THE ISOLATION, PRODUCTION AND DEVELOPMENT OF DERMATOLOGICALLY ACTIVE CONSTITUENTS IN COAL TAR

A Thesis Submitted

by

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ABSTRACT

The treatment of psoriasis largely involves the use of steroid and coal tar therapy. The latter offers certain therapeutic advantages over the steroids, but is generally not so cosmetically acceptable. This is discussed and other methods of treatment are reviewed. With a view to improving the cosmetic acceptibility of coal tar and also increasing its specificity, tars were fractionated and an animal screening technique was adopted to evaluate these fractions and their parent tars. The mouse tail skin was used as a model for the application of coal tar fractions in different formulations and their effect was monitored by determination of changes in the epidermal thickness, induction of granular layers, histochemical changes and the keratin fluorescence of the mouse tail skin.

Screening revealed that the neutral compounds in coal tar produced epidermal thickening without inducing a granular layer. They were omitted from further testing since it was considered that they would have no therapeutic value and they would be undesirable in formulations for psoriasis. Basic compounds were not screened. The greatest therapeutic promise was shown by high-boiling tar acids of the boiling range 280-340^{o}C. These appear to be largely alkylated mono- and di-hydric phenols and indanols, which are more abundant in low-temperature tars.

The economic viability for the commercial production, by the National Coal Board, of a therapeutically active phenol fraction has been examined. It is concluded that toxicological and clinical evaluation is required before definitive statements regarding the economic feasibility of producing a more active fraction on a commercial scale can be made.

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PART 1

I
COAL AND COAL PRODUCTS

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1. THE ROLE OF THE NATIONAL COAL BOARD

On January 1st. 1947, the National Coal Board was set up to manage Britain's coal industry (H. M. S. O., 1946). Prior to this date, the industry consisted of over one thousand individual collieries, all privately owned. The first task for the board was to co-ordinate all mining reserves; non-mining assets were sold off. The disruption of the war years had placed a great strain on the country's fuel reserves; the coal industry was in urgent need of modernisation to provide coal in the quantities required by expanding British industry and by the domestic markets.

The N. C. B.'s "Plan for Coal" had to be structured for flexibility to meet the varying and wide range of requirements of its customers. One of the fundamental difficulties facing management in the coal industry is the long-term nature of any expansion compared with that of an industry in the manufacturing sector. For example, a factory is built in about 2 to 3 years, it being possible to estimate the market fairly safely over the first five years of production. To commission a new pit, however, takes 9 to 10 years; consequently a great deal of careful planning and organization is necessary to predict the requirements of industry and the domestic markets to ensure that their corresponding needs will be met at the right time. A newly-commissioned pit is expected to "pay-off" over a period of fifty years, (N. C. B., 1957).

Not only was quantity crucial, but there was an increasing need for quality control of products suitable for use in technologically advancing industry. Within the first three years of the N. C. B.'s institution, the five thousand different types of coal mined in Britain were analysed and

classified. This enabled, for example, calorific values to be assigned to each type of coal (N. C. B. 1957).

Britain's coal industry is now almost 100% mechanised. The increased efficiency of mechanised methods has resulted in a doubling of output per manshift since 1947. The industry is now technologically equipped to continue coal mining under land and off the coasts of Britain for over a century more at present rates of production.

The principal financial aims laid down in the Coal Industry Nationalisation Act of 1946, (H. M. S. O., 1946) are essentially different to those of private concerns, which are usually judged by their annual profits. The N. C. B. is required to break even over an average of good and bad years in the task of supplying coal to the nation. There are large total reserves of coal, but the limits of coal production are governed by the capacities of collieries, available manpower and overall production costs. Production becomes increasingly difficult as the "easier-got" reserves are exhausted, (Browne, 1955; Valéry, 1973).

The National Coal Board is now one of Britain's and Europe's largest industries, employing over 300, 000 people and having a turnover of £900 million per annum, compared with £371 million in 1947 (N. C. B., 'A', 1972). Most of this income is from coal sales and more than half of this is paid out in salaries, wages and related benefits.

2. PRODUCTS OF THE COAL MINING INDUSTRY

(a) COAL

Coal is thought to be formed from the incomplete decay of plant life, (Parks, 1963). It has provided the largest source of mechanical energy in England since the Industrial Revolution, (Bronowski, 1957). It is of course the major product of the industry, the total output of the year 1970/71 being 142.2 million tons. The major outlets are shown below:

	Million Tons	
Power stations	73.5	
Coke ovens (mainly iron and steel works and N.C.B.)	24.7	
Factories, offices, hospitals etc	18.5	
Domestic	15.7	
Collieries' Miners' coal	4.4	
Manufactured fuel works	4.2	
Other inland uses	3.8	
Gas works	3.5	
Exports	3.0	(N.C.B. 'C', 1972)

The 73.5 million tons used by power stations generated almost three-quarters of our electricity , (N.C.B. 'A', 1972). With the demand for electricity having quadrupled in the years 1950-70, this figure compares well with the 27 million tons used in 1947; even as competition from nuclear power, imported oil and natural gas increases, British coal fulfils almost one half of Britain's energy requirements, (N.C.B. 'B', 1972). It is capable of supplying more than this since the recent discovery of the massive coal reserves at Selby, (Valéry, 1973). Coal fuels will long outlast the total reserves of oil and will be cheaper, (Clutterbuck, 1973).

In 1970/71, 3 million tons of coal and 437,000 tons of coke and breeze were exported, the biggest customer being West Germany, buying 1·3 million tons. Over 90% of the exports were to Common Market and EFTA countries, (N.C.B. 'A', 1972).

(b) COKE

Coke is the solid fuel residue formed when coal is heated in the absence of air at over 1000°C (in a "high-temperature" process) or at 500°C (in a "low-temperature" process). This coal carbonization produces not only the essential coke, but also a wealth of economically important primary and secondary by-produces such as gas, benzole and tar.

Prime coking fuel is required in steel manufacture. Coke was produced as a by-product in the manufacture of coal-gas. With the introduction of natural gas, a valuable source of coke has been greatly decreased, and there is a world shortage of prime coking coal. This has necessitated the development of smokeless fuels "tailor-made" for industrial and domestic purposes (Jones, 1956). The N.C.B. mines naturally smokeless fuels, notably anthracite and dry-steam coals, but these are in limited quantities. Manufactured smokeless fuels were necessary for implementation of the Clean Air Act of 1956 (H.M.S.O., 1956); there is now a wide range of smokeless fuels for use in industry and in the home.

The production of coke is usually carried out at a "high" temperature of about 1200°C or at a "low" temperature of about 500°C. An example of a high-temperature process is the N.C.B.'s Avenue Tar Works at Chesterfield; in the production of "Sunbrite" coke the oven temperatures reach over 1400°C. "Coalite" is produced by a low-temperature process by Coalite and Chemical Products Ltd. The temperature of carbonization

and the blends of coal used determine the nature of the products - this is particularly important in the production of tars and it will be shown later how this is related to their medical efficacy.

(c) PRIMARY BY-PRODUCTS OF CARBONIZATION

The four main by-products of coal carbonization are ammonia, benzole, gas and tar. In the year 1970/71, the N. C. B. produced 45,000 tons of ammonium sulphate and other ammonia products constituted 8,000 tons; these products were used in the manufacture of fertilisers and fire-proof materials.

In the same year the output of benzole was 16 million gallons. Crude benzole has many outlets, one of which is the production of high-grade benzene; it is refined and processed to produce toluenes, xylenes, naphthas and motor benzole. Phenol from refined benzole is used in the manufacture of caprolactam for Nylon 6. Benzole refinement gives rise to products which eventually find their way into plastics, photographic materials, drugs such as aspirin, dyes, saccharin, perfumes, food preservatives, metal and floor polishes, paints and varnishes.

The carbonization of coal for coal gas has decreased with the introduction of natural gas; however in the production of smokeless fuels, in 1970/71, the N. C. B. produced 33,000 million cubic feet of gas from coke ovens; this is now being piped and sold directly to industries and hospitals near the coking plants.

The Board owns 13 coking ovens, which carbonize about 6 million tons of coal each year, producing about 256,000 tons of tar. The crude tar is used in road tars, for binders and electrode pitch, and is also subjected to extensive fractionating and processing to produce tar acids and

bases, creosote and many individual compounds such as anthracene, naphthalene and pyridine. Naphthalene is especially important for the manufacture of phthallic anhydride, which in turn is used to make paints and plastics.

So many and varied are the constituents of coal tar that it is not surprising to find refined and processed 'fractions of fractions' in quite diverse manufacturing industries. Even fairly crude fractions have a wide range of uses, which include pitch, fuel oil, lamp black, sheep dips, disinfectants, water-proofing materials, metal and wood preservatives and winter washes for gardeners.

These ancillary processes of coal carbonization and economically viable utilization of the concomitant products directly support and help cut costs of the main product coal (N. C. B. 'A', 1972). In January 1963, the N. C. B. set up its Coal Products Division to manage the ancillary activities; in 1970/71 this earned £ 7. 3 million operating profit, the second highest since its institution. In pre-nationalisation days, private colliery owners ran subsidiary plants for tar, benzole and various chemical refining but these were found to be run-down and not particularly consumer-related. The Board has, on its own and by joint ventures with for example the steel industries, found ways of converting the crude fractions into more useable products.

3. COAL TAR - A VALUABLE BY-PRODUCT

Up until the mid-nineteenth century, tars were troublesome and unwanted by-products in the production of coke, (Moore and Hall, 1939). The discovery of chemicals such as aniline and benzene began the ever-continuing search for economically important chemicals in tar, (Robinson, 1937). Although coke is still the primary product, tar yields and possible uses are considered before initiating a new method of carbonization. The largest and most important non-fuel use of coal is the extraction of chemicals from the by-products of carbonization.

Numerous methods have been introduced, patented, and either discarded or developed to obtain the optimum conditions for carbonization. These methods are usually divided into "low" temperature carbonization with an average temperature of 500°C, and "high" temperature, where oven temperatures often exceed 1000°C. The cokes produced are very different. In low temperature carbonization, coal is rather incompletely carbonised, yielding a dull grey "char", which has an open porous structure, with still a high volatile content, causing it to burn easily and this has found widespread use as a domestic fuel, e.g. "Coalite". It is softer and has a lower density than high temperature cokes, (Wilson and Clendenin, 1963).

Karr (1963) states that a true low temperature carbonization should have an upper temperature limit of about 500°C, since considerable aromatization occurs about 600°C, which is often the upper limit quoted by other authors. The tar produced varies widely with the coal used and the conditions of carbonization. These tars are more sensitive than high temperature tars to temperature, length of residence time in the oven and the actual

vessels employed. There are far more qualitative and quantitative differences in these tars than high temperature (coke oven) tars, (Pichler et al, 1970). In general, low temperature tars contain very small amounts of a large number of individual components, (Combes, 1944). Only in very rare cases do the amounts approach 1% of the total tar, whereas coke-oven tars contain, for example, up to 10% of naphthalene.

Because of the serious shortages of fuel in the second World War, Germany and Japan used fuels obtained from low temperature tars. When petroleum is plentiful, this source of fuel is not wanted, (Kusy, 1970), but the concept of using such coal fuels in the future is indeed a viable possibility.

There have been many reports of the content of low-temperature tars, and the greatest number of studies have been on the acid content since the extraction of phenolic products is of great economical importance, (Bristow, 1947). These are sold for use in phenol-formaldehyde resins, plasticizers, wetting agents, inhibitors, disinfectants, antiseptics, insecticides and for ore-flotation. Plastics have been the most important outlet, and second only to this in terms of profit is the use of tar products in the pharmaceutical industry. Phenol itself is used as a disinfectant and antiseptic, forming the basis of many "medicated" preparations,(Wechsler, 1962).

High temperature carbonization leads to an increase in pitch, naphthalene and in the density of the tar. Tar acids are decreased and consequently low-temperature tars have become the major sources of all types of tar acids. There is a large number of individual components in low-temperature tar, whereas there may be up to 10% naphthalene in

high-temperature tars, (Combes, 1944).

A great deal of work has been carried out on the nature of high temperature tar pitch, which is used for road tars and various binders. There are over 5, 000 constituents and so far less than one hundred have been identified. All are condensed polynuclear ring compounds. Recovery of individual tar acids has been the subject of many studies (Harris et al, 1953, 1956; Wood and Phillips, 1955; Wood and Wilman, 1958); an example is the difficult separation of the cresol isomers. Quinoline and isoquinoline are retrieved from the tar base fractions.

The newly formed tar is usually reheated to about 300 - 400°C to drive off the volatile oils which condense and are collected in separate vessels, leaving the pitch residue. Some of these oils are "washed" to obtain the acids and bases. These various fractions find their way into all sorts of industries.

An example of a high-temperature coking plant is the "AVENUE" process at Chesterfield. Coal is provided by 7 local collieries in the Derbyshire area, and is blended in the following proportion:

Glapwell	5.0%
Bolsover	10.8%
Clipstone	10.8%
Sutton	16.2%
Renishaw	16.6%
Silverhill	19.0%
Rufford	21.6%

(Fere, personal communication).

1 ton of blended coal produces : 71% COKE (60.5%)
BREEZE(10.5%)
104 lbs. CRUDE TAR

(Cont'd)

4 galls. CRUDE BENZOLE
58 galls. AMMONIA
13, 000cu. ft. GAS

Daily charge to ovens = 2, 680 tons.

The crude tar is reheated to about 360°C, the pitch separating as a liquid which is run off for collection, and the vapourising oils passing over into a fractionating column. The amounts of oils fractionated are shown below together with their uses :

1 ton CRUDE TAR produces :	0. 008 tons	OVERHEADS	for benzene, toluene production
	0. 012 tons	CARBOLIC OIL	resins, paints
	0. 11 tons	NAPHTHALENE OIL	phthalic anhydride production
	0. 13 tons	BENZOLE ABSORBING OIL	Flux oil, or benzole or naphthalene absorption
	0. 05 tons	ANTHRACENE OIL	dyes, road tars, carbon black
	0. 15 tons	BASE OIL	Road tars, carbon black
	0. 54 tons	PITCH	Road tars, briquettes.

One used which doesn't usually occur in standard texts on tar utilisation is that of coal tar in ointments and creams as a topical therapy in various skin diseases. The tar is usually bought from tar distillers or formerly directly from gas works. Normally this use is not a direct concern of the mining industry. However tar has long been praised in dermatological therapeutics, yet the basis of its actions has still not been elucidated. This study has been designed to find active fractions, with a view to their identification and possible production for dermatological use.

II

COAL TAR AND SKIN

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1. THE SKIN AND PSORIASIS

The skin is the largest organ in the body and is itself composed of several vital organs. Fig. 1, a diagrammatic vertical section through the skin, shows that it is composed of a dermis and an overlying epidermis which protects the body from the outside world. The dermis contains sweat glands and capillaries which are essential for temperature regulation, a lesser part being played by the hairs. These organs are supported in a network of collagen, in which are also found fibroblasts (probably involved in collagen synthesis), mast cells (important in tissue damage for histamine and heparin release) and various phagocytosing cells from the blood and the defence system of the body, the reticulo-endothelial system.

Fig. 2 shows a diagrammatic representation of the main elements of the epidermis. Basal layer cells divide, and daughter cells move up through the epidermis to appear in the horny layer as "dead" cornified structures. Two processes are evident : there is a destruction of cell contents by endogenous enzymes and a synthesis and laying down of the fibrous and resistant protein keratin. The modus operandi is obscure; the cells are now far away from a blood supply, but this cell death is certainly not passive, (Bern, 1952). It seems there is an organised release of hydrolysing enzymes at the level of the granular layer, there being a delicate balance between the degree of destruction of the cell and keratin synthesis to produce the most suitable type of integument for the part of the body which it is to protect, (Jarrett and Spearman, 1964).

