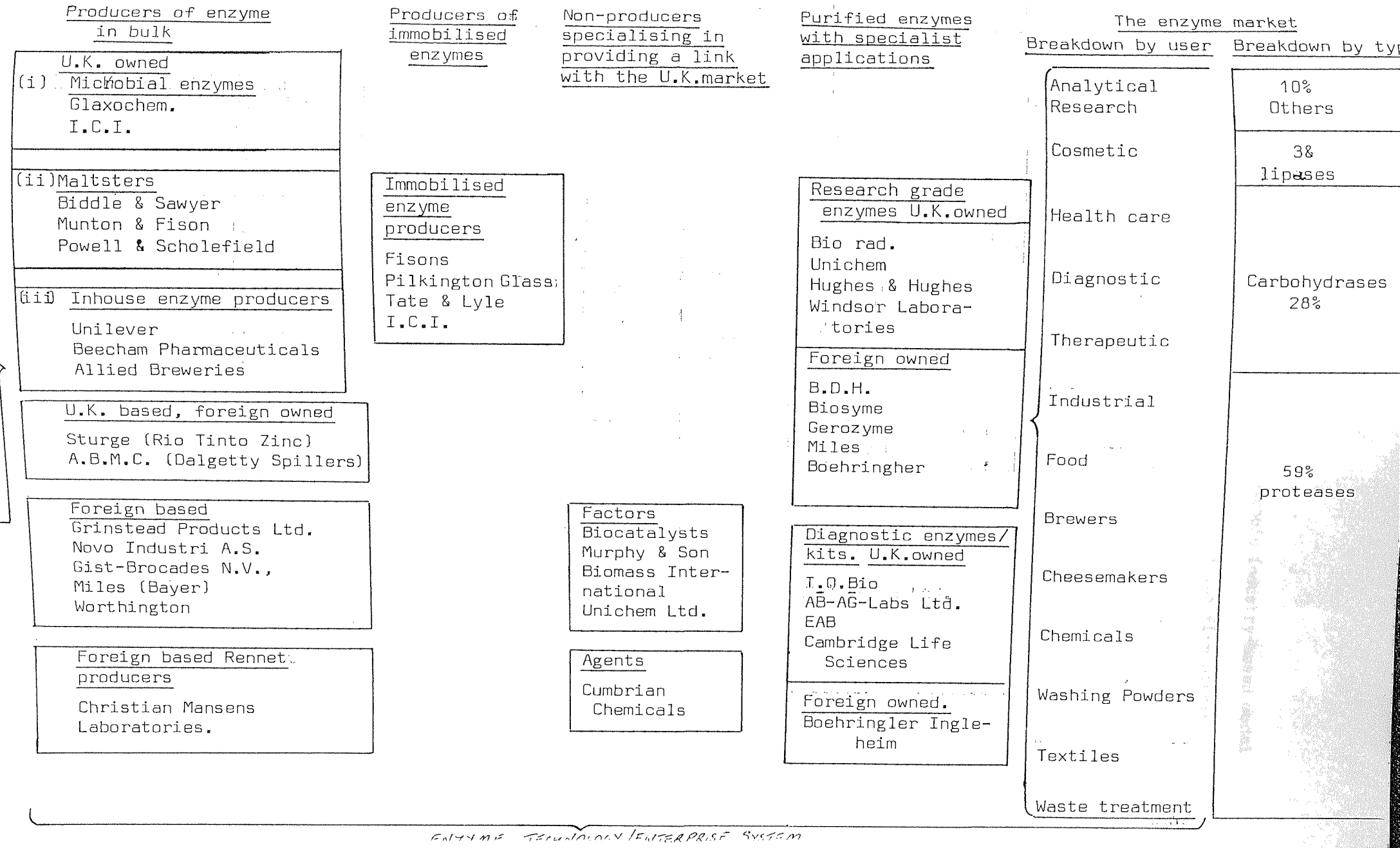
1. Factors related to the environmental system of which the organisation is a part, such as market conditions, economic conditions, legal environment, available technology, etc.
2. Factors related to perceived inducements for innovative activities, such as perceived market needs, perceived urgency in demands for a product/process, perceived opportunities for innovations, etc.
3. Factors characterising the firm, such as its technological capabilities, its communication system, its risk propensity, its organisational climate, etc.
4. Factors related to the firms policy subsystem, such as investment policy, project selection criteria, prior experience with other innovations, etc.

Fig. 9.13 Technological innovation as a result of complex interactions



Source: Twiss, B., Managing technological innovations (42)

Figure 9.14. Summary of U.K. Enzyme Science Technology Enterprise System.



It is essential therefore that a microscopic industry-based model of this process reflects the state of development of the firm within the industry as a whole. More extended models of innovation such as devised by Rosenbloom above or by Twiss[42] would seem most appropriate. (See Fig. 9.13 above).

Pavitt[43] in an attempt to explain sectorial differences between patterns of technical change points out that most technological knowledge turns out not to be information in the scientific sense but knowledge that is specific to firms and to applications. It is cumulative in development and varied amongst sectors in source and direction. He considers therefore that these observed characteristics and variations in patterns can be explained in terms of a three-part taxonomy based on firm type :

1. Those that are supplier-dominated
2. Those that are production-intensive
3. Those that are science-based.

In the case of the enzyme industry such a specialisation of firms appears to have taken place also. (See Fig.9.14) above. I have sub-divided the types of firms further into sub-groups which refer to the principal market they serve, since it is likely that each group of firms will then react differently from the other groups with respect to changes in market conditions and different market forces.

It is now possible to see why certain firms such as ABM have run down their R & D facilities since they were tied to large scale manufacture of enzymes, and competing with other bulk enzyme producers, so were unable to respond to new market opportunities and scientific developments with the speed of some of their competitors. I have also

included in this model companies which factor enzymes, and do not manufacture, but have a knowledge of the market and could therefore become manufacturers provided a suitable breakthrough in the science occurred.

Such a holistic industry-based model of the innovative process is capable of giving different answers to different inputs from science, which the simpler models have not and cannot account for. I shall now consider the SRC Enzyme Chemistry and Technology initiative in terms of this model in the next chapter.

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CHAPTER 10 : U.K.SCIENCE POLICY WITH SPECIAL REFERENCE TO ENZYME TECHNOLOGY

This section draws on material published as reference 1.