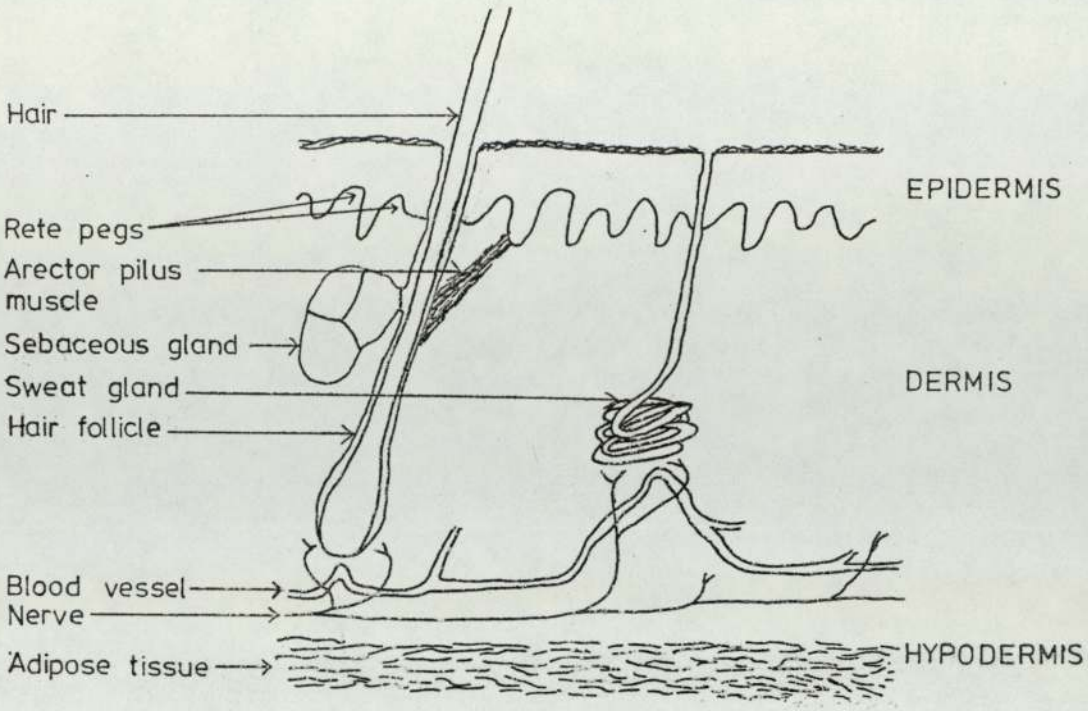


Fig. 1

Diagrammatic representation of Normal Human Back Skin (Vertical section)

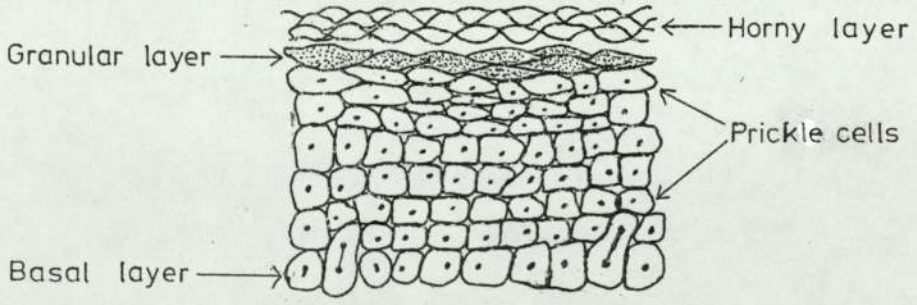


Fig. 2

Diagram of the Epidermis

The Production of Epidermal Cells

There is a constant renewal of the epidermis. Epidermal cells, (keratinocytes), formed from basal layer cell division, are thought to move outwards, (Pinkus, 1970). Mitoses are sometimes seen to occur at higher levels in the epidermis, but they always involve basal layer type cells, (Leblond et al., 1964). Epstein and Maibach's work (1965) suggests that mitoses and subsequent migrations are random.

The production of keratinocytes follows a diurnal rythym, i.e. mitotic activity is greater during sleep and less during awake periods, (Bullough and Laurence, 1956). Activity is at a maximum in wound healing. The control of this mitotic process has been the subject of many studies by Bullough and Laurence. From studies in mouse skin, they have found (1960, 1964) that stimulation of mitosis seems to be due to the lack of an inhibitory substance, "the epidermal chalone". The chalone seems to be a basic glycoprotein of molecular weight 25,000, and seems not to be species or even class-specific, (Bullough et al., 1970). The chalone requires adrenaline to function, (Bullough and Laurence, 1964; Marrs and Voorhees, 1971), and is thought to form a complex which breaks down in conditions of low adrenaline levels, (e.g. sleep), and mitosis ensues.

Estimates for the time required to completely renew the epidermis, (epidermal turnover time (E.T.T.)) have ranged from 7-258 days. (Epstein and Maibach, 1965). After reviewing previous work, Halprin (1972) states that total E.T.T. is probably about 52-75 days. The time required for cells to travel through the horny layer is taken as about 14 days, (Rothberg et al., 1961; Baker and Kligman, 1967).

The Granular Layer and Keratinization

The granular layer is a charateristic of mammalian skin, (Spearman, 1964). Skin sections stained with haematoxylin show layers of various thicknesses consisting of cells containing basophilic granules. These have been called keratohyalin granules as they are still thought by some workers to be keratin precursors, (Brody, 1962), although very little evidence remains to support this idea, (Bern, 1952). The nature of the granules is obscure, (Blank, 1952). Smith and Parkhurst (1949) found the granules of guinea-pig skin similar to the granules found in the thymus gland, which is also an epithelial tissue capable of keratinization.

Evidence against the granular layer being part of a passive keratinocyte cell death comes from Leuchtenberger and Lund (1951), who point out that these cells lack the transition stages so commonly associated with degenerating and necrosing cells. Indeed the cells show great activity. Jarrett and Spearman report the following enzymes present in the granular layer: acid phosphatase, β-glucuronidase, 5-nucleotidase, deoxyribonuclease (DNase), ribonuclease (RNase), non-specific esterase, and alkaline phosphatase, (Jarrett et al, 1965). Metabolites from the relevant substrates can be extracted from keratinized structures, (Jarrett and Spearman, 1964; Downes et al, 1966). It seems that the granules could represent breakdown products of the epidermal cell contents, (Hueck, 1935; Maximow and Bloom, 1952), formed from the activities of the enzymes.

Jarrett and Spearman (1964) suggest that the processes occurring in the region of the granular layer mostly determine the nature of the horny layer produced. For example, if there is little hydrolysis by

granular layer enzymes of keratinocyte contents, then the laying down of the keratin polymers will result in a fairly rigid structure. However, extensive hydrolysis of keratinising cell contents will produce a more flexible cornified cell, and hence flexible horny layers. The appearance of such a structure after routine histogical processing has prompted the title of "basket-weave keratin" for normal human horny layers found, for example, in back skin, (Spearman and Riley, 1967). The enzymes released in the granular layers cannot affect the newly formed keratin, since this is a highly inert and resistant protein protected by disulphur bonds, (Jarrett et al., 1965).

The workers, (Jenkins and Tresise, 1969; King, 1949; Liss 1965) who believe that keratinisation begins in the basal layers with "tonofibrils" which gradually polymerise on their way to the horny layer mostly base their views on electron microscope demonstrations of epidermal fibrils. However, Jarrett and Spearman (1964) pointed out that these are more likely artefacts of the fixation process necessary for electron microscope work.

It is known that at least some of the granular layer enzymes are released from intra-cellular bodies known as lysosomes, and it is possible that an ordered rupturing of these structures could form the basis of a co-ordinated and pre-determined keratinization process (Jarrett and Spearman, 1964). Acid hydrolases are thought to be progressively released when the membrane is ruptured, (Pearse, 1968).

PSORIASIS

Psoriasis is a chronic inflammatory skin disease involving abnormalities in keratinization. There are two main defects in the epidermis:

1) there is a great increase in the rate of cell division in the basal cell layer
2) there is an absence of a granular layer.

This results in the production of too many epidermal cells which do not undergo their full autolysis before keratin formation takes place. These abnormally keratinised cells collect into parakeratotic scales which have a silvery-white appearance. Stankler (1970) very aptly noted that "the irony of the patient with psoriasis is that whilst he strives to live, his skin has difficulty in performing the normal process of dying".

Psoriasis has been known at least since Greek times (Nardelli, 1959). It is a chronic disease, which is incurable due to its latent and hereditary nature, (Steinberg et al, 1951; Novotny, 1966; Kimberling and Dobson, 1973). Occurrence of lesions varies from small plaques which cause little discomfort to extensive involvement of the body, and this grossly interferes with the working and social life of the patient. In rare forms, generalised pustular psoriasis, the involvement is so great both in the skin and systemically, (Baker, 1971; Braverman et al, 1972) that this type can be fatal.

Psoriasis is often found in association with some forms of arthritis, (Bollet and Turner, 1958; Bunim et al, 1962). Baker (1967) states that psoriasis is 2-3 times more common in poly-arthritic patients. Controversy remains over a possible association between psoriasis and diabetes mellitus, although Burns and Whitehouse (1973) recently found impaired glucose tolerance in some cases.

Clinical and Histological Features

Psoriatic plaques have a silvery-white appearance, due to air

spaces between the scales, (Burks and Montgomery, 1943). The capillaries within the lesion become tortuous and dilated which gives the skin a red appearance. Lesions can occur on any part of the body, except for the mucous membranes, which are rarely affected, although the buccal mucosa of psoriatic patients shows an elevated mitotic rate, (Kaidbey and Kurban, 1971).

A common characteristic of psoriasis is pitting of the nails, which also have an accelerated growth rate, (Dawber, 1970). There are many disturbances of nail growth, but these do not seem related to the severity of psoriasis, (Calvert et al, 1963).

The composition of the scale keratin seems a relatively constant feature, (Flesch and Jackson Edoda, 1958). Flesch et al (1962) report the following characteristics:

(a) increased soluble proteins, uracil, mucopolysaccharides, sulphydryl-containing proteins, total phosphate, pentoses.

(b) decreased free amino nitrogen.

The histological picture is as follows:

there is marked epidermal hyperplasia; the rete pegs (see Fig. 1) are elongated. Typical of a classical inflammatory response, there is vasodilatation and leakage of lymphocytes and polymorphonucleocytes (Jarrett et al, 1966). This is why psoriasis has been described many times as a chronic inflammatory disorder, but Jarrett et al state that this cellular infiltration could be due to the abnormally large amounts of leucotactic lipids present in the psoriatic epidermis. These cells sometimes collect together to form Munro abscesses, which are sterile until complicated by a secondary infection. In the rare type of pustular psoriasis,

very large Munro abscesses are formed. As stated above, scale-like keratin is produced in which the horny layer cells still retain their nuclei. There is absence of a granular layer.

Telner and Fekete (1961) think that the vascular changes preceed all other events. A sudden activation of catechol-0-methyl transferase causing vasodilation by metabolism of vasoconstrictor catecholamines is suggested by Bamshad et al (1970). Capillaries often remain dilated, (Freedman et al, 1963) and abnormal in response after clearing of the lesions, (Millberg, 1947; Illig, 1966; Reid and Jarrett, 1967).

Clinically normal skin shows histological and enzymatic evidence of altered metabolism, an example being the elevated pentose phosphate shunt system which is increased in activity, (Comaish, 1963; Gordon and Johnson, 1967; Ruzicka et al, 1969). However, Halprin and Ohkawara (1966) found no difference biochemically between lesion-free psoriatic skin and normal skin, except for a decreased ability to form cholesterol esters. This was also the finding of Comaish (1963) and Gara et al (1964).

Epidermal Mitosis and Keratinisation in Psoriasis

In psoriasis, the mitotic rate of the basal layer is increased about 7-fold, reducing total E.T.T. to about 8-10 days, (Halprin, 1972). The cause of the greatly increased mitotic rate is unknown, but Voorhees and Duell (1971) suggest a defective chalone mechanism.

It is likely that the E.T.T. is inadequate for the keratinocytes to form a granular layer, (Weinstein and Van Scott, 1965; Van Scott, 1966), the absence of which causes abnormal keratinisation, (Jarrett and Spearman, 1964; Kaku et al, 1964). However the mitotic rate and the state of the granular layer are not so easily related, as has been

shown especially in the ichthyosiform dermatoses,(Frost et al, 1966). Fry and McMinn (1968) showed that in the healing lesions the granular layer often reforms before a significant fall in mitotic rate. Vitamin A can induce a granular layer, but also produces mitotic stimulation, (Jarrett and Spearman, 1964). However these effects can be explained by the tendency of Vitamin A to lyse lysosomes; in the upper epidermis, this would cause enzyme release (the formation of a granular layer) and in the lower epidermis this could cause mitotic stimulation of the basal cells, (Jarrett and Spearman, 1970).

It seems that in the region of the granular layer, there is insufficient release of hydrolytic enzymes to destroy cell contents whilst the cell is keratinized. Jarrett and Spearman, (1964) report the occurrence of many incompletely metabolised constituents in the psoriatic horny layer, and also the presence of the hydrolytic enzymes normally found in the granular layer. Steigleder and Raab (1962) found DNase activity decreased under the horny layer and suggested that this could explain the retention of the nuclei in the horny layer. The enzymatic changes though are not specific for psoriasis, and occur in other inflammatory dermatoses, (Fry, 1968). The lack of the enzymes normally appearing in the granular layer could be explained by deficient lysosome rupture, (Jarrett and Spearman, 1964; Rees, 1967).

It will be seen later that coal tar is a very valuable treatment particularly in psoriasis, as well as other inflammatory, particularly chronic, dermatoses. Realisation of tar's complex nature and also that of the skin makes apparent the wide range of interactions between tar and the skin - from the causation of skin cancers to the healing of psoriasis.

2. SKIN DISEASES CAUSED BY COAL TAR

Coal tar is probably more renowned for its ability to cause skin diseases, rather than for its therapeutic effects. Veteran tar workers are very familiar with the propensity of tar to cause dermatoses. The incidence of these diseases was higher years ago before the introduction of regulations, which have now drastically reduced their occurrence. One of the many actions of tar is to stimulate epidermal cell division, giving rise to many types of tar tumours, as for example, tar warts.

There have been many attempts to find the cancer-producing factor in coal tar. Kennaway, in 1924, reviewed the difference types of tar and their tendencies towards carcinogenicity. Gas-works tar, lignite tar and some forms of petroleum were well-known to produce cancer; coke oven tar was also implicated. Kennaway suggested that the carcinogenic fractions were contained in the higher-boiling fractions, i.e. between 250-500°C. Blast furnace tar was not carcinogenic. This tar contained many more phenols, but less phenol itself, than did gas works tar. This characteristic, as well as the presence of higher paraffins and a small amount of benzene, naphthalene and anthracene, it shared with low temperature tars.

Bloch and Widmer (1926) also found that distillates obtained at temperatures less than 230°C (at 1.15 mm. pressure) did not produce cancer in mice. At temperatures above 250°C, the substances produced had a stronger carcinogenic action than the crude tar or the total distillate. They suggested that these carcinogenic substances were probably high-molecular weight cyclic hydrocarbons, and that the carcinogenic

action of tar is contained in not one, but in several compounds.

Wood (1929) reviewed the investigatory work in this field and concluded that tar warts were caused by high temperature tars and not by gas works tar or low-temperature tar. Phenols seemed to cause dermatitis, but did not seem to be implicated in the production of papillomas. The heavy oils from high-temperature tars caused a more pustular reaction (perhaps these are then implicated in "tar acne"), but still did not cause papillomas. Combes (1954a) reported the carcinogenic fraction to be in the range 230 - 400°C - i.e. the anthracene and heavy oils.

Kreyberg (1927) stated that the production of tar cancers seemed related to its hyperaemic effects - i.e. the dilated blood vessels could supply more oxygen and nutrients. Berenblum (1929) made a hitherto unexplained observation that the mustard dichloroethyl sulphide inhibited the development of tar tumours.

The use of tar fractions as wood preservers caused hyperkeratosis in cattle, cats, dogs and rabbits wearing wooden collars (Köhler, 1954). These preservatives contained chlorinated benzene and naphthalene derivatives. However, in mice, naphthalene, anthracene, phenanthrene and pyrene were found not to induce hyperplasia or carcinogenesis. These disagreements between workers maybe in part due to the different susceptibilities to coal tar of different species (Woglom, 1926).

In 1953, Fisher published a study of tar ailments in tar distillery workers. Tar was found to affect the hair follicles, and cause acute and chronic erythema, pigmentation, chronic tar dermatoses and warts and epitheliomas. Inflammation of the follicles was common, causing "tar

acne" - it was also common for a wart to form around one of these inflamed follicles. There was also a close connection between susceptibility to tar warts and acute tar erythema. 70% of the tar warts occurred on the head and neck; 28% occurred on the forearm and hands; 1.7% were found on the scrotum and 0.2% were described as "else-where". The incidence of warts was definitely related to exposure.

Combes in 1944 stated that the middle and heavy oils and naphthalenes are capable of producing erythemas and papular dermatitis, and that the tar acids in the middle oils, i.e. phenol, cresols, cresylic acids and xylenols may cause burning, erythemas, pruritis and ecchymoses (a type of interstitial haemorrhage). Cases of contact of occupational dermatitis were well known in the production of synthetic resins from this fraction. However, alleviation of itching by low boiling fractions which mainly contain phenols was reported by Rothman and Shapiro (1949). Obermayer and Becker (1935) also wrote that it is generally assumed that the anti-pruritic effects are due to the phenols and cresols, although they do say that these are irritant in high concentrations. These workers report naphthalene as being one of the irritants in tar.

Tar can also be absorbed through the skin to produce systemic toxicity. Babes and Lazaresco-Pantzu (1928) found extensive lesions of rabbit spleens after painting the skin with tar.

Davidson (1925) produced liver damage by painting ethereal coal tar solutions on rabbit ears. In some cases there was regeneration of the tissue. Liver damage was also reported by Grigor'ev (1959) along with spleen and kidney lesions, which he attributed to systemic absorption of the phenols. However, the tar was applied to the skin in benzene,

which itself is known to be highly toxic,(Malten et al, 1968).

The results above only represent a small fraction on the work carried out on the adverse effects of coal tar on the skin, from which have developed stringent regulations for tar workers, leading to the drastic decrease in the incidence of tar dermatoses. It is now common knowledge that exposure of the unprotected skin to neat coal tar can be very dangerous both in terms of direct effects to the skin and systemically through exposure over a long period leading to absorption through the skin.

However, use has even been made of the hyperplastic action of tar. Combes (1944) stated that a good coal tar paste properly applied was one of the best remedies for industrial (or contact)dermatitis. Gross et al (1954) suggested that it caused the horny layer to be thickened; this would give added protection against external irritants.

3. SKIN DISEASES TREATED BY COAL TAR

(a) INTRODUCTION

The use of coal tar as a dermatologic therapy is reported to be over 2000 years old (Kerr and Plein, 1953). In 1894, Fischel made the first report of the specific use of coal tar to treat skin diseases (Combes, 1947); the therapy became very fashionable and further reports of its use followed, (Sack, (1896); Leistikow, (1900) and Dind, (1906)). In 1909, Brocq used a low temperature tar to treat lichen simplex, a chronic scaly inflammatory dermatosis and also neurodermatitis. Following this, White, (1921) wrote very enthusiastically of the use of coal tar in a variety of skin conditions, including eczemas, pruritis ani et vulvae, neurodermatitis, etc. Brocq had used neat coal tar on weeping lesions, but White found that this often caused smarting and burning. This was avoided by reducing the concentration to 5% and incorporation in a zinc oxide and petrolatum base. However White used a high-temperature tar (Combes 1947) and the effects of different modes of production were already evident in their different efficacies. In the treatment of widespread psoriasis, the dangers of systemic absorption following too great body coverage were stressed. It was thought that this could be due to the high phenol content.

(b) COAL TAR AND ULTRA-VIOLET LIGHT

It became apparent that in cases such as scaling diseases like psoriasis, the use of ultra-violet (UVL) light therapy in conjunction with coal tar treatments was very beneficial. Goeckerman in 1925 described such a regimen which has been extensively used and modified to this

day, (Young, 1972). In the original treatment, White' s coal tar ointment is applied to the skin for 24 hours; the excess is then removed with olive oil, but ensuring to leave a film of tar through which the patient is irradiated with UVL. The remaining debris is removed in a soap and water bath (or an oatmeal or soda bath if the skin is sensitive). This treatment is repeated daily. Muller and Kierland (1964) report that this treatment has shown no toxicity in 38 years of use.

With the exception of a small minority, most psoriatics experience improvement with increased sunlight. The idea arose to sensitise the skin artificially and Goeckerman tested several fluorescent substances such as eosin, sodium chloride, quinine and rose bengal; all were found to be useless. Coal tar was the only efficacious material, none of its fractions being as good as the crude coal tar, (Goeckerman, 1931). To exclude vehicle effects, olive oil, petrolatum, cod liver oil, and ergosterol were tested for possible photo-sensitisation, but they had no effect. Obermayer and Becker (1935) suggested that acridine in the tar maybe the photo-sensitiser. However, Everett and Miller (1961) stated that the wavelengths required for such sensitisation are out of the ranges of hospital lamps. Muller and Kierland (1964) found that coal tar actually filtered UVL instead of sensitising for it, although Herrick and Sheard (1928) found that irradiated coal tar showed increased transmition to UVL.

Even with extensive body coverage, Goeckerman reported no systemic toxicity. O'Leary (1943) recommended the Goeckerman regimen, condemming the use of treatments such as X-Rays and potassium arsenite (Fowler's solution), which produced side-effects worse than

the disease itself. The Goeckerman regimen was found to be free of such side-effects - O'Leary states that the worst effect was blistering from the ultra-violet ray treatment and that this did not constitute a large inconvenience. In 1968, Perry et al described their successes with the Goeckerman regimen both in terms of patient satisfaction and control of the disease. These patients were severely afflicted, the disease and their treatment necessitating hospitalisation. The most frequent and troublesome side-effect described by these workers was folliculitis, which subsided rapidly on interruption of the treatment.

A modified form of the above regimen was initiated by Ingram (1953). His 'Ingram' regimen started with a coal tar bath, followed by UVL therapy. The ointment applied contains dithranol, (an anthracene derivative), in Lassar's paste. Ingram also agreed that as well as coal tar's own antipsoriatic effect, it probably potentiates the light therapy.

(c) THE SEARCH FOR THE THERAPEUTIC FRACTION OF COAL TAR

Coal tar has certainly stood the test ot time as a dermatologic therapy; however, its unwanted side-effects still remain. It is smelly and messy, immiscible with water, and therefore difficult to remove from the skin, clothes and bath, (Combes, 1947). It is also irritant in some cases, (Carney and Zopf, 1955). There have been many attempts to isolate the therapeutic fraction of coal tar in a hope that this will cut out side-effects such as folliculitis and skin staining, and also to produce a more cosmetically acceptable product. In some cases a systematic search for a more specifically active fraction was undertaken;

others seemed to involve a random selection of coal tar constituents and formulation of these into a cosmetically acceptable form. Towle, (1921) advocated washing the tar before formulation to remove the irritant effects, but the problem is certainly far more complex.

Combes, (1944, 1954) related the story of the "Diener" at Jadossohn's and Neisser's Clinic who himself always went to the gas-house to ensure he obtained tar from the bottom of the tank - "In der Tiefe liegt die Wahrheit". Combes (1944) suggested that a possible reason for its greater efficacy was that fixed gas, aromatic light oils, ammonia, naphthalene and other irritating aromatic compounds and liquors could be expected to rise to the surface of the barrel avoiding inclusion in the tar. A slightly different explanation was put forward by Kinmont (1957), who stated that the tar would contain less light oils and tar acids and a higher amount of middle and heavy oils, naphthalene and anthracene oils.

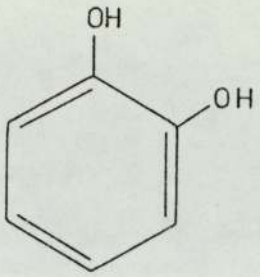
Jaffrey's (1928) work showed that fractions containing the greatest concentration of tar acids were most effective, and a low naphthalene concentration reduced irritation; this described the features of a low temperature tar. Combes (1954) did not agree with the finding about naphthalene; he found the naphthalenes to be beneficial and non-irritant in acute vesicular dermatoses, acting as keratoplastic agents. Obermayer and Becker (1935) suggested that only low-temperature tars should be used in the treatment of skin disease. They pointed out that high-temperature tars contain large amounts of free pitch and carbon, rendering the tar thick and heavy. These workers tried three different methods of analysing tar effects. They used fractionally distilled

mixtures (i. e. separation on a temperature basis), fractions separated by solubility properties in ether and by use of individual constituents of coal tar. All were formulated at 5% in White's original formulation. Some higher concentrations were used to monitor irritant effects.

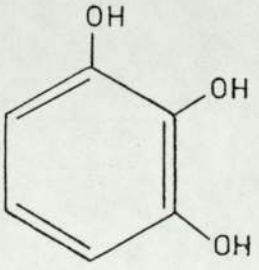
The results obtained from clinical trials were as follows: the low-boiling fraction collected at 150°C was inferior to coal tar in 50% of the patients, caused irritation in half of the cases and was more effective when used in conjunction with UVL therapy. The fraction collected at 250°C was definitely less efficacious than the crude coal tar; combined with UVL it caused a small improvement. The most favourable fraction was the highest boiling distillate, which also was more effective with UVL treatment. Effects of the pitch were indefinite but it was irritating.

Ether soluble and non-soluble fractions were found to be equal in effects to the tar, but there were inflammatory complication in their use. No single chemical tested equalled coal tar (see Fig. 3). Catechol and 8-hydroxyquinoline (the latter in some cases being irritant) had "a decided effect", although no sensitising with UVL occurred. Pyrogallol and leningallol were only slightly inferior to crude coal tar but the former caused irritation, and is known to be systemically toxic, (Goodman and Gilman, 1970). β-naphthol seemed to check the spread of parakeratosis.

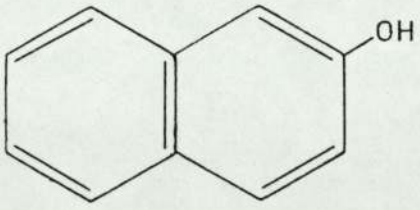
Obermayer and Becker themselves warned to interpret the results from such clinical trials with caution, since even simple routines are known to cause dramatic improvements in hospitalised patients. They concluded that the irritating compounds in the tars seemed to decrease in amounts with an increase in boiling point of the fraction. Since



CATECHOL



PYROGALLOL



β-NAPHTHOL

Fig. 3

fractional distillation and separation by solvents did not specifically locate very active fractions, they suggested that future work should be concentrated on the effects of individual compounds found in coal tar, for example, catechol and 8-hydroxyquinoline.

Downing and Bauer (1948) tested several different low - and high-temperature tars in a variety of dermatoses, and concluded that high-temperature tars were useful in the treatment of chronic scaly dermatoses such as psoriasis, but that these must not be used in the acute stages of skin diseases. Combes (1954) offered an explanation for these findings on a tar acid/ naphthalene content basis; he says that in general, a high tar acid content would cause irritation in the eroded skin in an acutely inflamed condition. Tars with a low tar acid and medium naphthalene content relieved pruritis and acute vesicular eruptions; tars with high acid and little or no naphthalene were most effective in chronic scaly dermatoses. Morley (1970) maintained that the best treatment for chronic psoriasis was crude gas works tar; this refers mostly to low temperature tar.

Nelson and Osterberg (1927) fractionated their tar by steam distillation and reported the steam distillate as effective as coal tar in infantile eczema. This preparation was also reported to be non-staining and did not cause folliculitis. Such a distillate probably contained a large proportion of phenols.

The production of individual fractions did not provide a coal tar preparation which was widely accepted as being superior to crude tar in either efficacy or cosmetic acceptibility. "Synthetic tars" were proposed: Guy et al (1939) listed the main constituents of the light, middle,

heavy and anthracene oils, of a coke oven tar. These oils comprised just over 50% of the tar, the pitch forming 44.7%. A "synthetic tar" was made up, bearing in mind the original structure of the tar.

Anthracene	1·10%
Naphthalene	10·90%
Phenanthrene	4·00%
Carbazole	2·30%
Picoline	0·58%
Quinoline	0·58%
Pyridine	0·58%
Phenol	0·70%
Cresol	0·75%
Petrolatum to	100·00%

Butterworth (1950) reported its use in lotions, baths and at full strength for psoriasis of the scalp.

Similarly Kinmont (1957) tested a synthetic tar of the following composition:

Anthracene	4·17%
Naphthalene	41·66%
Phenanthrene	16·66%
Carbazole	10·43%
Picoline	2·08%
Quinoline	2·08%
Pyridine	2·08%
Phenol	4·17%
o-cresol	4·17%
Toluene	8·33%
Xylene	4·17%
	100·00%

In a clinical trial, the synthetic tar was reported to be better than Prepared Coal Tar B.P. in its efficacy, cosmetic acceptibility and lower incidence of side effects. Yarrow and Thorn (1966) used the above formula in their tar preparation. A synthetic tar was also described by Saunders and Davis (1947) for the treatment of eczema.

In looking for a better tar preparation, combinations with other agents, reported to be therapeutic, have been developed. Bleiberg (1958) used a 5% commercial "extract" of tar with 2% allantoin as a lotion and found that this improved previously resistant cases; Clyman (1957) found 50% of patients experienced marked improvement of their lesions or complete clearance in some cases.

An allantoin and coal tar cream was described by Singer (1962) to be without any undesirable side-effects, clearing lesions in one to four weeks. Omission of either the coal tar or allantoin rendered the cream ineffective; in this case concomitant UVL therapy did not enhance the therapeutic effect of the cream. There are also combinations with steroids available.

To try to reduce the irritant effects of conventional coal tar ointments, Carney and Zopf (1955) added a surfactant to reduce particle size in the tar. They had found particles up to 100μ in diameter and thought that this large particle size could be a reason for irritation. The surfactant reduced the size to about 3μ. This preparation was found to be clinically effective and the incidence of irritation was reduced by about 75%. Surfactants have been used by other workers to produce washable ointments. Pflag and Zopf (1951) formulated a washable tar preparation and Fanburg (1952) described a "cosmetic", washable and non-staining tar preparation for ointments and scalp application. Goldstein (1953) also formulated a washable ointment using coal tar solution. Lloyd and King (1959) found alcohol-soluble tar extracts in water-miscible bases the most therapeutically and cosmetically acceptable preparations. Attempts have also been made to reduce the staining by coal tar prepar-

ations. Thambiah (1938) suggested formulating tar as a solution in acetone and reported that this did not cause bad staining of underwear, as routine coal tar ointments do. Kerr and Plein (1953) finding all the attempts to isolate therapeutic fractions to be without success, suggested that it could only be improved through vehicle effects.

In his review of anti-psoriatic drugs, Champion (1966) stated that the active constituents of coal tar had still not been found despite many attempts; commercial tar treatments seemed to differ more in cost than efficacy. Although there have been some promising looking fractions reported, in most cases these "new" tars make only the one appearance in the literature; the fact that despite many attempts, the use of crude coal tar is still recommended reinforces the notion of the "mysterious harmony" between tar constituents mentioned by Rothman and Shapiro (1949).

(d) SUGGESTED MODES OF ACTION

It is almost impossible to ascribe a mode of action to tar in the skin, because of the complex nature of both systems. However, it is known that a granular layer re-forms in psoriatic skin after tar treatment, although Fry and McMinn (1968) found it impossible to determine whether this was the primary site of action.

Freedberg (1965) showed that 15% coal tar in cotton seed oil applied 3 times each day for 4 days inhibited incorporation of labelled amino acids into the epidermis. Ruzicka et al (1969) found that tar pastes temporarily inhibited the elevated pentose phosphate pathway in psoriasis.

(e) DISEASES OTHER THAN PSORIASIS TREATED BY COAL TAR

Especially in the past, coal tar has been used to treat a wide range of skin lesions, but now about 50% of coal tar prescriptions are for psoriasis, 15% for eczema and "dermatitis", and 5% for seborrheic dermatitis (the latter disease is often classified as psoriaform); the remaining 30% is accounted for by "a large number of relatively minor dermatological conditions", (I. M. S., Ltd.,* 1972). Mention has already been made of its use in some acute inflammatory dermatoses (Brocq, (1909); White, (1921); Nelson and Osterberg, (1927)), but nowadays steroids are usually prescribed for an acute stage of a disease, and the use of coal tar is often restricted to the chronic phases. Clyman (1957) described the use of coal tar "extract" in combination with hydrocortisone to treat atopic dermatitis. Such a combination is recommended by Rook (1966), since the use of coal tar alone is to be avoided.

Tar was highly recommended for ringworm of the feet by Pardo-Castello (1944), who painted neat coal tar between the toes!

* Intercontinental Medical Statistics Ltd.

SUMMARY

Coal tar preparations are well-established as safe long-term treatments for psoriasis. Its therapeutic constituents remain unknown and attempts to improve its cosmetic acceptibility usually have resulted in a loss of potency.

To highlight the obvious worth of coal tar, other drugs used in psoriasis are reviewed in the following section.

III

A REVIEW OF OTHER ANTI-PSORIATIC TREATMENTS

III

A REVIEW OF OTHER ANTI-PSORIATIC TREATMENTS

1.(a) Anthracene Derivatives

DITHRANOL

Dithranol (1, 8, 9 - trihydroxy-anthracene) is chemically unstable. It has been used as a reasonably safe treatment for psoriasis over the last fifty years, (Swanbeck and Liden, 1966), and together with UVL is one of the most widely used treatments for psoriasis in Europe, (Ippen, 1966).

In the last few years its use to control chronic psoriasis has been widely recommended (Shuster and Comaish, (1966); Waddington, (1968); Dahl, (1971) and Pegum, (1972)). However it should only be applied under medical supervision, as it is very harmful to the eyes and can cause irritation, which in some cases can be severe; dithranol also stains the skin. It has been found that its anti-psoriatic activity is very much enhanced by incorporation into Lassar's paste, (Maclennan and Hellier, 1961). The Ingram regimen which involves the use of dithranol has already been described (see page 26); the staining of the lesions is a great drawback, since it appears that as this is finally fading, the psoriatic lesions re-develop, (Jarrett, personal communication). Bowers et al (1966) found this regimen extremely beneficial; their experiments confirmed the beneficial effects of dithranol and UVL, but the role of the tar baths was uncertain. Dithranol was found to be safe and free from systemic toxicity in the routine doses used in psoriasis, (Gay et al, 1972).

TRIACETOXYANTHRACENE

This compound was used to try and reduce the staining and burning which occurs with dithranol, and which necessitates hospital supervision. Hellier and Whitefield(1967) found it was less potent but more acceptable to patients, because of the reduction in staining and irritation. It cleared or greatly improved 25 out of 41 cases who treated themselves, whereas dithranol must be applied by a trained nurse. In some cases they reported dramatic improvement. Hodgson and Hell (1970) found triacetoxyanthracene to be less potent and slower to act than dithranol, and it did cause some staining and burning. Double blind trials in these cases are not possible, since dithranol quickly stains the skin making its presence obvious. The staining, too, may not be purely chemical dyeing, since the stained skin around dithranol-treated plaques has shown some increased melanocytic activity. Dahl (1971) also reported it less potent than dithranol.