10.1. Introduction

In the previous chapters I have described scientific, technological and economic aspects of enzyme technology. What emerges is a picture of a series of technical changes and possibilities capable of affecting and linking many industrial and agricultural sectors. Enzyme technology is thus described as a generic technology occupying a 'heartland' common to a range of hitherto unrelated technologies and appears to be typical of other biotechnologies.[1] However the rate at which these developments are occurring and their rate of diffusion to the various economic sectors is uneven. For example, the pharmaceuticals industry appears to be innovating more rapidly than other sectors and thus is attracting more investment. A study of current biotechnology investment in the USA showed that in 1982 61% was in health care, 23% in agriculture and 16% in 'other' developments. In the long-term, investment forecasters believe that the relative proportions will change, c.f. Table 10.1. The share taken by enzyme technology is also likely to vary between these sectors, and will be related to development of critical markets and new enzyme technologies, capable of competing successfully with rival technologies within them. The problem of forecasting the future is exacerbated by the lack of reliable information about the actual market size, and in this case the volume of enzymes sold and their value.

This has obvious implications for science policy, the coupling of state funded science research with national economic development and the development of the enzyme sector in particular. Competition with

other advanced industrial nations is carried out by competition at the market interface. However despite the economists credo of perfect competition and free markets, competition today involves reconciling the needs of multinational corporations, with the needs for regional and national development. Competition under the circumstances enjoins the whole state, science, industry, society complex. Technological innovation therefore has become an imperative in sustaining the viability and economic growth of the manufacturing sector and science policy an important fact underpinning a nations ability to produce manufactured goods.

I shall now proceed to review and evaluate past and present policies for science especially those directed at stimulating applications in the development of a U.K. based high technology base utilising enzymes catalysts.

Table 1. World Market for Biotechnology in 2000 (forecast)

Industry	US $(million)	Share %
Energy	16,350	25.2
Foods	12,655	19.5
Chemicals	10,550	16.3
Health Care	9,080	14.0
Agriculture	8,546	13.2
Others	7,670	11.8
Total	64,851	100.0

Source : Biotechnology International Update 1982

10.2. The Development of the U.K. Science Policy for Enzyme Technology

10.2.1. Introduction

Enzyme technology forms a part of the UK's policy on biotechnology which in turn represents a sector of the overall policy on new technology. The 'Spinks' report[2] marks an acknowledged watershed in the development of a UK policy for biotechnology. It is convenient, therefore, to examine the pre-Spinks period, the Spinks Report and the recent White Paper.[3]

10.2.2. Landmarks in the pre-Spinks period stretch over at least 20 years

In the 1950's the British Commonwealth Scientific Conference[4] stimulated international collaboration on microbial culture collections of industrial importance. The SRC in the late 1960's and early 1970's[5] identified enzyme chemistry and technology 'as worthy of special encouragement in the belief that its development could lead to a new era in pure and applied chemistry' and reported difficulties in university/industry communications in the field, the subject of this thesis and to which I shall return. In July 1974, the 'Emery' Report (SRC/Institution of Chemical Engineers),[6] charged with examining the state of development of university biochemical engineering, published recommendations as to its improvement. In 1974, the publication of the Berg letter[7] calling for a moratorium for recombinant DNA studies until the potential hazards were understood was immediately upheld by the Medical Research Council.[8] An investigation of these hazards was charged by the Government to the Ashby Committee, whose report[9] was delayed to coincide

with the publication of the Godber Committee's findings on behalf of the Health and Safety Executive[10] were quickly followed up by a Government Counsellor in the shape of the Williams Report[11] which led to the setting up of the Genetic Manipulation Advisory Group (GMAG). GMAG was a departure from the traditional regulatation of science by scientists - its membership includes representatives of public organisations such as trades unions as well as industrialists, civil servants and scientists. Since its inception, there have been pressures from academic and industrial groups engaged in research to reduce its framework of controls. This has now come about.

10.2.3. Aspects of the SRC's policy of selection and concentration

In 1967 the SRC established a joint Panel of the Biological Sciences, Chemistry and Chemical Engineering and Technology Committees to examine aspects of the enzyme field in order to discuss areas where new or more research could be profitably undertaken, to consider how best to foster any desirable research activities and to make recommendations for appropriate SRC support.[12]

The Panel first met in February 1968. Consideration was given to views of workers in the field of enzyme chemistry and technology and to reports of meetings of members of the Panel with other interested parties.

Replies were received from 45 workers from 35 universities and colleges and government departments.[13] The existing and potential industrial uses of enzymes were surveyed. Liaison was

maintained with bodies who also had an interest in the field, in particular the Medical Research Council, the Agricultural Research Council, the then Ministry of Technology and the National Research Development Corporation.[14]

The Panel reported on a large measure of unanimity in the views presented to the Panel on what is scientifically desirable in particular answers to four questions on :

(i) the main directions and promises of research in the enzyme field

(ii) the major hindrances to progress in a particular area

(iii) the progress required in related fields to facilitate their own research

(iv) the desirability of a more collaborative and coordinated research effort.[15]

The argument for setting up the enzyme Chemistry and Technology Panel was introduced under two headings :

(i) The need for better understanding of enzyme chemistry and

(ii) Their potential value to industry.[16]

The need for interdisciplinary work was recognised as a consequence of developments in basic sciences that occurred in structural and functional analyses (proteins made possible by X-ray crystallography through the work by Kendrew and Perutz: the amino-acid sequencing work of Sanger, which led to the determination of the three dimensional structure of lysozyme,

by Philips.) It was felt that better understanding of the high catalytic rates and specificity of enzymes would also be of use, and that this could be achieved by spectroscopy (ultraviolet, infra red, nuclear magnetic resonance, electron-spin resonance and Mossbauer). It was also argued that understanding of enzyme catalysis at this level would help in the reproduction of enzyme activity in model systems and in the development of new catalysts.

The panel's recommendations centred on the need to support enzyme technology made on the basis of the wide value of enzymes to industry. In support of their argument they pointed out the wide range of enzyme applications in food, fermentation, textile and pharmaceutical industries and that these uses were based largely on partially purified extracts of hydrolytic enzymes. Unspecified Company reports, market surveys, specialist articles and extensive enquiries were also used as sources of data.[17] The value of enzymes used by British Industry for 1966; was estimated to be about £900,000. The report pointed out that at that time (1969), the utilisation of enzymes as catalysts had increased considerably especially in the cosmetic and detergent industries and that the current expenditure was probably in excess of £1.5M per annum. The report also pointed out that most of the industries cited above relied on a semi-empirical approach in their use of enzymes, and that a movement towards more scientifically based processes had begun. By way of example, the Panel quoted the trend away from soaking material in stagnant water infected with microbes, now carried out with partly purified bacterial amylases. (A trend discussed in Chapter 4 above as being more or less complete by the

1930's!).