Mode of Action of Anthracene Derivatives

Hammar (1970) showed that dithranol interfered with the enzymes of carbohydrate metabolism. This may account for its well-known anti-mitotic action, (Krebs and Schaltegger, 1965).

Swanbeck and his co-workers have produced a great deal of data about this anti-mitotic action. It seems that dithranol inhibits replication of DNA by intercalation of base pairs, (Swanbeck and Thyresson, 1965). Swanbeck and Lundquist (1972) suggest that dithranol interacts with mitochondrial DNA causing production of mitochondria insufficient to provide the energy needs of the fast growing epidermis. Raab and

Paterman (1966) also showed interference with mitosis by inhibitory effects on the oxidative processes of dividing cells. Dithranol was found to inhibit mitosis in the basal layer in psoriatic plaques; this was preceeded by an improvement in the granular layer. Fry and McMinn (1968) made similar observations.

Jarrett and Spearman (1967) suggested that dithranol could be rupturing lysosomes, causing a release of hydrolytic enzymes, which seems to be in some way blocked in psoriasis. The concept of lysosome rupture could also explain why the use of dithranol in the acute phases of psoriasis can cause severe aggravation and irritation.

Hellier and Whitefield(1967) have proposed the following mechanism of action for dithranol and a rationale for the greater cosmetic acceptance of triacetoxyanthracene. It has been found that dithranol is more stable as its keto tautomer (see Fig. 4). The resultant CH_2 group is highly reducing, abstracting oxygen from the skin at a rate which causes irritation and burning. The anthraquinone produced can react to form a reduced anthraquinone dyestuff, which on oxidation, forms a quinone, which is permanently attached to the skin causing staining. It is thought that this is the way in which dithranol exerts its anti-mitotic action, by blocking oxygen receptor sites and thereby reducing the energy available for mitosis. It can be seen that the related compound triacetoxyanthracene can only exist in its enolic form, and so none of the above reactions can occur. It is probably activated by hydrolysis at the plaque site, where there is increased enzymic activity.

(b) Steroids

The adrenal glands produce steroids which are divided into

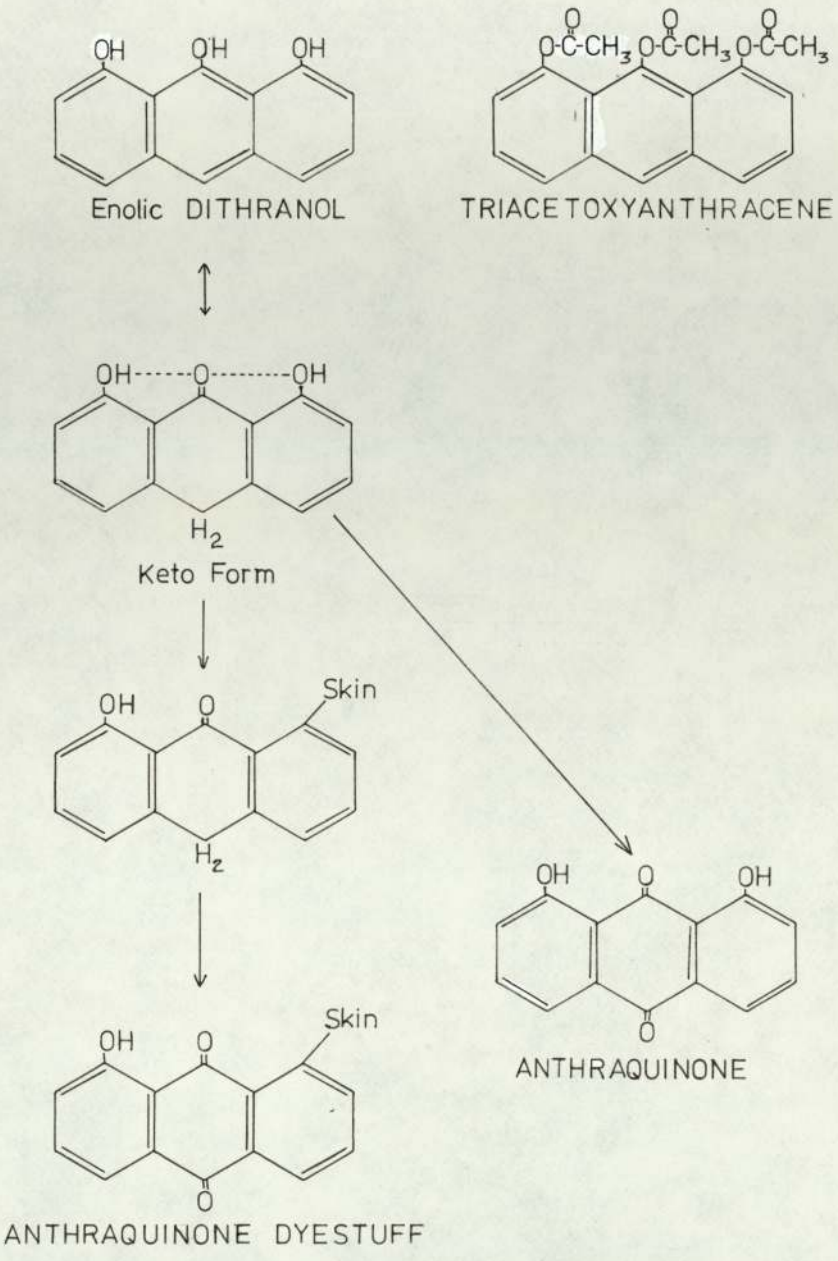


Fig.4 PROPOSED METABOLISM OF DITHRANOL

(Hellier and Whitefield, 1967)

mineralocorticoid and glucocorticoid groups. The former modify electrolyte and water loss and are essential in maintaining a homeostatic balance. Glucocorticoids have a wide range of actions, notably affecting protein catabolism and carbohydrate metabolism; the anti-inflammatory and anti-mitotic properties of steriods are made use of in diseases such as psoriasis, which have an unknown, but non-infective, cause.

Steroids have been widely used in supra-physiological doses to suppress inflammation, especially in the connective tissue diseases such as rheumatoid arthritis. However concomitant with their potent ameliorative properties, they have serious side-effects when treatment is prolonged, as it usually is in psoriasis. These effects are due to direct actions of the steroids themselves and also are a result of indirect effects of interfering with endogenous steroids. The systemic effects arise because the drugs have to be given in increasing doses when used in such a chronic disease, and percutaneous absorption becomes significant, (Morley, 1970). This systemic absorption occurs to a greater extent in children, (Feiwel, 1969). After large doses of steroids, the following severe systemic effects have been recorded:

(i) excessive protein breakdown (gluconeogenesis) leading to muscle weakness and osteoporosis.

(ii) carbohydrate disturbances, which may trigger off diabetes mellitus.

(iii) electrolyte disturbances, the most common being oedema and potassium deficiency (sodium being retained). This may be a contributary factor to the mental depression which is often found in steroid-treated patients.

(iv) abolition of the pituitary-adrenal stress response, which occurs on high doses, (Bowman, Rand and West, 1968).

(v) suppression of the adrenal-pituitary feedback mechanism by endogenous steroids, leading to adrenocorticoid atrophy, (Scroggins and Kliman, 1965; Carr and Tarnowski, 1966).

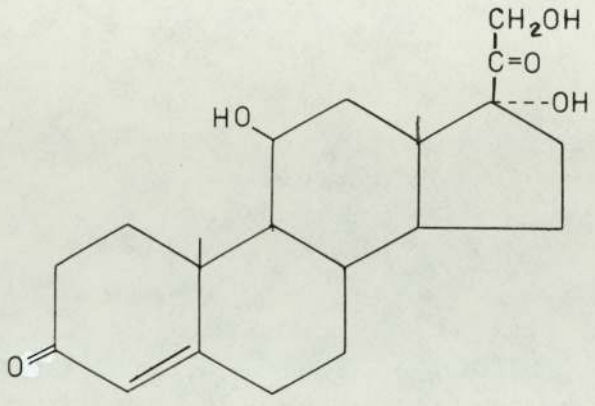
(vi) large doses of topical corticosteroids on pregnant animals has caused foetal abnormalities, and such therapy has been discouraged in pregnant women, (H.M.S.O., 1972)

Interruption of steroid therapy in chronic psoriasis often results in a rebound condition, (Rook, 1966), which is usually worse than the original condition and which does not respond to any anti-psoriatic therapy for quite some time, (Farber and Peterson, 1961).

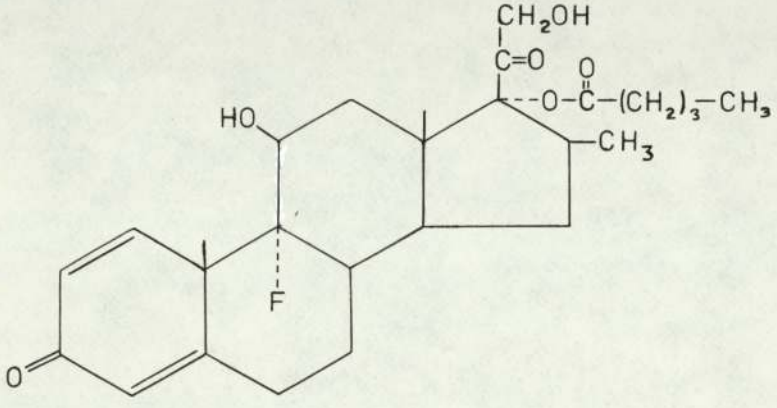
Their potent anti-inflammatory properties have been increased by synthesising especially halogenated derivatives. Any subsequent skin infection is masked by the suppression of the inflammatory manifestation. This makes, for example, some fungal infections impossible to diagnose, (Ive and Marks, 1968). Some steroids frequently used in psoriasis are shown in Fig. 5. opposite

Many attempts have been made to synthesise anti-inflammatory drugs based on the steroid structure, but minimising their mineralocorticoid and other unwanted side-effects. There are numerous reports on the use of steroids in psoriasis, using a wide range of doses and routes of administration. Treatment usually begins with topical application, followed if necessary by systemic administration.

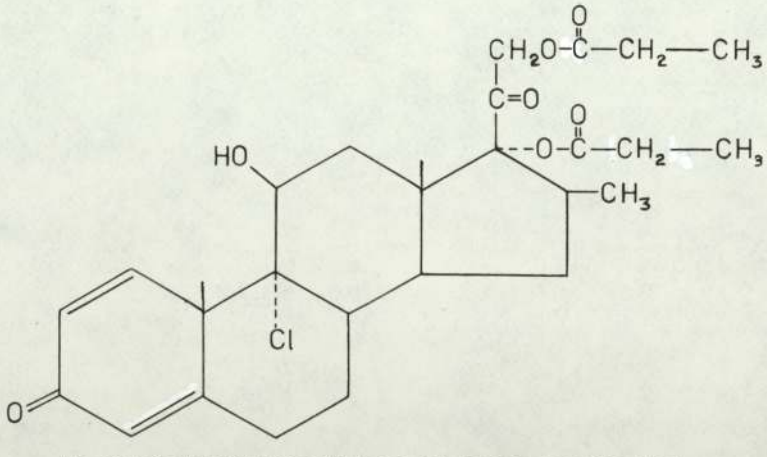
Pearn and Rendle-Short (1970) listed the above systemic side-effects among others which appear in children, in some cases leading



HYDROCORTISONE



BETAMETHASONE-17-VALERATE



BECLOMETHASONE-17, 21-DIPROPIONATE

Fig. 5 SOME STEROIDS USED TO TREAT PSORIASIS

to dwarfism, which has been seen in steroid-dependent asthmatics, (Falliers et al, 1963).

Weidman (1963) reported clearing in 27 out of 43 psoriatics by intra-lesional injections of triamcinolone acetonide with no serious side-effects, except one case of cutaneous atrophy. This atrophy causes scarring with may occur with steroid injections (Freedman et al, 1963); one such case following injection of triamcinolone into the superficial dermis is described by Tanenbaum and Becker (1964), and yet another case of atrophy at the site of injection by Komisaruk et al (1962). Sneddon (1969) warns of collagen atrophy following the use of fluorinated steroids in the treatment of rosacea; side-effects included rebound oedema, redness, papules and the 'Moon face', so typical of patients on steroid therapy. An effect of collagen atrophy is that major skin damage is caused by minor mechanical trauma (David, 1972). A simple knock may raise a flap of non-viable skin.

Shuster (1969) considers the cutaneous atrophy a very serious side-effect and is strongly against the use of steroids in psoriasis. He points out that fungus infections are masked by the anti-inflammatory activity of the steroids. On the face they rapidly produce unsightly reactions such as flushing and telangiectasia (capillary dilatation). Steroid-induced atrophy and telangiectasia are also reported by Stevanoić (1972). Steroids are physically addictive in that withdrawl causes severe rebound conditions to develop due to the adrenal atrophy they produce, (Burry, 1973); the halogenated steroids are especially rapidly absorbed into the systemic circulation when they are used for whole body treatment. They are also psychically addictive in that it is often difficult to stop the

patient taking the drug, which does clear the lesion, and which when compared to coal tar or dithranol, is cosmetically far superior.

Ryan and Baker (1969) published a disturbing report in which they surveyed 104 cases of generalised pustular psoriasis, treated with systemic corticosteroids and folic acid antagonists (see page 46). There were very severe side-effects, and increasingly adverse lapses on attempted withdrawl of the steroids. Only 12 out of the 73 treated had satisfactory control of their disease with minimal or no side-effects. In these 73 cases 17 patients died; all were on steroid treatment at the time of death and in 9 cases the disease was uncontrolled. Steroids were only directly implicated in 2 cases. In only one patient was the cause of death uncontrollable psoriasis. In the others it was suggested that the corticosteroids played an important role in the deaths. There were certainly more deaths and fewer lasting remissions in the steroid-treated group than those treated with folic acid antagonists. Generalised pustular psoriasis may be fatal, but complete and spontaneous remissions are also possible. Hence it is impossible to find out whether the steroids reduced the mortality rate or not.

Mode of Action

Halprin et al (1969) found interference with enzyme systems involved in energy producing processes, reducing the amount of energy available for the critical stages of mitosis, (Belsan et al, 1965). In the case of hydrocortisone, Bullough and Lawrence (1968) found that it acts with their epidermal chalone-adrenaline complex to suppress mitosis.

Fry and McMinn (1968) found fluocinolone induced granular layers together with a fall in mitotic counts. However they also pointed out that

steroids do not inhibit mitosis in wounded skin, which has a similar rate to that of psoriatic skin, so steroids must have some additional anti-psoriatic effect.

Some steroids have a vasoconstrictor action which may help reduce the capillary dilation in the dermis in psoriasis. Use has been made of this phenomenon for steroid essays (Garnier 1971) and to show percutaneous absorption of steroids, (McKenzie, 1962; Thune, 1971, 1971a). Katz and Poulsen (1972) point out that potent vaso-constrictors could drastically reduce their own rate of clearance from the skin.

The Use of Occlusive Dressings with Steroids

Steroids when applied topically are often covered by occlusive dressings, which greatly enhance their cutaneous absorption, (Goldman et al., 1963). This has been attributed to the increased temperature and hydration of the skin which accompanies their use. In fact there are reports of the formation of a granular layer and a slight but significant fall in mitotic rate by occlusion alone, (Fry et al., 1970; Baxter and Stoughton, 1970). Halprin et al. (1969) showed that polythene occlusions suppressed the enzymes of carbohydrate metabolism.

(c) Anti-Metabolites

The following drugs have been developed for use in neoplastic diseases, but have been used in severe psoriasis in an attempt to reduce the high epidermal cell turnover; their use is restricted to hospitalised patients. They will be considered according to the classification of Goodman and Gilman (1970).

(i) Folic Acid Analogues

METHOTREXATE

Newbold (1972) has reviewed the many cytotoxic properties of methotrexate, one of which is interference with folic acid metabolism. Folic acid is a dietary constituent which is reduced to tetrahydrofolic acid (THF) by the enzyme dihydrofolic acid reductase (DHFR). THF forms many derivatives which act as co-enzymes in pathways which function to carry 'one-carbon' units through biosynthetic pathways in cells. Methotrexate prevents formation of THF by binding to DHFR (see Fig. 6). Its affinity for this enzyme is 100,000 times that of folic acid, (Coe and Bull, 1968). One main consequence is that DNA synthesis is blocked, (Rees et al, 1967). However it is now clear that methotrexate also interferes with several other enzyme systems which contribute to its potent cytoxicity, (Newbold, 1972).

Methotrexate is used when patients are resistant to all other forms of treatment (McDonald and Bertino, 1969), and its use is now standard practice for severe psoriasis in the U.S.A., (Rees et al, 1967). However this drug is highly toxic to normal cells too, and especially cells which are rapidly dividing, e.g. cells of the buccal and gastro-intestinal mucosa, and the bone marrow cells. Common side-effects are severe anaemias, reduction in platelet counts, and the fatal agranulocytosis, stomatitis, anorexia, vomiting and gastro-intestinal ulcers. Occasional side-effects are hair loss (alopecia), various skin rashes, pleuritic chest pain, localised peritonitis and fever, (Coe and Bull, 1968). Other side-effects have been reviewed by Berlin et al (1963). No changes are detected in normal skin since mitosis is interfered with only for a few days. Any

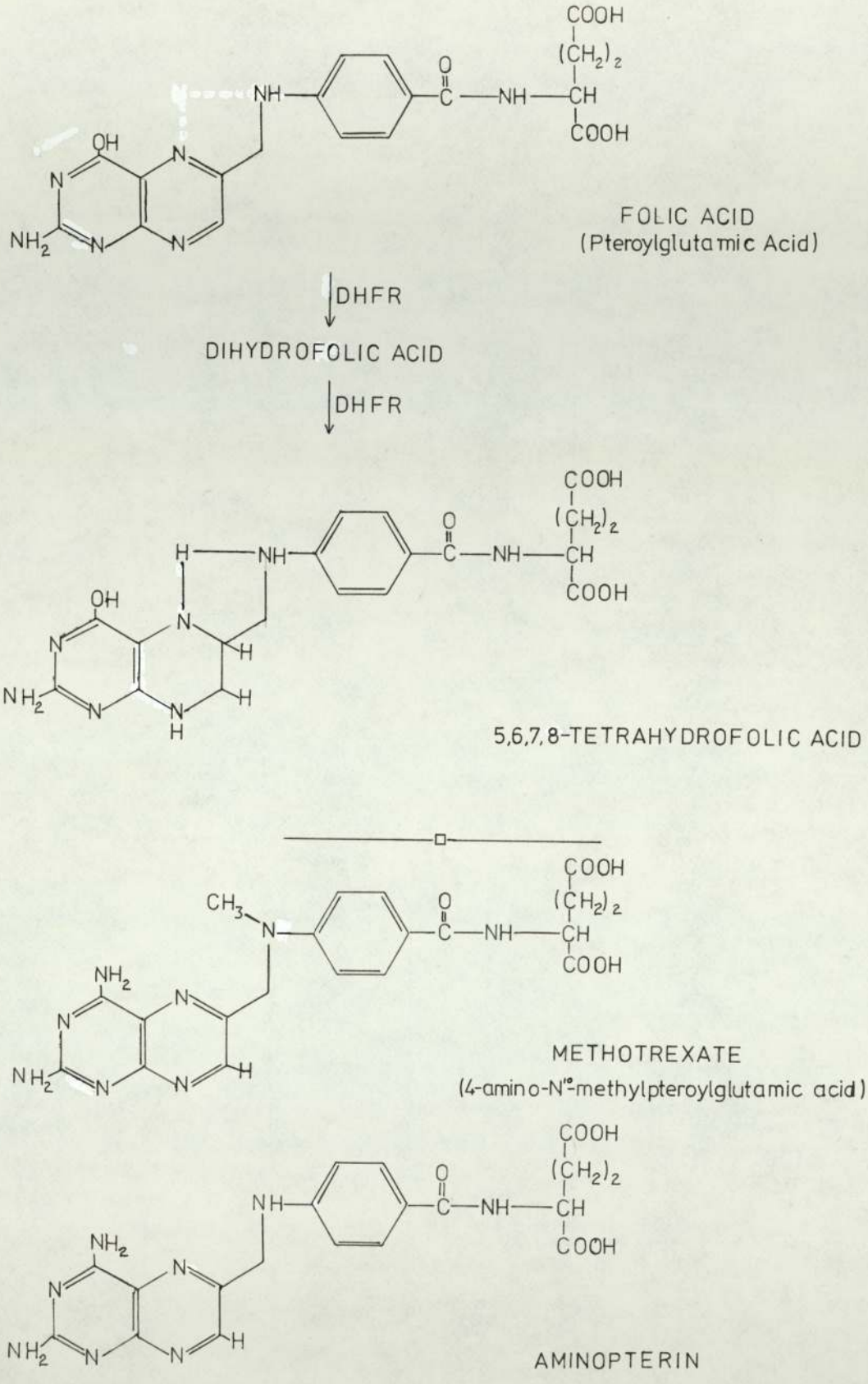


Fig. 6 FOLIC ACID and ANALOGUES

eruptions during this time will probably be confined to the small blood vessels. However, the mitotic rate of the buccal mucosa lies between that of the psoriatic epidermis and normal epidermis, and so mouth ulcers are common in methotrexate treatments. Intestinal mucosa turnover time is approximately equal to the mitotic rate of psoriatic skin and hence gastro-intestinal disorders are common. One of the most sensitive organs is the hair root. The rhythmic hair growth is most intense when a hair is being formed, (Bullough and Laurence, 1958). Abnormalities occur in the shape of the hair bulb under methotrexate therapy; when mitosis is arrested constrictions appear in the hair shaft, and if they are sufficiently great, the hair breaks at this point and there is temporary alopecia, (Van Scott et al, 1957). However this is reversible.

There have been many reports of successful treatment with methotrexate. Biro et al (1967) injected 50 mg. intra-muscularly every 7 - 12 days into psoriatic and normal skin and found that the major change after 24 hours was a marked decrease in mitotic index.

Roenigk et al (1969) reported that the use of methotrexate reduced the time normally required for other treatments; for example, the Goeckerman regimen was reduced from four to two weeks. It also significantly improved nail lesions and arthritis associated with psoriasis.

Black et al (1964) report its use in psoriatic arthritis, and found that it was successful in reducing joint tenderness and increasing joint motion. The afore-mentioned side-effects occurred, so these people recommended restricting the use of methotrexate to only very severe cases.

Ryan and Baker (1969) found that methotrexate cleared the pustules in many cases of generalised pustular psoriasis. Patients previously treated with steroids did not respond so well but methotrexate helped in "weaning" the patients off steroid regimens. In comparing methotrexate and steroids, they decided that methotrexate was responsible mainly for the short-term side-effects and corticosteroids for the long-term ones.

Recently much evidence has gathered on the incidence of liver damage due to methotrexate therapy, (O'Rourke and Ekert, 1964). (Epstein and Croft, 1969). A disturbing aspect is that damaged livers can still give normal results in liver function tests, (Dubin and Harrell, 1970). It is therefore necessary to take liver biopsies during such therapy. Greaves et al (1971) found that biopsies taken from patients with bullous pemphigoid (a severe blistering disease) when on conventional doses of methotrexate showed that 50% of patients on methotrexate for 3 years would have cirrhosis of the liver or some degree of hepatic fibrosis.

Dahl et al (1971) wrote that the doses used for psoriasis have only recently been recognised as hepato-toxic. In their patients, hepatic fibrosis was present in 50% of the cases and unfortunately unlike other side-effects of methotrexate, hepatic fibrosis is potentially irreversible and may lead to cirrhosis. They also point out that liver function tests, as they stand at present, are not adequate to show such liver damage (also shown by Muller et al, 1969). Biopsies and histological examination are necessary. However liver malfunction as a possible feature of psoriasis itself has yet to be evaluated, (Newbold, 1972), and it has been

suggested that the above hepatoxic effects are not all due to methotrexate, (Roenigk et al, 1971).

It seems that the toxic effects of methotrexate can be in some way reduced by parenteral as opposed to oral adminstration, (Auerbach, 1964; McDonald and Bertino, 1969). Single large doses also seem to be less toxic than a continuous low dose regimen, (Dahl et al, 1972; Condit, 1960; Van Scott et al, 1964; Berlin et al, 1963; Carpenter and Jolly, 1967; Almeyda et al, 1972).

However even with breaks between doses, there is still cumulative toxicity, (Coe and Bull, 1968). Methotrexate is essentially irreversibly bound to DHFR, and so stays in the cell until death and removal from the body, (Berlin et al, 1963). Werkheiser (1962) found that it can persist in mouse liver for up to 8 months after a single injection. It seems that all the DHFR is blocked and this is what causes the toxicity, (Coe and Bull, 1968). There does seem to be some sort of resistance to this phenomenon, which was reviewed by Goodman and Gillman, (1970). It is interesting that there are increased levels of DHFR in leucocytes of patients treated with methotrexate, (Bertino et al, 1962). These workers wondered whether their results were due to selective destruction of cells with low levels of enzymes or due to a definite effort to produce this enzyme by another mechanism.

A derivative of methotrexate, aminopterin (see Fig. 6), has also been used with great caution in psoriasis, although some workers believe its high toxicity should preclude its use, (Rees and Bennett, 1959). Toxic effects can be reduced by adminstering aminopterin in single or weekly or biweekly doses, rather than in a continuous low dose, (Van Scott et al, 1964).

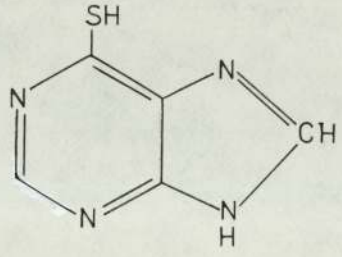
In conclusion, this drug should only be used in the treatment of psoriasis, when the disease has proved intractable to all other treatments, for example some cases of generalised pustular psoriasis. Khan et al (1972) recommend the use of methotrexate in these cases in preference to steroids. It should never be used in women of child-bearing age; adequate renal clearance is vital, and it should never be prescribed when there is any sign of liver malfunction. It is also necessary to ensure that the patient is not concomitantly taking vitamin preparations which may be contaminated with folic acid as this will compete with methotrexate itself, (Van Scott et al, 1964). Also, malnourished people will be particularly susceptible to the toxic effects if their folic acid stores are low. Methotrexate is protein bound in the plasma and its potency will be dangerously increased by drugs which will replace it from this binding, such as the salicylates, the sulpha drugs and para-amino benzoic acid.

The drugs which follow are not in such widespread use as those described above. Some of their properties and reports of their use in psoriasis are briefly discussed.

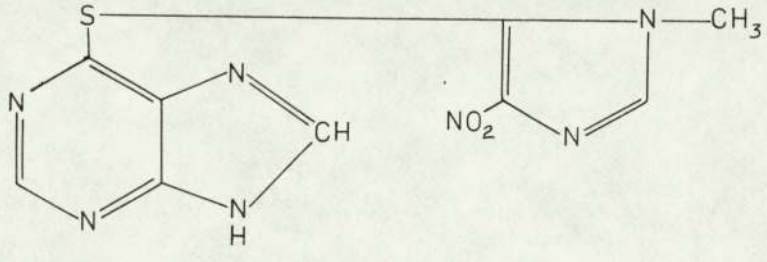
(ii) Purine Analogues

6 - MERCAPTOPURINE

It is thought that this drug takes part in "lethal synthesis" although the exact mode of action is not clear. Kravetz and Balsam (1961) reported "good results with minimal toxicity" from 24 courses of therapy in 12 patients over $3\frac{1}{2}$ years. Leucopenia was the most serious side-effect. They recommended a complete blood count every two weeks. Fredricksson et al (1967) had less certain results from their study of 17 cases over three



6-MERCAPTOPURINE



AZATHIOPRINE

Fig. 7 PURINE ANALOGUES

weeks. 6-mercaptopurine did not influence the frequency of relapse and haematologic complications were common. They conclude that it should only be used for very resistant cases and there must be continuous control.

AZATHIOPRINE

In a review of cytotoxic drugs used in psoriasis, Finzi (1969) found that azathioprine, (see Fig. 7), was the best tolerated and quotes a success rate of 86%. He also states that the anti-metabolites are thought to be effective in psoriasis not only for their anti-mitotic activity, but for their immuno-suppressive action as well. Dawber (1970a) found that azathioprine reduced the rate of finger-nail growth, which had been shown to be increased in psoriasis.

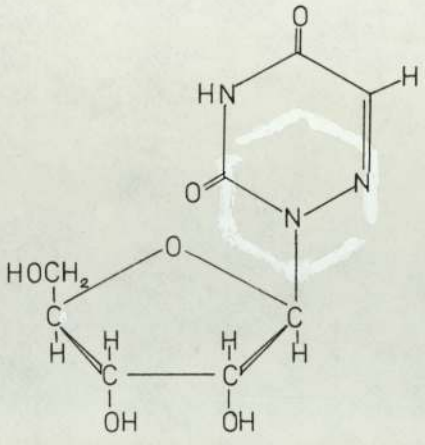
(iii) Pyrimidine Analogues

AZAURIDINE

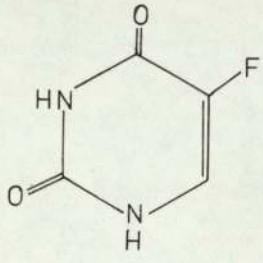
Azauridine compounds interfere and prevent the synthesis of uridylic acid, essential in pyrimidine synthesis. The acetylated form is used orally for psoriasis. Large doses are required but the drug seems to be fairly localised to the lesioned area and side-effects have been limited to moderate anaemia and temporary neurological distrubances, which have both been reversed on withdrawl of therapy, (Calabresi and Turner, 1966). In a clinical trial carried out by Turner and Calabresi (1964), there was no oral, intestinal or skin ulcerations and no alopecia.

5-FLUOROURACIL

Topical application of this compound, (see Fig. 8) causes painful



AZAURIDINE



5-FLUOROURACIL

Fig.8 PYRIMIDINE ANALOGUES

erosion of the skin and considerable skin damage, (Tjuji and Sugai, 1972). However, keratinization seems to return to normal, although, after such drastic treatment, exaccerbation of the psoriatic plaque would be expected, (Nurse, 1963).

(iv) The Alkylating Agents

(a) NITROGEN MUSTARDS

MECHLORETHAMINE

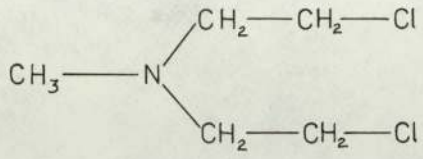
The alkylating agents are thought to react with bases on DNA, and especially with guanine. Bifunctional groups can intercalate for example, two guanine residues on each side of the sugar-phosphate backbone of DNA, and thus prevent its fission and hence replication. These agents have other wide-spread enzymatic inhibitory effects in the cell as might be expected from their great reactivity. The alkylating agents are therefore very potent cytotoxic drugs.

Epstein and Ugel (1970) applied topical mechlorethamine (see Fig. 9), to 12 cases of resistant psoriasis. 10 experienced significant improvement, with no systemic toxicity. However the developement of hypersensitivities prevented its use in the long-term. Mandy et al (1971) found only one case of contact dermatitis out of 7 treated and reported liver function tests normal. They state that it should not be used for routine management of psoriasis. Zackheim et al (1972) also found that the high incidence of sensitising reactions precluded its use in routine treatments.

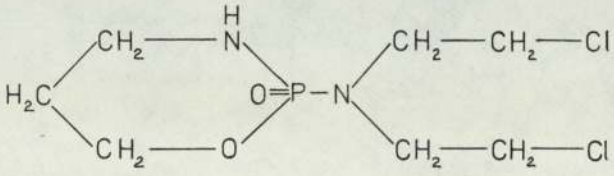
CYCLOPHOSPHAMIDE

This drug was designed to be more selectively concentrated by

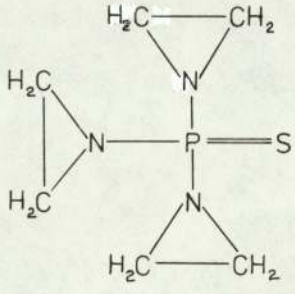
(a) NITROGEN MUSTARDS



MECHLORETHAMINE

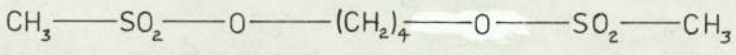


CYCLOPHOSPHAMIDE



TRIETHYLENETHIOPHOSPHAMIDE

(b) ALKYL SULPHONATES



BUSULPHAN

Fig.9 ALKYLATING AGENTS

the lesions, so cutting down the toxicity. The rationale is as follows: the drug only becomes active when the cyclic group is removed at the nitrogen-phosphorous linkage by the enzymes phosphatases or phosphamidases, It is known that certain neoplastic cells contain high levels of these enzymes, and so a more selective and greater activation of the drug was expected by the neoplastic cells. However plasma and liver can also activate the drug, and so toxicity can be widespread, (Greenwald, 1967). In psoriasis, Finzi (1969) quoted an 80% success rate for cyclophosphamide, being second only to azathioprine.

TRIETHYLENE THIOPHOSPHAMIDE

Triethylenethiophosphamide (Thio-tepa) as described as a non-irritating alkylating agent, (Heydendreich, 1971). Eleven out of fourteen patients experienced complete clearing of previously intractable plaques. However in 2 cases, treatment had to be terminated prematurely due to the developement of leucopenia.

(b) ALKYL SULPHONATES

BUSULPHAN

There is some controversy about the mode of action of busulphan, ("Myleran"); it may not intercalate base pairs at all but react with thiol groups on proteins, (Greenwald, 1967).

Möller and Waldenström (1970) used busulphan and found improvement in 5 out of 9 cases; 4 were uninfluenced by the drug. The usual topical therapy was continued and regular blood counts were made. There were no serious side-effects during the two-month trial except for slight depressions of thrombocytes and leucocytes. The ameliorations were not

correlated to the age, sex or type of psoriasis or to previous effects of methotrexate treatment. Side-effects of this treatment are similar to those described for the folic acid antagonists; they include pulmonary fibrosis, Addisonian-type hyperpigmentation, weakness, anorexia and weight loss.