The Panel also discussed the increasing use made of enzymes as catalysts in biochemical assays of analytical reagents, gave examples of sophisticated medical analyses developed as a basis for medical diagnosis but did not feel that specific recommendations were called for at that time in regard to these techniques. Lastly the lack of applications of enzymes as chemical catalysts were discussed, and that this development could be hastened by making enzyme catalysts more stable, for example, by covalently attaching them to porous supports (a decision to immobilise enzymes by chemical reactions that produced covalent bonds between the enzyme and the carrier). The Panel concluded that on the base of the evidence supplied it was evident that support should also be provided for research involving the exploration of new fields in which the catalytic properties of enzymes may be exploited. Furthermore that results, even of a primitive and tentative kind, would awaken interest in both academic and industrial laboratories, and would lead to the wider consideration of feasible uses of enzyme catalysts!

The Panel however expressed some caution in that research programmes of this kind are bound to be speculative but they stated that it was this kind of speculation which could lead to the development of new technologies. They considered that it was platitudinous to remark that a willingness to seek and to exploit such opportunities was one of the chief reasons for many of the technological advances that occurred in America and implied that it was for these reasons that enzyme technology was very much more advanced in the U.S.A. and Japan than in the U.K.[18]

The Panel further speculated that significant advances in enzyme technology could well arise from entirely new developments, such as for example, in their use in reactions for which there is no direct chemical analogy. They felt that such new lines of development were most likely to emerge from the support of speculative research work in these areas in universities and industry.

It is interesting to examine the areas of activity that the Panel considered to be important for the development of the area and which also had science policy implications.[19] Their scientific objectives are beyond dispute. These included the need to prepare and purify large quantities of enzymes by extending facilities for enzyme purification on the laboratory scale, for the encouragement of biochemical engineering centres to investigate the scaling up of laboratory procedures, and for the exchange of information and personnel between centres where experience of the isolation of particular enzymes existed. They also suggested a need to investigate scientific areas where the efficiency and specificity of enzymes could be fully exploited by industry. Above all they suggested that groups working on structural studies of enzymes were to be of an adequate size and have access to adequate computing facilities.

That a 220MHz (or higher) nuclear magnetic resonance machine (NMR) was to be made available to workers in the enzyme field, and that groups involved in peptide synthesis and on developing amino and sequencing techniques should be encouraged.

That organic chemists worked on the mechanism, specificity and control of enzyme action, and that physical organic and synthetic chemists should be encouraged to engage in work on inter- and intra-molecular catalysis.

The remaining policy recommendations were of an administrative nature.

It was also felt that in order to maintain present levels of support this would involve a rising budget in the region of £250,000 p.a. over the next 2-3 years and that Grants awarded by the Enzyme Chemistry and Technology Committee during its first 2 or 3 years would be between £400,000 and £500,000. The Panel then considered areas of activity that needed support. These include the following :

1. enzyme isolation
2. structural studies
3. electron microscopy
4. spectroscopic techniques
5. peptide synthesis
6. amino acid sequencing
7. protein modification
8. inter and intra molecular catalysis
9. kinetic studies
10. enzyme technology.

Finally, the Panel recommended the formation of an Enzyme Chemistry and Technology Committee of the Council's University Science and Technology Board, to keep continuously under review the state of enzyme chemistry and technology and to be responsible

for the support of research in this interdisciplinary area. This committee initially set up for an experimental period of 2 years was re-appointed by the Science Board until 30th September 1973.[20] Subsequently it was re-appointed for a further 2 years until 30th September 1975.[21]

During the period 1973-4 the committee set up an industrial study group to look into the unsatisfactory state of collaboration between the Universities and Industry in the field field of Enzyme Technology, which had led to a lack of awareness on the University side of the real needs and problems facing industry and a corresponding lack of awareness on the part of industrialists of work being carried out in the Universities and its relevance to their problems! It set out to define the problems which had caused this situation and sought ways to stimulate and foster a fuller dialogue and closer contact between the two parties which could lead to a beneficial exchange of ideas.

This is perhaps a convenient point at which to formulate a model of the flow of knowledge from science to industry implicit in the SRC Enzyme Technology Panels and Committees' statements.

That model can best be described as the benevolent science push model used by Blackett and discussed in Chapter 9 above. Implicit in this model is the belief that it is the duty of the industrialists to pick up any commercially useful scientific discoveries, and to apply them. Adherence to such a model and belief in it as a system of innovation enables the scientific community to place the blame for the lack of development of any discoveries on industry. It also supports the belief that thrcugh

the pursuit of disinterested but directed research, discoveries may come about that will revolutionise the commercial use of enzymes!

By 1973 these flaws in this initiative were beginning to be recognised but the blame was placed largely with industry for its inability to make a commercial success of the discoveries that the research scientists had generated as a result of this programme.

It is possible to identify reasons for this failure by comparing the science push model with the holistic industry based model developed in Chapter 9 above which represents the way enzyme science appears to couple to enzyme technology and through it with the commercial environment and the market.

1. The extended holistic industry based model of innovation recognises the important role that a company already trading within the market plays in providing the necessary vehicle in which new technology can develop. In the U.K., as the survey of patent activities indicates, such an innovative organisation did not appear to exist. Consequently any commercially worthwhile discoveries emanating from science would be hard put to find a home for their development and commercial exploitation. A rigid policy of allowing only U.K. companies to commercialise the technology and refusal for political reasons to license the technology to foreign based companies placed the U.K. science based discoveries at a disadvantage from the marketing end of the innovative process.

2. The holistic industry based model recognises the importance of market forces in the development of a new

technology. This vital element appears to be missing from this policy initiative. In the past (see Chapter 4) new enzyme applications came about only when new market opportunities occurred. Neither the SRC's Enzyme and Technology Committee nor the enzyme Panel had an idea of what was commercially feasible and which markets to pursue and attempted to redress this by suggesting research in this area and through a working group of the Panel making recommendations to remedy the lack of science and industry coupling.

3. The Panel used trend extrapolation of raw projected market sales data as evidence of a growing market for enzymes by assuming that such growth could continue to take place for new developments which could be the result of as yet unknown new discoveries.

4. Although the Panel and Committee implied that they had discussed the need for research with industrialists, the weight of evidence collected, came from academics or was based on other observations of industrial activity and therefore was unrepresentative of the then industrial situation and of current commercial needs.

5. No assessment was made of the social or political dimensions of the industry or of any technology. It is quite fortuitous that any commercial application came from the initiative. The characterisation of and preparation of enzymes chemically attached to the inner surface of tubes from commercially synthetic polymers, and the development of the necessary procedures whereby these

materials may be used in biochemical automated analyses. (W. Hornby at the Biochemistry Dept. of the University of St.Andrews had moderate success as a glucose analyser, using the enzyme glucose oxidase). A similar instance occurred with the development of an analyser for blood cholesterol. This analyser had a very large potential market in the U.S. where clinicians and public alike were increasingly worried about the association between coronary heart disease and levels of Cholesterol in the blood. However this market for an analyser of blood cholesterol, never materialised, because a panel of American doctors and scientists felt that they could not establish a causal link between the incidence of cancer and the level of cholesterol in the blood.

6. The Panel also carried out an analysis of bottlenecks which largely constituted blocks to the scientific understanding of enzymes as catalysts and not blocks to the development of enzyme technology. It is interesting to compare this analysis with a survey completed for the EEC by Thomas[22] (see the patent survey discussed in Chapter 3 above). Here Thomas identifies stabilisation of enzyme catalysts and cofactor regeneration as two bottlenecks preventing the substitution of chemically catalysed processes by enzyme technology.

7. Both the Enzyme Science and Technology Panel and the Enzyme Science and Technology Committee relied extensively on the National Research Development Corporation, to develop

any commercially useful research. In this respect the NRDC acted as an agency or broker facilitating the transfer of new ideas into the commercial sector. It was the same organisation that was instrumental in involving Whatman Biochemicals Ltd., as a company to produce high value enzymes for the diagnostic kit market.

The lack of marketing knowhow by the company of this area was a major factor in the slow exploitation of a large health care motivated market. In effect the role of the NRDC was such that it appeared to wish to develop scientific discoveries with small potential markets by interesting larger companies or organisation to enter into joint venture enterprises. In this case the lack of market knowhow and contact with potential customers proved a major stumbling block. (see also 1 above)

Lack of market perception by the SRC committee, lack of industrial involvement, lack of coordination of effort all appear to have played a part in crippling this laudable initiative.

I shall now discuss the Spink's[23] and post Spink's[24] policy initiatives.

10.2.4. The Spinks report

1976 saw the establishment of an Advisory Council for Applied Research and Development (ACARD). Concern at high political levels over the uptake of biotechnology as exemplified by the

SRC's enzyme initiative and by British industry in general prompted the setting up of a Joint Working Party drawn up from members of ACARD, the Royal Society and the Advisory Board for Research Councils 'to study the industrial applications of biological knowledge'. Its terms of reference requested a review of existing and prospective science and technology relevant to industrial opportunities in biotechnology and to recommend action by Government or other bodies to facilitate British industrial development. The report[25] focused on those aspects that appeared to have greatest commercial potential in the 80's (genetic manipulation, enzymes and enzyme systems, monoclonal antibodies, waste treatments, cell culture, SCP and the production of fuels). Constraints on the development of biotechnology were identified as being principally: availability of trained staff, lack of long-term research support, unduly stringent safety legislation and regulations and availability of finance for industrial development and coordination of effort.

In a list of 24 recommendations, the Spinks' report set out the need for a coherent, co-operative approach to stimulate biotechnology from industry, government, Research Councils and universities, involving a commitment at best comparable to that of the U.K.'s major industrial competitors. It urged the need for industry to grasp the opportunities to develop new products and processes and to supply these to home and overseas markets. It requested that support for biotechnology should be increased to £3 million a year, that the Government should develop a coherent programme of industrial R & D with an expenditure of £2.5 million a year, and that the NRDC should play a more entrepreneurial role in support of

innovation in this area. It also suggested that NEB and NRDC should consider using public funds to set up a research-orientated bio-technology company. The report recognised the need for inter-action between universities, industry and government and requested a modification of the customer/contractor principle for government financed research and development. Scientists, and others involved in the field, welcomed the report as the basis for a sensible policy. In many respects the report appeared to underwrite the need for a continued science/technology push. Since this model was not implemented and it is similar to the pre-Spinks policy, I shall not discuss it any further.

10.2.5. The post Spinks' report development

In March 1981, HMSO published the long awaited government response to the Spinks' Report. The 'White Paper'[26] indicative of the governments science technology policy showed a lack of enthusiasm both for Spinks' diagnosis and his recommendations. The reception by the scientific community reflected this disappoint-ment. To many in the field, the White Paper had poured cold water on their hopes and expectations. Many also felt that there had been no consultation in the interim period. To members of the Education, Science and Arts Committee, 'the most careful reading of the White Paper did not reveal a single clear, unambiguous response to any of Spinks' 24 recommendations'. The White Paper consigned a key economic role to biotechnology, but not until the next century.[27] It reveiwed the developments in U.K. companies and stressed that the main responsibility for turning concepts and discoveries of underlying science rested with industry. The role

of the government's financial and economic policies was to provide an environment in which private initiative and enterprise would be encouraged to take advantage of opportunities as they emerged. The White Paper stressed that there was a role for small firms in this area and that government was committed to fostering their growth. On the question of research and development, the government considered that there was much expertise in both private and public sectors, in R & D labs, CAMR, and university departments. It recommended that biotechnology should have a high priority among university research activities. It confirmed that the research councils had set up simple and informal machinery for coordinating their activities. It suggested that selective support should be given from the Research Councils which, when combined with university resources, would be suitable for supporting university-based research in biotechnology. The government felt that companies could form profitable partnerships with Government Research Establishments, CAMR, universities and Research Councils.

On the question of education and training, it was felt that the higher and further education systems could take account of future manpower needs, but they should respond only to clear signals from the employment market. It felt that no substantial concern had been expressed about technician training by employers.

On the issues concerning safety and health, it supported the need for maintaining a balance between safeguards to public safety, health and the natural environment and a need to foster innovation; however, GMAG's procedures should continue to be relaxed whenever new scientific knowledge showed it to be safe to do so. The White Paper encouraged international collaboration

between industrial firms, governments and academic institutions. By way of a concession to industry for the occasions when public bodies might have a contribution to make, it proposed that the facilities and expertise of the Centre for Applied Microbiology Research, CAMR should be made available for both basic research and development of processes to production stages.[28] To sum up, there was evidence that government sought to begin to coordinate national research activities on, and provide support for, biotechnology, but it would have to be in response to market needs rather than, as Spinks suggested, from a science/technology push.