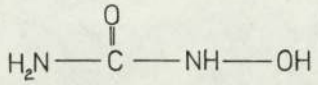
MISCELLANEOUS DRUGS USED IN PSORIASIS

HYDROXYUREA

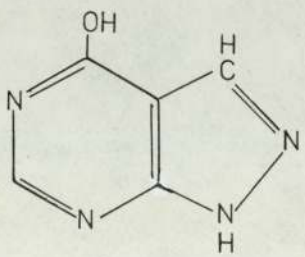
Hydroxyurea, (see Fig. 10) seems to interfere with DNA synthesis, but not with RNA or protein synthesis, (Young and Hodas, 1964). Leavell and Yarbro (1970) found that the lack of gastro-intestinal toxicity permitted oral dosage and they report that it is useful in the management of refractory psoriasis. At high doses there was impairment of renal function and the bone marrow was depressed; the latter is however rapidly reversible. Dahl and Comaish (1972) report lesion clearance with hydroxyurea, but found it more toxic than methotrexate. They suggest its use should be restricted to cases where methotrexate has failed and become too toxic. Hydroxyurea causes macrocytosis, decreases in haemoglobin and in some patients leucopenia and anaemia. Moschella andGreenwald (1973) found that its withdrawl can cause a rebound condition which is intractable to treatment. Combination with methotrexate has been suggested by Sauer (1973).

ALLOPURINOL

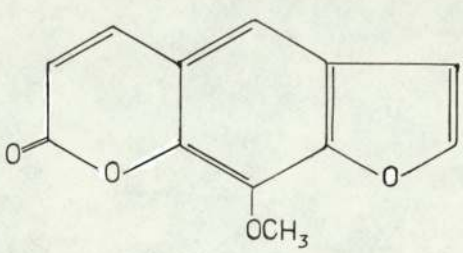
This xanthine oxidase inhibitor, (see Fig. 10) is reported as low in toxicity and successfully treats psoriasis with no rebound conditions on withdrawl, (Newbold, 1972). However, Feuerman and Nir (1973)



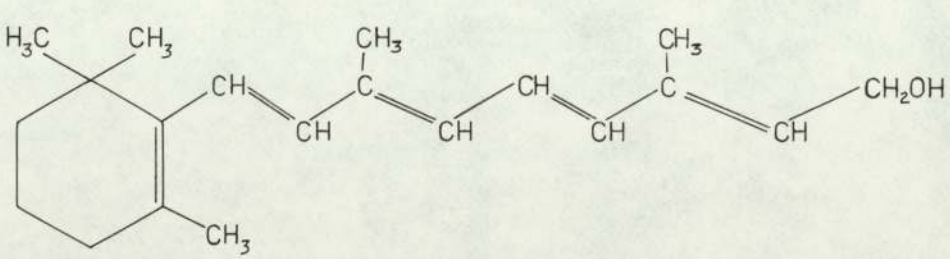
HYDROXYUREA



ALLOPURINOL



METHOXSALEN



VITAMIN A ALCOHOL (RETINOL)

Fig.10 MISCELLANEOUS DRUGS USED IN PSORIASIS

found allopurinol to be no better than a placebo.

ACTINOMYCIN D

This crystalline antibiotic binds with DNA to prevent RNA synthesis by RNA polymerase, (Goldberg, 1965) Actinomycin can damage hair roots and other rapidly proliferating cells, and may induce severe skin rashes. Finzi, (1969), states that this is one of the most active and cytotoxic drugs used in psoriasis.

METHOXSALEN

Photo-excited methoxsalen is known to be cytotoxic, and was used successfully in combination with dithranol in dithranol-resistant patients (Willis and Harris 1973). It is thought to react with DNA and to a lesser extent with RNA.

VITAMIN A

Vitamin A has been shown to induce granular layers in skin which previously was without this layer, thereby influencing the type of keratin produced, (Lawrence and Bern, 1958;Jarrett and Spearman, 1964). Jarrett and Spearman used this in combination with triamcinolone in the treatment of psoriasis. Its ability to cause granular layer formation has been attributed in part to its property of lysosme rupture, (Dingle, 1961; Jarrett and Spearman, 1964; Reid and Jarrett, 1967; Rees, 1967).

On the hypothesis that retinoic acid is the form in which Vitamin A acts, Fry et al (1970a) applied retinoic acid to the skin of 12 psoriatic patients. 6 experienced reformation of the granular layer. However, the treatment time was only 2 weeks, and a longer-term study of 3 months showed it to be irritant and of no therapeutic value, (Macdonald

et al, (1972). However it was also found to be of value in combination with steroids by Macdonald and Fry (1972) and Orfanos et al (1973).

AMMONIATED MERCURY

This old and not very effective treatment is now rarely used, (Champion, 1966), because of the known dangers of mercury poisoning from systemic absorption. Due to its rapid reaction with sulphhydryl groups, Samitz (1958) suggests an interference with keratinisation. However the high reactivity of mercury with many enzyme systems probably results in many of these being blocked in the psoriatic process.

DAPSONE

Dapsone, diaminodiphenyl sulphone, is used in the treatment of leprosy, but was shown to be of value in psoriasis, (Corrales-Padilla, 1966). Recently Macmillan and Champion (1973) found it to be life-saving in one patient with long-standing psoriasis which had become intractable to other treatments.

FUSIDIC ACID

Fusidic acid has recently been reported as being beneficial in some cases of psoriasis, (Voetman, 1971), It has a steroid structure (Natarajan and Paily, 1971), and these workers found it more efficacious when used in combination with a keratolytic. However there is disagreement on its efficacy, (Hall-Smith, 1971) and placebo effects have been suggested, (Levantine and Baker, 1971; Jackson, 1971).

IODOCHLORHYDROXYQUINOLINE

This compound has been reported as efficacious especially in

combination with a steroid. Its anti-bacterial and antifungal properties make it especially useful in flexural psoriasis, (Champion, 1966).

SUMMARY

The above discussion of some of the many drugs used in psoriasis highlights the poor state of knowledge about the disease itself and the drugs used to treat it. Very few can claim to have a specific effect, perhaps lest of all the most complex and yet safe therapy, coal tar.

2. The Cost of Anti-Psoriatic Treatment

The incidence of psoriasis has never been precisely determined (see Section 2), but in Great Britain it is commonly taken to be 2%, (Ingram, 1964). This forms a market size of about 1.3 million; this number of patients can expect to be treating their disease on and off for most of their lives. Lesions may be so limited as not to warrant any treatment or they may be sufficiently severe to merit hospitalization. Different therapies will be employed at different stages in the disease, but for the most part, treatments are required for the protracted chronic stages of psoriasis. This is where psoriasis becomes an economic problem too, both in terms of costs of treatment and in days lost from work. About 1.5% of the working population has annually at least one period of absence due to skin disease, (O.H.E, 1973), although no figures are available for psoriasis itself.

Psoriasis is a type of chronic disease where typically patients themselves will try many treatments to alleviate their condition, and many of these are available without prescription. This makes it very difficult to judge what sort of medicaments patients are buying for self-treatment, and also to evaluate the efficacies of these preparations. The best indicator of the drugs most commonly recommended and used is the amount of prescriptions issued for a particular drug. Of all drugs prescribed for psoriasis and related disorders, 60% are topical corticosteroids, (this includes those combined with an anti-biotic). Coal tar preparations at 15% form the second largest group of drugs prescribed. The new steroids are the most expensive treatments, their cost being mainly due to the large expenditure in the research and developement for their production,

their synthesis and testing and in their formulation.

Calder (in Stankler, 1970) priced commonly used anti-psoriatic drugs according to the Drug Tariff and showed that coal tar and dithranol treatments were far cheaper than were equivalent amounts of corticosteroid preparations. Coal tar could be up to 30 times cheaper than the new synthetic steroids. Commercial tar preparations are of course more expensive, particularly when special extracts are used, these often being very poorly qualified. Examples of these have been chosen which do not contain other known anti-psoriatic agents in significant proportions. Their descriptions and prices are shown in Table 1.

Prices have been taken from MIMS (1972, 1973). MIMS states that the prices shown are mostly wholesale, and they do not bear any relation to retail price or to a price which may be charged for a private prescription. They are quoted for purposes of comparison. Prices do not include the pharmacist's allowance on cost, dispensing fee of container allowance, all of which are variable and which together with drug price form the cost to the Health Service, (Jepson, personal communication).

Table 1

MAIN CONSTITUENT	TRADE NAME	MANUFACTURER	PRICE OF 1g (p) 1972	1973
COAL TAR	Tardrox	Carlton	0.47	0.63
COAL TAR SOLUTION	Carbodome	Dome	0.33	0.57
COAL TAR EXTRACT	Alphosyl	Stafford-Miller	0.32	0.32
COAL TAR DISTILLATE CETYL ALCOHOL	Tar-Biosone	Berk	1.00	1.00
COAL TAR DISTALLATE	Pragmatar	S.K.F.	1.08	0.51
PURIFIED COAL TAR	1. Pixcyl	Fisons	0.4	0.48
	2. Sebigen	Fisons	0.40	0.40
TRIACETOXY-ANTHRACENE	1. Exolan cream	Dermal	1.76	1.80
	2. Exolan paste	Dermal	1.61	1.64
STEROIDS				
BETAMETHASONE	Betnovate	Glaxo	1.35	1.35
TRIAMCINOLONE	Ledercort	Lederle	1.26	1.26
FLUCLORLONE	Topilar	Syntex	2.00	2.00
BECLOMETHASONE	Propaderm-Forte	A.H.	16.60	14.40
FLUOCINOLONE	Synalar-Forte	I.C.I.	17.20	17.20

1. Coal Tar Solution, U.S.P.: 20g. coal tar, 5g. polysorbate, 75 ml. ethanol. (Dome Laboratories, communication).

2. From a high-temperature tar to Coal Tar Solution U.S.P. standard, using i.m.s. instead of ethanol. (Stafford-Miller, communication).

3. From a high-temperature tar.

SUMMARY

Psoriaform diseases are extremely common. There are very few people in temperate climates who have not experienced at least a very mild form, dandruff, which at most is an embarrassing inconvenience. Seborrheic dermatitis is also very common and the itching often associated with it can be quite discomforting. However psoriasis itself, even in its milder forms, is an extremely distressing disease, often intefering quite drastically with the patient's working and social life. Its capricious tendency to spontaneously remit or exacerbate to serious proportions makes treatment even more difficult. Psoriatics understandably become very depressed with their disease and this in itself is not conducive to possible remissions.

The treatments reviewed leave much to be desired. Steroids have certainly lost their almost "panacea image" on the discovery of serious side-effects when used in the long-term; the long-term treatments known to be safe are coal tar preparations and dithranol. With dithranol it has been said that the silvery-white and red patches of psoriasis are merely exchanged for a brown-stained skin, (Jarrett, personal communication). Its use also necessitates carefully controlled supervision in hospital. Coal tar also stains the skin and in some cases produces irritant reactions Baer et al (1955). It is also messy and has a smell which is far from pleasant. However in a review of the recent advances in the treatment of psoriasis, Pegum (1972) advises the use of steroids by day and the use of coal tar and dithranol by night, the latter two being more "likely to produce a more sustained improvement."

It is obvious that coal tar does contain constituents which are highly efficacious in this disease and their isolation for use in psoriaform disease may provide a long-awaited and safe treatment. This isolation may cut out constituents which stain and irritate. On the other hand it may well be that the therapeutic effect is quite non-specific, and isolation of these compounds may reveal very toxic effects, when out of the tar environment. However, this seems an unlikely possibility.

It is therefore undoubtedly worthwhile to have a closer look at coal tar in the hope of finding those constituents which are both therapeutically active and cosmetically acceptable in the treatment of psoriasis. The N.C.B. is not directly involved in the production of coal tar for this use, there being numerous other economically viable outlets. However when the possibility exists for a public corporation to provide a much-needed treatment from what would be an almost unnoticeable proportion of the total amount of annual tar production, the normally employed economic criteria of profitability are not the major considerations.

PART 2

I
INTRODUCTION

I. INTRODUCTION

1. AN ANTI-PSORIATIC MODEL : THE MOUSE TAIL TEST

The previous attempts to uncover the therapeutic constituents of coal tar have been many and varied, and have met with little definite success. Several critisms can be made:

1) There has rarely been a well followed scheme of testing of fractions with a logical underlying theoretical rationale.

2) The test situations have usually consisted of clinical observation of various types of psoriatic lesions, which have tended to be subjective.

3) The use of psoriatic lesions for routine screening of many fractions is not adequate. Classification of lesions is almost impossible without taking an unacceptably large number of biopsies to determine the stage of the disease, and psoriasis is certainly a disease which requires certain distinct types of therapies at different stages of the disorder.

4) Evaluation of drugs in clinical situations is difficult, due to the high incidence of spontaneous remissions especially under hospitalisation, (Samitz, 1958).

5) The history of these various attempts is sadly lacking in histological evaluation of the effects of the tar.

Proposing the testing of many fractions of coal tar and many combinations of fractions obviously requires a routine screening test, capable of producing reproducible results. An animal model which would allow standardisation by species, strain, age, sex and weight would be preferable. One such model is suggested by the work described by Jarrett and Spearman, (1964). These workers used mouse tail scale epidermis as a "model" for psoriatic epidermis.

Jarrett and Spearman pointed out that the mouse tail epidermis,

unlike the rest of the mouse body skin, is histologically and biochemically very similar to psoriatic skin. The mouse has a similarly flexible keratinising epidermis to humans over most of its body; it is of course more hairy. The epidermis is thin with a granular layer, which is about one cell thick. However, the tail skin looks scaly and histological examination shows that this horny layer is produced without a granular layer. Fig. 11 shows that groups of hairs are sparsely scattered among the scales, (Spearman, 1964). It is only in areas of hair production that a granular layer appears on the mouse tail, and only here is the scale keratin absent, and thin flakey basket-weave keratin can be seen, (Fig. 12). Jarrett and Spearman (1964) have shown that the two types of keratinising epidermis have many features in common with normal human epidermis and that found in the parakeratinising epidermis in psoriasis. There also seem to be a number of enzymes in mouse tail scale horny layer that are found in the parakeratotic horny layer of psoriatic skin. Also there is incomplete autolysis of the epidermal cell contents in mouse tail scales, so that a comparatively solid horny layer is produced.

It has already been discussed (see p. 14) that the presence of a granular layer seems to be a pre-requisite for orthokeratinisation, (Jarrett and Spearman, 1964). This layer is absent in psoriasis and in mouse tail skin, and both produce a more solid and parakeratotic horny layer. It has been shown that certain compounds, such as Vitamin A, which induces a granular layer in psoriatic epidermis, can also induce this layer in mouse tail scales, (Lawrence and Bern, 1958; Jarrett and Spearman, 1964). The occurrence of scale keratin in the mouse tail,

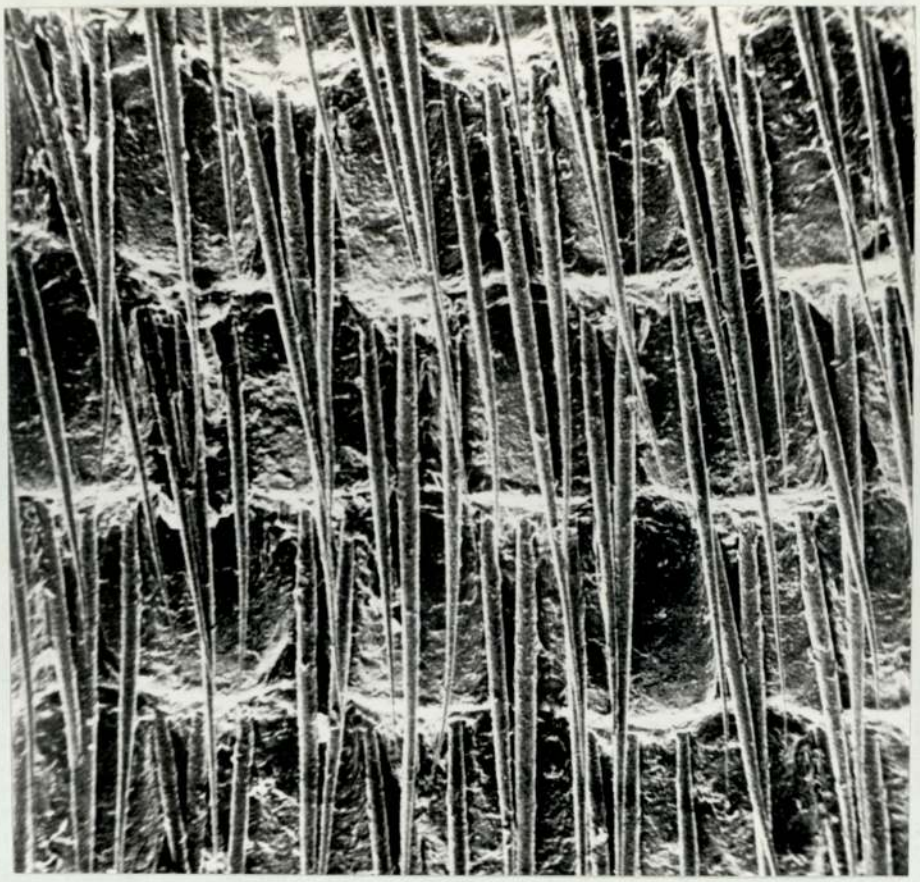


Fig.11a MOUSE TAIL SKIN
Electron micrograph of surface.
(By courtesy of Miss Inez Bolderson)

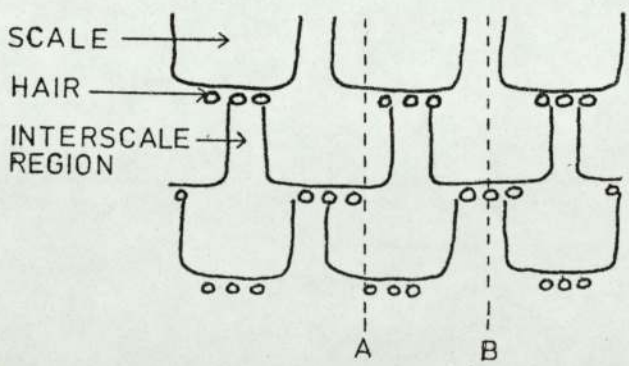


Fig.11b MOUSE TAIL SKIN
Diagrammatic represention of (11a)
(From Spearman and Garretts,1966)

A Sections selected for tests
B Sections rejected: interscale regions have granular layers

Fig. 12

NORMAL MOUSE TAIL SKIN

(a) Vertical proximo-distal section to show main features of scale and hair follicle regions:

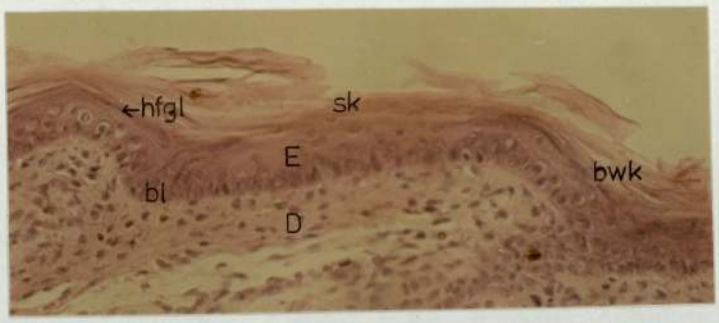
E	:	Epidermis
D	:	Dermis
bl	:	basal layer
bwk	:	basket weave keratin
hfgl	:	hair follicle granular layer
sk	:	scale keratin

(b) Similar section to show detail of the hair follicle granular layer with overlying basket-weave keratin; scale epidermis shows the lack of a granular layer and strands of scale keratin.