10.2.6. The Current Situation in the U.K.

The "Interim Report"[29] on biotechnology from the House of Commons Education, Science and Arts Committee published in 1982 is the most significant report to appear since the White Paper. This report, its Minutes of Evidence,[30] and the subsequent response by the government, entitled Memorandum,[31] together provide a useful guide to the forms and scale of government policy and support for biotechnology.

This Interim Report was particularly concerned with problems faced by biotechnology researchers in the universities - we are promised a wider study of the future of biotechnology at a later date. Most of its recommendations were therefore to do with universities. The Committee argued that :

1. the UGC should be involved in a more formal manner with official committees dealing with biotechnology;

2. steps should be taken to mitigate the effects of UGC cuts in areas and university departments contributing to biotechnology research;
3. links between the universities, D.T.I. and industry should be improved;
4. the training of biotechnologists should be given more support, and so forth;
5. they also made recommendations about the need to review the monopoly rights of both the British Technology Group (BTG) and Celltech to research council funded discoveries;
6. they suggested a review of tax incentives for research and development to industry.

For some reason the government chose not to reply to the House of Commons in the form of a White Paper - something which concerned the Select Committee - instead they sent a Memorandum to the Committee. In it they attempted to reassure certain "over-anxious politicians"[32] that the government was providing sufficient encouragement for biotechnology. The Interim Report,[33] whatever its shortcomings, served to open up to the public eye a series of issues about biotechnology and brought together information about the form and scale of government policy and support for biotechnology. It did not, however, provide a critical discussion about the potential impact of biotechnology - which was implicitly assumed always to be essentially beneficial. First I shall review the current biotechnology scene in the U.K. and in particular the programmes of support for biotechnology that have emerged from government departments, research councils and the UGC.

A key theme in the Spinks Report was the need for coordination and coherence in the national system of support of biotechnology. The Select Committee's criticism focused on this, arguing that the lack of coordination in government activities in relation to biotechnology noted by Spinks seemed to be but a reflection of a greater lack of coordination in the management of science policy generally. The overriding impression I derived was of a loosely coordinated structure which may indeed serve well enough for the exchange and sharing of departmental views, but which, at the same time, appears to lack any sense of strong, central guidance. Their views bore out similar though earlier conclusions of the House of Lords Select Committee on Science and Technology.(34)

The Government, through the Memorandum argued that coordination of their efforts to support biotechnology had been greatly strengthened, and reaffirmed that the D.T.I. was the lead department for biotechnology, providing overall coordination and promotion. Of particular importance in this regard is the Interdepartmental Committee on Biotechnology (ICBT), set up in April 1982 in response to the decision of the Select Committee to investigate biotechnology. Coordination between the Research Councils is sought through the Inter-Research Council Coordinating Committee in Biotechnology (IRCCCOB) established by the Heads of the Research Councils in November 1980.

A complex government committee structure for biotechnology has been established in the post-Spinks period.(35) Table 10.2. below lists the five key committees, their terms of reference, membership and links with other committees; only GMAG existed

pre-Spinks. As already indicated, ICBT provides the main focus for biotechnology in the government, coordinating the interests of government departments and related bodies as well as providing a point of contact for those outside government. It is claimed that it operates flexibly, allowing departments and bodies which are not formally members "to receive papers and attend as necessary". It has been argued that it is too early to say how well it will work in practice but some indication of its success can be gauged by examining its role in the light of the holistic industry based model presented in this thesis. The Interim Report expressed concern that "too many committees are growing up", and there was a danger of key people spending too much time at committees to the detriment of their research!

There are, as Table 2 shows, linkages through cross-membership of committees. ICBT has links with relevant Sector Working Parties of NEDO as well as links with non-governmental committees set up by the CBI, Society of the Chemical Industry and the British Coordinating Committee for Biotechnology. The Select Committee Interim Report felt it was a serious weakness that the UGC was not formally linked with ICBT; in the Memorandum, the Government says that the UGC would be informed when matters relevant to their interests were being tabled. However, those who feel that the UGC 'cuts' in 1982 bore scant relationship to any sensisble assessment of national industrial priorities might think that a more formal linkage would ensure that in the future the UGC was more closely acquainted with D.T.I. priorities. On the other hand it might be argued that it is important that for scientific development that the universities be protected from too close an

TABLE 2 GOVERNMENT COMMITTEES CONCERNED WITH BIOTECHNOLOGY.

Committee	Abbreviated Title	Terms of Reference	Chairman/Membership	Links with Other Committees
Inter-departmental Committee on Biotechnology	ICBT	To assist DOI in planning and executing action in those areas of biotechnology that do not fall clearly in specific Departmental responsibilities by providing the necessary co-ordination within the U.K. and internationally. To stimulate the exploitation of biotechnology in U.K. industry by actively identifying and encouraging support for specific projects. To provide biotechnology in Government with a visible focus for outside enquiries and suggestions.	CH Government Chemist Membership flexible including DOE, MAFF, DEn, DHSS, HSE, MRC, ARC, SERC, PHLS/CAMR, PHLS, MOD(PE), BTG, Scottish Office. Others attend as necessary.	Cross membership with IRCCCOB, MRCB, Links with UGC, BCCB, SCI, CBI.
Inter Research Council Co-ordinating Committee on Biotechnology.	IRCCCOB	To advise the Heads of the Research Councils on the development of biotechnology research programmes within the Research Council system. - any co-ordination or rationalisation that may be desirable between the programmes of different councils. - any new work that should be initiated as a consequence of inadequate coverage of recent discoveries.	CH MRC Membership; MRC, ARC, SERC, NERC.	Cross membership.
Materials and Chemicals Requirements Board (and its Chemical Manufacture and Biotechnology Executive Committee)	MCRB (CMBEC)	The DOI's research and development requirements boards determine the objective, composition and balance of the DOI research and technology programme. The MCRB covers mineral resources, mineral processing, metals extraction, chemical manufacture, biotechnology process plant, reclamation and engineering materials. CMBEC covers chemical manufacture and biotechnology.	CH Dr B C Lindley, Dunlop Ltd, Membership made up of senior industrialists, academics and government	Chairman of ICBT sits on MCRB AND CMBEC Director of SERC Biotechnology Directorate sits on CMBEC.
SERC Biotechnology Directorate Management Committee	BTMC	To advise and report to the SERC's Engineering and Science Boards on the development of the Biotechnology programme. To approve expenditure within the powers delegated to it. To oversee the programmes and encourage the participation of industry, academic institutions and government in both the research and training aspects of the programme. To guide the Director on the content, balance, implementation and exploitation of the programme.	CH: Dr A T James (Unilever) membership senior industrialists and academics and Government Chemist.	Director of SERC Directorate sits on ICBT, CMBEC and IRCCCOB. Government Chemist
Genetic Manipulation Advisory Group	GMAG	To advise research workers and others on the risks involved with recombinant DNA experiments and the appropriate safety and containment precautions.	CH : Sir R Williams, (Formerly Director of PHLS). Membership made up of senior industrialists, academics and government.	Contacts with HSE and Advisory Committee on Dangerous Pathogens

involvement with industry at the level of the UGC.

A fairly cursory appraisal of the social and institutional origins of the membership of these committees shows that besides civil servants, leading members of the scientific and technical establishment of biotechnology, only certain large industrial organisations are well represented. Only one of the key committees GMAG, allows for representatives from trade unions and the broader social constituency.

10.2.7. Role of Research Councils

In November 1980 the Heads of the Research Councils formed IRCCCOB to advise them on biotechnology research.[36] The Science and Engineering Research Council[37] established the Biotechnology Directorate (see Table 10.2. above) in late 1981 with its own funds for financing research and taking initiatives (grants of almost £4.million had been awarded by early 1983, 24% in the field of fermentation/microbiology and 21% in recombinant DNA studies). SERC also spent £1.6million on biotechnology in 1982/83 and expects to increase this in the future. SERC's general approach to university research and university/industry links is dealt with in the next section. However, it should be noted that the Biotechnology Directorate has engaged in some strategic thinking. They adopted the Spinks definition of biotechnology and identified six priority areas where they believed it was necessary to maintain a national capability of fundamental expertise that could be tapped by industry. These were: immobilised enzymes and cells; plant genetics and biochemistry; large scale animal and plant cell tissue

culture; fermentation technology and microbial physiology; new reactor design and downstream processing technology; sensors and bioelectronics. These are generally similar to the areas that the Spinks Report identified as having great commercial potential.

The Agriculture and Food Research Council (AFRC)* [38] began recombinant DNA and gene transfer research in 1978, and has adopted a narrow definition of biotechnology: "the application of molecular biology to the agricultural, horticultural and food industries". Expenditure under this heading for 1981/82 was £2.4million out of a research budget of £92million. Obviously, a great deal of AFRC research might be expected to be caught by a broader and more conventional definition of biotechnology and they estimate a further £2.7million could be added to the first figure. Areas being studied include: genetic manipulation of plants and animals, micropropagation of plants; monoclonal antibodies and animal vaccines; immobilised cell systems for ammonia production, high value flavour compounds; biological control of pests and disease; photosynthesis. Since 1982 laboratories in particular have suffered cuts in their staff and the rate of expansion and effectiveness of their biotechnology research would seem to depend on how quickly they are able to sort out reorganisational problems stemming from their cuts.

AFRC are exploiting genetic manipulation techniques for the agriculture industry through a new venture capital company with support from BTG along the lines of Celltech's relationship with the Medical Research Council.

* The Agriculture Research Council became the Agriculture and Food Research Council in 1981.

The MRC [39] has approached biotechnology research differently. Past MRC funded research has made major contributions to the development of recombinant DNA research and to other significant fields such as monoclonal antibodies. They see no reason to change an approach to research which has worked so successfuly in the past. They have not earmarked funds for biotechnology, apart from some studentships and fellowships for training in recombinant DNA techniques. They estimate that in 1980/81 they spent £1.7 million on biotechnology research. To avoid diverting money and skills from fundamental research they have sought to make their genetic engineering and hybridoma research available for development and commercialisation through Celltech Ltd.

Celltech, [40] the research company alluded to in the Spinks Report, was created at the end of 1980, with a share capital of £12 million. Share-holders include NEB (now B.T.G.) Technical, Development Capital, Prudential Assurance, the Midland Bank and the Commonwealth Shipping Company. Celltech's five year agreement with MRC gives it rights to the first option to commercialise MRC's research has been attacked as other firms have realised that medical biotechnology offers the surest return on research investment. It has been argued by its proponents that it provides the time and institutional framework for MRC scientists and industrial interests to develop a new understanding and productive relationship. Its contract also contains the degree of monopoly protection needed for a British firm to grow and mature sufficiently to compete on the world market with leading foreign genetic engineering companies. [41]

The National Environmental Research Council (NERC) has a fairly small role in this area compared with the other research councils.[42] It funds the major culture centre of algae and protozoa and is pursuing a small programme of improving tree stocks by cloning techniques.

10.2.8. Biotechnology in the Universities

I am not aware of any detailed quantitative analysis of the size of the biotechnology research base in the British University system. Until relatively recently biotechnology was not a word which one found in academic departmental/institutional titles. There are however now several university units and centres using biotechnology in their titles. The First Report of the IRCCCOB[43] listed 24 universities with biotechnology-related scientific and engineering expertise in 1982. The list showed a very diverse range of activities and, significantly, few combined strength in science, e.g. molecular or cell biology, with strength in process engineering; yet it is at the life science/process engineering interface that one might expect important biotechnological research to occur.

After several years of "running down" of university funds and the 1981/82 cuts the dual support system is in disarray. Such a period is not an auspicious one for new developments. The University Grants Committee (UGC) cuts have bitten deep and seemingly at random and disciplines important to biotechnology have been cut along with the rest; e.g. 12% of biochemistry staff in the universities have been lost. The multi-disciplinary nature

of biotechnology makes it particular vulnerable to a weakening of the dual funding system. This worsening of conditions could, it is feared, lead to a (braindrain' of leading biotechnologists, and SERC has sponsored an investigation which reported in 1984 that the effects of a brain drain were minimal.

The UGC[44] deals with biotechnology through joint discussions of its Biological Sciences and Technology sub-committees - an arrangement which has had its critics; e.g. the Select Committee called for "a more specific decision making structure" to deal with strategic decisions about biotechnology.

The UGC has attempted to mitigate the effects of the cuts in areas thought to be in the national interest in at least two ways - by earmarking funds and through the 'new blood' posts scheme. The UGC biotechnology initiative allows for £900,000 to be made available to selected universities. Some have questioned whether these funds can do more than restore in the short term what the cuts took away. The first stage of the 'biotechnology initiative' awarded £100,000 each per year for 3 years to three universities (University College, London, Birmingham and UMIST). The second stage will make grants to selected universities.