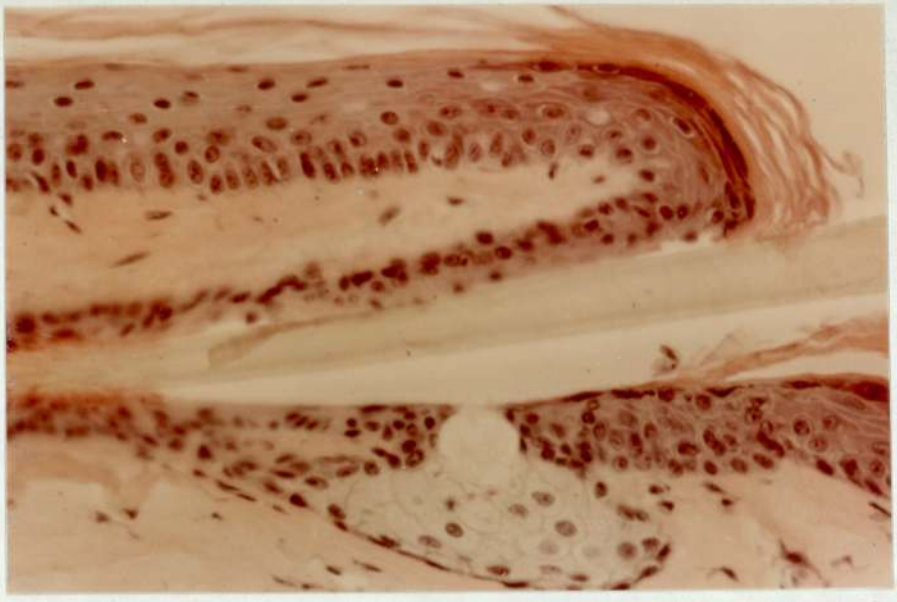
(c) Same section as (b): viewed by phase contrast to show clearer structure of sebaceous gland (sg) emptying into the hair follicle (hf).

Fig. 12 NORMAL MOUSE TAIL SKIN

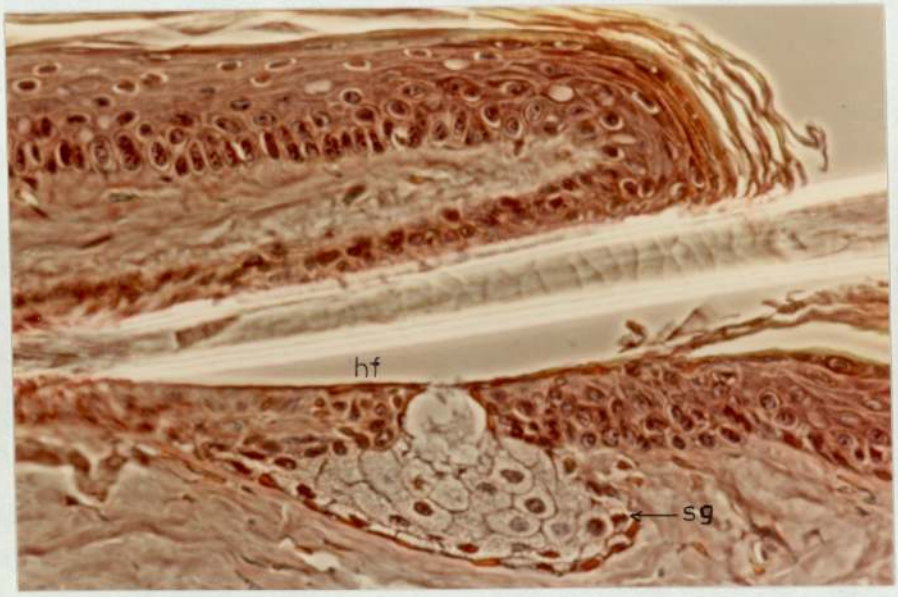
(a)



(b)



(c)



especially with orthokeratotic areas which could be monitored, suggested a very useful experimental model for screening possible anti-psoriatic tar compounds for a specific effect of granular layer induction. This was thought to be a suitable parameter in looking for a specific anti-psoriatic treatment for the following reasons:

1) Although there is disagreement over whether the primary disturbance in psoriasis is dermal or epidermal in origin, (Burks and Montgomery, 1943), many workers believe from histological studies that the epidermis is the primary site of the psoriatic lesion, (Pinkus and Mehregan, 1966; Braun-Falco, 1963). Hence it is not unreasonable to concentrate on epidermal changes as representing an important feature of psoriasis.

2) Inducement of the granular layer has been shown to be one of the first events in healing psoriasis, shown in the following drug trials:

   (a) A wide range of anti-psoriatic treatment produced granular layers first, (Van Scott and Reinertson, 1959).

   (b) Komisurak et al. (1962) felt that the lack of a granular layer was an important defect in the disease, because it was one of the first histological improvements after intra-lesional injection of triamcinolone.

   (c) Freedman et al. (1963) reports that the earliest and most consistent finding in psoriasis treated with corticosteroids was the induction of a granular layer followed by a loss of parakeratosis.

   (d) Within 2 days of using topical methotrexate, the first sign of histological improvement was reformation of the granular layer, (Fry and McMinn, 1967).

   (e) Fry and McMinn (1968) also found that when psoriasis was treated with methotrexate and dithranol, there was improvement in the granular layer before a significant fall in mitotic counts.

The changes induced in mouse tail scale epidermis by anti-psoriatic drug combinations, (Jarrett and Spearman, 1964), indicated that this skin could provide a guide to what other possible anti-psoriatic drugs may do in the psoriatic lesion. Although the mitotic rate of mouse tail skin is unknown, Epstein and Maibach (1965) stated that epidermal turnover time is similar for mice, men and rats.

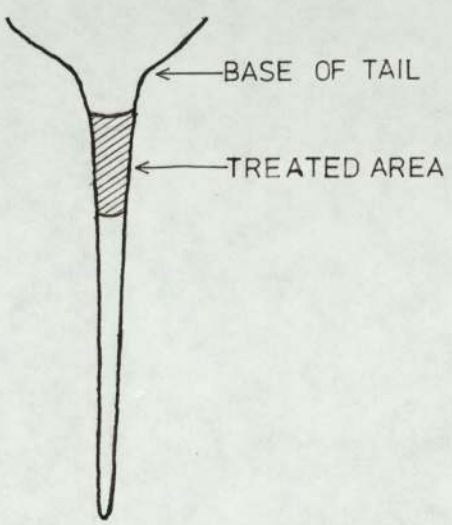
It was decided that this mouse tail model would provide a test system in which a large number of tar fractions could be screened without putting patients through the discomfort and possible danger of repeated applications of previously unknown fractions.

The aim therefore, was to find which fraction of tar produced the most well-defined granular layer in mouse tail scale skin with the minimum of side-effects such as thickening of the skin, skin staining and general unspecified irritant reactions, in order to produce a safe and cosmetically improved long-term treatment for psoriaform diseases.

2. CHARACTERISATION OF THE MOUSE TAIL TEST

(a) METHODS

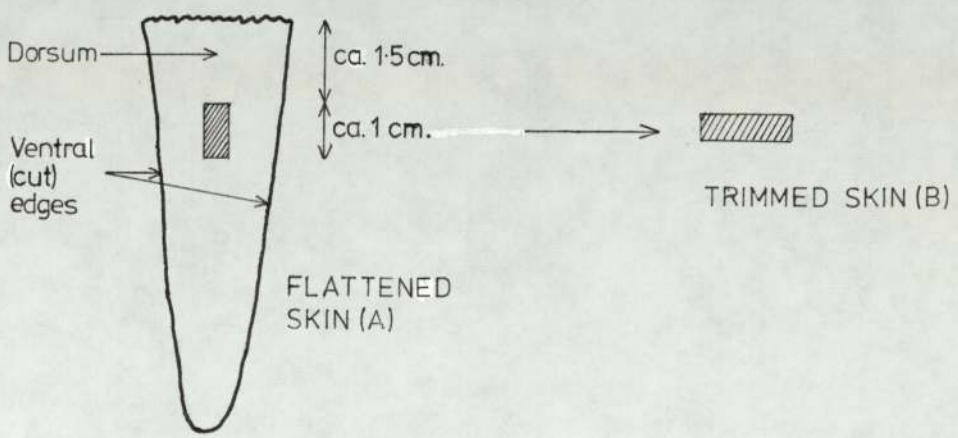
Each fraction of tar tested was formulated at a known concentration with one of the vehicles described on p. 88 . The preparations were applied daily at the same time each day, (about 14.00 hrs.), all around the tail, over an area extending about one centimetre from the base of the tail (proximal end) and about $2\frac{1}{2}$ centimetres along the length, as illustrated below:



The preparations were applied with cotton wool or cotton wool buds, depending on the consistency of the ointment or cream. In later cases where the preparation was very runny, application was by hand covered with "TRU TOUCH" plastic surgical gloves, which ensured intimate contact between the preparation and the skin.

The duration of treatment was pre-determined at 1, 2, 3 or 4 weeks, but if irritation developed, the runs were stopped prematurely. Termination of a run was made 24 hours after the last application of tar fraction. Animals were killed by stunning and dislocation of the cervical

vertebrae. The tail skin was then cut along the ventral surface, overlying the ventral tail vein. After removing the whole tail, the skin was quickly stripped off and flattened (dermal side downwards) on glass in 70% alcohol for at least 10 minutes. The processing described by Jarrett and Spearman (1964) was then followed. The skin was fixed for 18 hours in 70% alcohol, after which it was trimmed as shown:



In trimming it was important to exclude skin near the base of the tail, since in this area the "soft" keratin of the back is merging with the "hard" keratin of the tail, and many of the scales have a granular layer, (Lawrence and Bern, 1958).

The tissue (B) was then dehydrated over 9 hours in 3 changes of absolute alcohol, cleared overnight in cedar wood oil and impregnated with "PARAPLAST" (m.p. 56-57^{o}C) or in "CERESIN" wax over 4 hours, during which time the wax was changed twice before final embedding in fresh wax.

Sections, 7μ in thickness, were cut on an M.S.E. Base Sledge Microtome and floated out on warm distilled water on prewashed slides. (No adherent was used to attach the sections to the slides; it was found

that the best adherence occurred when the sections had been cut with an extremely sharp knife, (see Appendix II), and when the slides had been cleaned in 70% acid alcohol and rinsed in running tap water before drying). About 100% adherence was obtained by leaving slides with mounted sections at 37°C overnight or by subjecting them to 60°C in an oven for 30 minutes.

In some cases, (where indicated), cryostat sections were used. Whole stripped skin was flattened on a spatula and frozen by immersion in liquid nitrogen (-197°C). The frozen tissue was quickly trimmed and mounted on a chuck in "TISSUE TEK" in a stream of carbon dioxide delivered from a "S.L.E.E." ‡ bench freezer.

Sections were cut on a Cambridge Rocking Microtome in a "BRIGHT FS/FCS" cryostat maintained between -30° - -20°C. The sections were mounted directly onto microscope slides, dried in air at room temperature for approximately one minute and then transferred to 70% alcohol for fixation (about 5 minutes). Routine haematoxylin and eosin staining followed rinsing with water, starting at (A) in the procedure shown below:

Wax sections were subjected to the following staining routine:

IMMERSION IN:	Xylene I	FOR:	2 minutes
	Xylene II		2 "
	Absolute Alcohol I		2 "
	Absolute Alcohol II		2 "
	70% Alcohol		2 "
	Tap water rinsing		2 "
(A)	HAEMATOXYLIN (Ehrlichs)		15 " *
	Acid Alcohol		5 seconds *
	"Blueing" in tap water		10 minutes *
	AQUEOUS EOSIN		5 seconds

* Repeated with variable times until nuclear staining adequate, (Youngs, communication).

‡ South London Electrical Equipment Co. Ltd.

The slides were then rapidly dehydrated by momentary immersion in 70% and absolute alcohols into xylene, from which they were mounted with cover slips in Canada Balsam.

In processing and cutting the skin, in most cases the horny layer was detached and lost. Double embedding methods were suggested, (Ayres, communication), but this did not result in retention of the horny layer. It was most probable that detachment occurred during cutting: approaching the knife from the dermal side seemed to keep this layer more intact, but this approach often resulted in shattering of the wax section, thereby making serial sections very difficult to obtain. This was probably due to the cutting of the resistant horny layer pulling apart the rest of the section. In an epidermal approach these stresses would be distributed through the skin and ensuing wax, and hence most of the sections were cut in this way with loss of the horny layer.

(b) EXAMINATION OF MOUSE TAIL SKIN SECTIONS

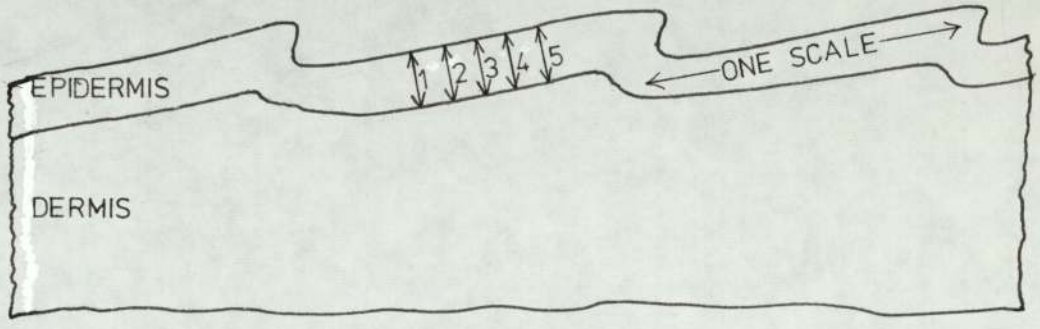
(i) Granular layer inducement

It was important to cut sections which would include only scale epidermis, e.g. along line 'A' in Fig. 11 . It is possible to obtain sections which include the naturally occurring granular layers found between scales, (Riley, 1966a; Spearman and Garretts, 1966) by cutting along a line 'B'. These granular layers would confuse the search for those induced by tar constituents. These sections were discarded for granular layer evaluation and for measurement of epidermal thickness, but increases in naturally occurring granular layers, when observed, were noted.

Similarly, the sections were examined for signs of extension of the hair follicle granular layers, as described by Jarrett and Spearman, (1964).

(ii) Epidermal Thickness

Several sections from each mouse tail were taken in order to provide one suitable for measurement. Sections were cut transversely through the skin along the length of the tail to include at least 10 consecutive measurable "scale" sections of epidermis, as shown below. Using a micrometer eyepiece, (previously calibrated with an eyepiece graticule), 5 measurements of epidermal thickness were made in the mid-scale region of each of the ten scales.