It is by no means certain that 'centres of excellence' and 'selective' policies provide more than short term expediencies. The Merrison Report[45] thought universities will need to concentrate research funds into selected areas. It seems to me that selective policies, in the absence of an adequate support for overall academic research (through UGC recurrent funding) are continuing to damage the U.K. research base in the long term, for

reasons such as the difficulty in providing future support for research in a new area. Further, this might reduce the level of exploratory research where new ideas are tried out that involve multidisciplinary dimensions and expertise. These reasons seem especially cogent for research involving multidisciplinary fields and expertise.

The D.T.I. and Research Councils[46] have evolved a number of schemes to try to encourage links between universities and industry as a general policy, e.g. awards schemes, teacher company schemes, industrial liaison officers, science parks, CASE awards, BTG/University Coordination. Biotechnology can obviously benefit from these. However, biotechnology has also been the subject of special attention since the British situation showed little of the academic entrepreneurial flair found in the U.S.A.[47] Following the Spinks report there have been significant efforts to overcome British 'backwardness' in this respect. I have already discussed Celltech and shall now examine the university-related institutional innovations. These have been varied in scale and scope, ranging from academic consultancies to new companies. (It appears that most of the leading university biotechnologists are paid consultants to one or more companies.)[48] SERC helps co-operative research ventures between university departments and industry in which the industry provides at least 50% of the necessary finance. At the beginning of 1983 there were 17 such ventures involving £1.7m. from industry and £0.7m. from SERC. Another approach can be exemplified by the University of Leicester 'Leicester Biocentre'[49] - this is a research institute involving collaboration between the University, the SERC Biotechnology

Directorate and five companies. Leicester University has also established a joint ICI-University laboratory which will be studying the industrial application of genetic manipulation techniques. Problems with obtaining entrepreneurial management led to the recent appointment of Prof.Higgins from Cranfield as director of the Leicester Biocentre.

Other universities have sought venture capital and established biotechnology companies. For example, Technical Development Capital Ltd. is associated with developments at Imperial College (Imperial Biotechnology Ltd.)[50] and Sheffield University (Plant Science Ltd.)[51]

Yet another approach is the establishment of consultancy and contract research organisations, e.g. South East England Biotechnology[52] (a consortium of Kent University, University College, London, and the Polytechnic of Central London). Science parks[53] are yet another route seen leading to a better transfer of knowledge from universities to industry.

Most of these initiatives are at their early stages of development; it is probably too soon to pass judgement on their chances of commercial success. Neither can one say how they will affect the universities and departments associated with them. Looking at these developments it is clear that most universities with sizeable groups working in biotechnology-related areas have now established commercial entities and linkages to exploit them. In the United States where such developments occurred earlier, academics have found problems arising from conflicts of interest

between academic work and ties with commerce and industry.[54] Academics also found that management of a research company is not like the management of a university research department. However at a time when university funds in the U.K. are in a parlous state industrial funds are too attractive to turn down. Industry, has its own need for a commercial pay-off from research and it might turn out that the U.K's universities had been encouraged to put too great an emphasis on application at the expense of fundamental research. Industry's requirement of confidentiality poses problems to academics used to and seeking open disclosure of findings to their peers.[55] The solution most often voiced is "to achieve the right balance", but who can say what this is today or in the future.

10.2.9. Government Research Establishments

The role, actual and potential, of government research establishments and institutes in developing biotechnology should not be overlooked. Three such establishments are closely involved with the Department of Industry's (DoI) Biotechnology in Industry programme. They are the Centre for Applied Microbiology and Research, (CAMR), Warren Spring Laboratory (WSL) and the Atomic Energy Research Establishment, Harwell, (AERE).[56]

CAMR is especially strong in fermentation technology; the scale-up of genetically engineer microorganisms is obviously a key area in biotechnology, strengthened by making available additional fermentation capacity and recently linked to a commercial company Porton International.[57]

Downstream processing, the separation of the product from water, biomass, etc. is another vital area of development. WSL and AERE Harwell have strong programmes in this area.[58] Together with D.T.I. support they run a multiclient cooperative R & D and technology transfer programme - BIOSEP.

There are strong feelings among some workers at Government Research Establishments (GREs) that the national biotechnology research effort has now swung too much towards academic institutions.[59] They argue that GREs are better designed for development work, and it is wrong to think that only the universities have good ideas. GREs are able to direct their staff to specific tasks and goals and more easily establish stable interdisciplinary teams. Further, their staff members, unlike university academics, do not have a wide spread of commitments and are more able to handle the industrial need for confidentiality.

However, innovative research is sometimes handicapped by the lack of funding of exploratory research in establishments working mostly to the customer/contractor principle. In a period of staff reductions and tight funds it is understandable why the 10% of contract funds earmarked for such research is subsumed and sequestered.

10.2.10. Government Initiatives for Biotechnology and Industry

Of particular importance for encouraging the industrial application of biotechnology are the British Technology Group (BTG) and the Biotechnology in Industry programme.[60]

The D.T.I. has sponsorship responsibility for BTG, which was formed in 1981 by the merger of the National Enterprise Board (NEB) and the National Research Development Corporation (NRDC). BTG's stated objective is "to facilitate the 'pull-through' of new technology from the laboratory to the market place". Prior to the merger NRDC often came under very bitter attacks for the manner in which it worked. Accusations from academics include missing out on patenting monoclonal antibody technology and causing the collapse of the original Leicester Biocentre scheme. In fairness to NRDC it should be pointed out that considering that they were bound to examine all proposals put to them they were grossly underresourced. Also they experienced difficulties in persuading U.K. companies to pick up new ideas which foreign companies were allegedly anxious to buy.

Within BTG, NEB is to have the catalytic role in developing new technology and NRDC is concerned with the commercial exploitation of inventions and developments resulting from publicly funded research and with the provision of risk finance for innovation. BTG set up Celltech, and has now launched its 'country cousin' Agricultural Genetics Company (AGC). BTG[61] has made about 40 investments totalling about £13m. in biotechnology. Further, these act as a catalyst for substantial private sector investment, e.g. the proposed phased investment of £5m. by BTG in AGC would be in association with £10m. of private money. Even so BTG is becoming a victim of the Treasury desire to see it become self-financing, and in 1983 its core budget has been halved. Forced by these commercial pressures to seek quick returns it is likely that it will be less willing to back the more risky long-term

ventures now and even less so in the future. From the holistic industry based model of innovation it is difficult to see how BTG can function successfully since it is still unable to guide research, but has to couple products of science to the commercial environment via industrial firms.

The 'Biotechnology in Industry' programme,[62] launched in November, 1982, is one of the D.T.I's nine special new technology support programmes. The £16m. over three years allocated to the programme compares with £150m. allocated to information technology, £90m. to production engineering, and £50m. to microelectronics applications. Dr. Coleman, the Government Chemist has helped to draw up the programme and believes this sum to be sufficient. He holds the view that at this stage to pour in larger sums of public funds would be a mistake, it being better to provide more modest sums to support projects that have been carefully selected.[63]

Through the programme, the D.T.I. is offering grants of one third of the costs of selected projects, processes and product developments (in some cases more); grants for, and a service of, consultancy studies at three levels - strategic studies, feasibility studies and problem solving; support from centres of expertise - CAMR, Warren Spring Laboratory, Atomic Energy Research Establishment Harwell, Biotechnology Institute and Studies Centre Trust, Patscentre International; support for culture collections, (to make national microorganism collections more accessible and useful to industry); and to encourage the improvement of access to biotechnology information. The Biotechnology Unit has been established within the Laboratory of the Government Chemist

Biotechnology Group charged to review and sort out submissions to these schemes. During the three months following its inception it received over 2400 enquiries for basic information about the scheme. In addition the Biotechnology Unit has appointed three consultants, seconded from industry, to help assess D.T.I. investments and look for gaps in British biotechnology. They are able to draw on broad advice from bodies such as The Industrial Biotechnology Liaison Group of the Society for the Chemical Industry, and BCCB. This availability of funding to industry sponsored projects ensures that some limited coupling takes place at the level of the firm. However little planning appears to have been undertaken in the assessment of the overall strategy apart from the selection of likely areas of research. Furthermore it relies very heavily on the firms to initiate such a programme and establish a joint programme of research.

10.2.11. British Industry, Venture Capital, and Biotechnology[64]

I have already discussed companies formed in association with Research Councils and universities. Here I can do little more than indicate some of the main trends. Interest in genetic manipulation can be seen by listing industrial companies represented on GMAG: Beecham, Ciba, Dista (Eli Lilly), ICI, Searle and Wellcome, all of whom have major pharmaceutical interests. Medical products generally offer the quickest return for biotechnology: e.g. in monoclonals we find Unilever, Sera Laboratories, Celltech, Montech; in interferon - Celltech, ICI, Searl and Wellcome. Important food/feed processes have been developed: ICI single cell protein Pruteen is a commercial reality, RHM's fungal protein - now backed

backed by BTG and ICI has begun production using the Pilot plant facility at Billingham. Unilever has developed techniques for cloning oil palms and a large cultivation programme is under way. Biotechnology process engineering offers opportunities for innovation e.g. John Brown Engineers and Constructors already have worldwide visibility in SCP project knowhow.

Venture capital - several groups have specialised in biotechnology. These include Advent Technology; Biotechnology Investments - an offshore venture capital company of N.M.Rothschild; Prutech - associated with Prudential Assurance and Technical Development Capital - part of the Finance for Industry Group (owned by Britain's big banks). Several City merchant banks have subsequently displayed less enthusiasm. It should be of some concern that Rothschilds claim (65) that they have been unable to find any U.K. projects in the area that meet their criteria for investment to date. Despite these couplings this appears to indicate the lack of appreciation of commercial criteria by fledgling U.K. scientist entrepreneurs and if anything confirms the status of large companies and their key role in the exploitation of biotechnology.

Conclusions

What is striking about all the above examples is that despite the adoption of a variety of mechanisms to bring about the integration of scientific research on the one hand with U.K. industry so that industry can capitalise on this new knowledge, the policy still appears to be largely optimistic in stressing opportunities for innovation without

paying sufficient attention to the details of the mechanism by which it can be brought about. Thus for example the government points to the opportunities for small firms to be innovative, provides financial support whilst cutting back on fundamental underpinning research in the hope to force out scientific entrepreneurs to venture into the commercial environment. A carrot and stick mentality that does not give sufficient emphasis to the important contribution that scientists can make and underplaying the importance of market knowhow and business acumen . By involving representatives from larger organisations on the scientific advisory panels, pure science based research initiatives have become directed towards providing the underpinning research that appears to be of use to large companies. The opportunities to develop new ideas emanating from pure research that small companies could make use of may be correspondingly reduced.

Providing that this coupling process is efficient then ideas emanating from science will be channelled directly to industry, as a result of this route. In that case then organisations such as the BTG will have a vanishing role to play. Evidence suggests that the BTG performs a valuable service in the U.K. and if this is the case, then clearly the measures used to bring about the coupling of science, industry and the market are little better than they were some 20 years ago, and science biased initiatives are likely to continue to fail in the U.K.'s commercial environment. Modeling innovation in terms of a holistic industry based model as proposed in this thesis appears to have a predictive value in appraising the outcomes of past science policies correctly suggesting reasons for their failure. It appears to have a predictive value in analysing present policies for science also identifying elements of the innovative process which have been poorly

developed or omitted altogether. Consequently this holistic model of the industry based innovative process should be a useful tool in the formulation of future policies for science.

Although this thesis has examined enzyme technology in detail, the discussions of biotechnology policy in this chapter indicate that as a technique it may provide a useful basis for wider policy analyses.

In conclusion this thesis and the modelling approach present an important methodology for understanding the complex mechanisms by which science relates to innovations. The thesis also provides a framework for the formulation of more effective science policies directed at the stimulation of innovation. It is further suggested that this approach would be of value to science policy makers, in order that some of the problems that have been encountered in the past will be overcome in the future, and past and present policies which have failed for the reasons detailed in this thesis will not be repeated.

The findings of this thesis however are restricted by virtue of the analysis to the situation found in the U.K., where the problems of coupling science and technological innovations continue to be especially acute.

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56. Outlines of biotechnology research programmes can be obtained from the publicity departments of these laboratories e.g. D.T.I. Warren Spring Laboratory 'Biotechnology' and PHLS Centre for Applied Microbiology and Research Information Sheet 'CAMR and Biotechnology'.

57. Fishlock, D., A £15m. landmark for Porton. Financial Times, 18 October, 1985.

58. Personal communication and interviews.

59. Ibid.

60. Op cit. pp.xlvi-xlvii 24.

61. 'No shortage of financial support'. Financial Times, 3 May 1983, p.28.

62. Op cit. 'Biotechnology in Industry'. (46)

63. Op cit. (61) (iii) see also Dol Press Release April 1983 'A stimulus for biotechnology in the UK'.

64. (i) 'Biotechnology' Financial Times survey, Financial Times 3 May 1983.
(ii) (Biotechnology Europe', Imsworld Publications, London, 1981.

65. Biotechnology Bulletin 2,(3), 5-6 April 1983. ISSN 0261-6904.