The horny layer (when present), was omitted from measurement, so that a constantly well-defined area from basal layer to the beginning of the horny layer could be measured (Bern et al., 1955). Hence 50 measurements were taken for each mouse.

Epidermal thickness never varied significantly within each mouse, except in cases where epidermal damage was so great as to render measurement impossible, e.g. 40% Coalite Oil Acids.

(iii) Calculation of Epidermal Thickness

The mean of 50 measurements was calculated for each mouse, and these were used to obtain the mean for the group under test. Standard deviations and standard errors were calculated for each group mean,

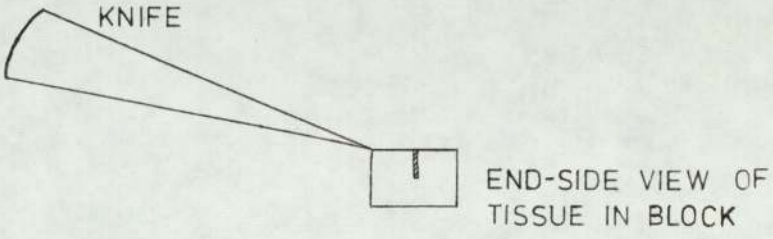
and groups were compared by the 'Student's' t test.

(iii) Alterations in the Horny Layer

When the horny layer remained attached to the epidermis, it was observed for changes in the normal scale keratin. It was hoped that the horny layer would show the "basket-weave" structure described by Jarrett and Spearman (1964).

(c) ERRORS IN MEASUREMENT

Due to the well-defined area of measurement, once under the microscope, sections were easily measured with no source of error of any consequence. However, the production of a good representative section of the animal's skin was dependent on the tissue sectioning procedure. Of the utmost importance was the orientation of the skin relative to the knife. As far as was possible, the tissue was orientated so that the knife cut sections perpendicular to the flattened epidermal surface:



Small errors in such an orientation would not have any significant effect on the epidermal measurements, as shown below in this simple calculation:

The most extreme error which could be made aligning the tissue relative to the knife without being visibly obvious would be 5^{o} either side of the desired perpendicular; this rather large error in 90^{o} is due to the small size of the short side of the tissue observed during orientation (see Fig. 13).

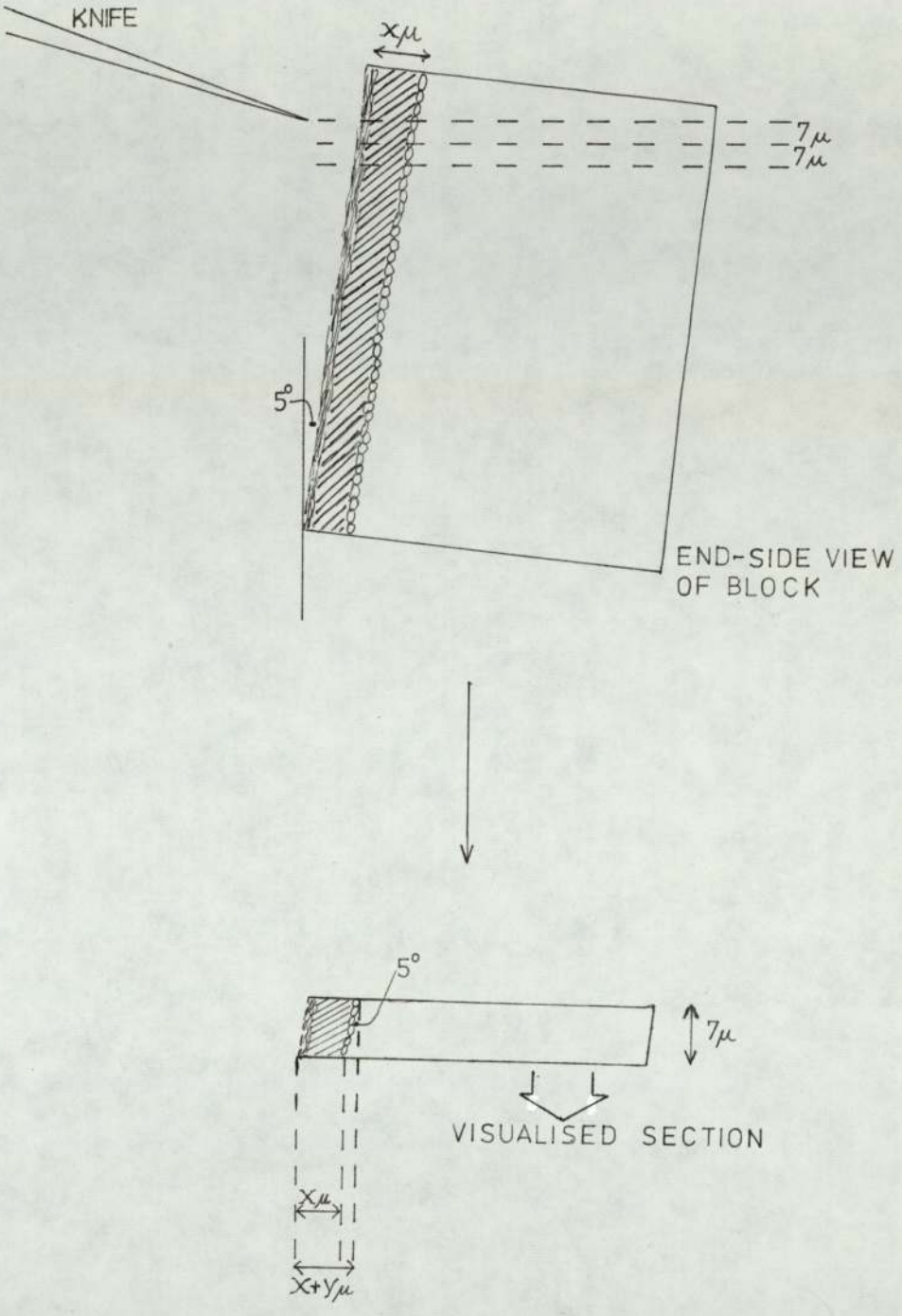
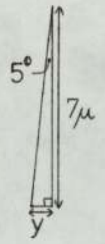


Fig. 13 Errors in Section Cutting

(Diagrams not to scale)

The true epidermal thickness 'x' will appear under the microscope to be 'x + y'

The error 'y' is:



$\tan 5^{o} = y/_{7}$

$y = 7 \times 0.0875\ \mu$

$= \underline{0.61\ \mu}$

The actual thickness could then be increased by about 0.6μ.

(d) CHOICE OF MICE

After initial experiments with 'A2G' mice, it was decided to use the 't. o.' strain as used by Jarrett and Spearman (1964). The source of supply varied between Aston University Animal House mice and those from Messrs. A. Tuck and Sons Limited. It was found that more consistent results were obtained from the latter source, and these mice were used continually in the experiments. The mice were maintained on a diet of '41 B' .

Although in their experiments with Vitamin A, Lawrence and Bern (1958) found no sexual difference in response, male mice only were used, as female mice would have varying hormone levels, and Bullough and Laurence (1964a) have shown that increased oestrogen levels seem associated with increased epidermal activity. Testosterone also increases the mitotic rate in castrated mice, but in intact male mice, testosterone levels are usually constant and consequently of no significance.

3. TAR PREPARATIONS EVALUATED

(a) TARS AND TAR FRACTIONS

The N.C.B. originally supplied samples of high-temperature and of low-temperature tars, these being 'AVENUE' and 'COALITE' tars respectively, (see p. 7). Samples of the tars had also been split into their pitch and oil fractions by heating the tars to about 360°C, (as described on page 10). The pitch was abandoned as, being a very brittle dense mass, it would have presented a very difficult formulation problem. It is also known to contain a high proportion of carcinogens, (Hodgson and Whiteley (1970)).

The whole tars and the oils were then used. Eventually these were both split into acid, neutral and basic fractions, by the following method. 500 g. of each tar and oil were used.

After preliminary heating and stirring to dissolve any suspended solids, 10% sodium hydroxide was added with vigorous shaking in a volume about four times that of the coal tar. In the case of the oils, the aqueous and organic phases separated (Stage (1)) with little interference from emulsion formation. However, in the case of the whole tars, these had to be diluted with an equal volume of solvent, e.g. benzene, toluene and/or ether, to minimise emulsion formation.

To the aqueous layer was added ether, and this was shaken to remove any oils and neutral substances which may have been carried down in the alkaline medium. The ether-washed aqueous layer was treated with concentrated HCl acid until all the tar acids had been

released from their salts. These were then extracted in ether and benzene (or toluene) and washed with tap water until there were no further traces of acid in the organic layer. The mixture was dried by shaking and standing over anhydrous $MgSO_4$ (or with anhydrous $CaCl_2$). The solvents were then removed under reduced pressure leaving the tar acids as a residue.

The basic and neutral fraction left at (1) was shaken with four times its volume or 10% HCl, the protonated basic compounds being run off in the aqueous layer, leaving the neutral organic layer (2). The aqueous layer was washed with ether, basic substances being reconstituted by addition of 10% NaOH. As with the acids, the bases were extracted in ether, which was later removed, after washing and drying, by distillation under reduced pressure.

The remaining neutral compounds (2) were dissolved in ether and benzene (or toluene) mixtures, washed, dried and recovered from the solvent. A sample of the neutral and base mixture was also prepared as above.

Samples collected were then:

TAR	Acids	TAR OIL	Acids
	Bases + Neutrals		Bases + Neutrals
	Neutrals		Neutrals
	Bases		Bases

In the case of Avenue whole tar, fractionation was not possible due to the inability to see the interface of the organic and aqueous layers. After several attempts, involving large dilutions of both layers, fraction-

ation in this case was abandoned.

Fractionating for High-Boiling Tar Acids

Acids were extracted from the following Avenue Tar Oils:

a) naphthalene oil (200 - 230/240°C)

b) creosote oil (225 - 300°C)

c) anthracene oil (290 - 330°C)

d) base oil (330 - 400°C)

e) a mixture of creosote and anthracene oils fractionally distilled into 12 x 10° portions (220 - 340°C)

In the case of extracting acids from these oils, one gallon of each were required to provide sufficient material for testing. In these extractions, it was found essential to pre-dissolve the oils in an equal volume of benzene and toluene, prior to addition of NaOH. Emulsion formation was a difficult problem, even when both phases were well diluted. Sudden freezing, addition of salts and filtering through Kieselguhr had little effect. Large amounts of resinous compounds were formed on addition of sodium hydroxide, and these had to be removed by filtration under vacuum.

The higher-boiling fractions of (e) were partly solid and had to be heated prior to dissolution in the solvents. Papkov and Pats (1969) pointed out that the efficiency of phenol recovery is reduced at high temperatures, but due to the high content of hydrocarbons such as naphthalene, they recommended 40-50°C as an optimum extraction temperature.

(b) OTHER SOURCES OF FRACTIONS

SOURCE	TAR FRACTION	DESCRIPTION
N.C.B.		Avenue Oil Acids:
	A.O.A. '175'	175-185°C
	A.O.A. '185'	185-190°C
	A.O.A. '190'	190-195°C
	A.O.A. '210+'	195-210°C
	A.O. '200'	200-230/240°C:Naphthalene Oil
	A.O. '225'	225-300°C :Creosote Oil
	A.O. '290'	290-330°C :Anthracene Oil
	A.O. '330+'	330-400°C :Base Oil
		Anthracene Oil Acids:
	A.O. '<230'	Below 230°C head temp.
	A.O. '230'	230-240°C
	A.O. '240'	240-250°C
	A.O. '250'	250-260°C
	A.O. '260'	260-270°C
	A.O. '270'	270-280°C
	A.O. '280'	280-290°C
	A.O. '290'	290-300°C
	A.O. '300'	300-310°C
	A.O. '310'	310-320°C
	A.O. '320'	320-330°C
	A.O. '330'	330-340°C
	Coalite H.B.T.A. *	As supplied to the Avenue Tar Plant
		Low-Temperature Tar Acids:
Coalite and Chemical Products Ltd.	Phenol	98% pure
	o-cresol	98% pure
	m/p-cresol	80% pure
	2,4-/2,5-xylenol	90% pure
	L.B.X.	Low Boiling Xylenols: approx. 50% m/p-ethyl phenol 25% 3,5-Xylenol, and smaller quantities of: 2,3-/3,4-xylenol, 2,4,6-trimethyl phenol 2-methyl-4-ethyl-phenol
	H.B.X.	High Boiling Xylenols: approx.: 3,4-/3,5-xylenol 2-methyl-4-ethyl-phenol
	X.L.	230-270°C (H.B.T.A.)
	X.X.L.	250-305°C (H.B.T.A.)

* High Boiling Tar Acids

		Low-Temperature Tar Acids:
Coalite and Chemical Products Ltd.	2, 3, 5-trimethyl phenol	
	2, 4, 6- trimethyl phenol	
	3-methyl-5ethyl-phenol	Individual H. B. T. A.
	4-indanol	
	5-indanol	
Coal Tar Research Association		H. B. T. A.
	L 14/13	227. 0-232. 0°C
	L 14/19	229. 5-239. 5°C
	L 14/47	241. 0-245. 0°C
	L 14/56	244. 0-247. 5°C
	L 16/38	253. 0-256. 5°C
	L 16/53	256. 0-260. 5°C
	L 16/70	260. 5-264. 0°C
	6-methyl-4-indanol	
	1-methyl-5-indanol	
	6-methyl-5-indanol	
Midland Yorkshire Tar Distillers	H. B. T. A. Grade 108	

(c) VEHICLES USED IN TAR PREPARATIONS

There have been numerous methods described for formulating tars and tar fractions, (Martindale, 1972). Since there are many thousands of compounds in coal tar, they will all be absorbed through the skin to different extents, thus making the choice of vehicle very difficult. But within the confines of this project, it was necessary to choose a "suitable" vehicle and compare different fractions within the same environment.

Vehicle 1 (OIL-IN-WATER CREAM)

Cetamacrogol '1000'	5%
Isopropyl myristate	22%
Wool fat	15%
Emulsifying wax	5%
Water	53%

(Reference: Hadgraft and Wolpert, 1960)

Vehicle 2 (OINTMENT)

Yellow Soft Paraffin	95%
Anydrous Wool Fat	5%

Vehicle 3 ('PLASTIBASE 50W' OINTMENT)

Liquid Paraffin	95%
Polyethylene	5%

'Plastibase' is formed by mixing 5 parts of polyethylene with 95 parts of liquid paraffin at 130^{o}C and shock-cooling the mixture. This produces an ointment of gel consistency. It is inert colourless, stainless, odourless and tasteless, (Robinson, 1955)

Lassar's Paste

This ointment was used in experiments with dithranol; no tar preparations were formulated with this paste.

Zinc oxide	24%
Salicylic acid	2%
with starch in white soft paraffin	

(Reference: B.N.F., 1968).

II
EXPERIMENTS

II. EXPERIMENTS

The main mouse - tail skin experiments to compare tar fractions are classified according to vehicles used, followed by the accounts of allied subsidiary tests:

1. Vehicle 1
2. Vehicle 2
3. Vehicle 3
4. Histochemical Evaluation of Tars
5. Other Anti-Psoriatic Treatments
6. Examination of Young Mouse Tails

1. VEHICLE 1

EXPT. V1(i) 5% AVENUE TAR : 21 days
5% COALITE TAR :

Results

Histological examination showed highly significant increases in thickness of the epidermis and cell hypertrophy in both groups. Similar thickening was also found in the mice treated with the vehicle alone, (see Table 2). A granular layer was not induced in the tail-scale region. Control animals were untreated.

EXPT. V1(ii) 10% AVENUE OILS : 21 days
10% COALITE OILS :

Results

Both oils caused epidermal cell hypertrophy and highly significant increase in epidermal thickness, with no granular layer formation.

Comment

It was found that the epidermal thickening in the case of the vehicle was caused by the vehicle itself, and not by the rubbing-in procedure, (see p.171). The wool fat and yellow soft paraffin ointment was adopted for the next set of experiments.

Table 2: Expt. V1(i)

Initial no. mice/group = 10; weight range = 28-32g. Duration = 21 days

	E.T.	S.E.	n	't'	p
CONTROL	35.0	0.90	17		
5% AVENUE TAR	41.6	1.50	9	4.04	0.001
5% COALITE TAR	44.9	1.71	10	5.71	0.001
VEHICLE I	40.4	0.77	18	4.61	0.001

Table 3: Expt. V1(ii)

Initial no. mice/group = 10; weight range = 28-32g. Duration = 21 days

	E.T.	S.E.	n	't'	p
CONTROL	35.0	1.47	6		
10% AVENUE OILS	52.7	1.77	7	7.54	0.001
10% COALITE OILS	48.0	1.80	7	5.50	0.001
VEHICLE I	43.0	1.40	8	3.88	0.005

E.T. Epidermal Thickness (μ)
S.E. Standard Error
n No. of mice at termination
t 'Students' t value
p Probability.

2. VEHICLE 2

EXPT. V2(i) 10% AVENUE TAR, AVENUE OILS : 14 days, 21 days
10% COALITE TAR, COALITE OILS :

Weights of mice were taken prior to killing and dissection of the tail in an attempt to correlate individual body weights with tail thickness.

Results

At the end of 21 days treatment, mouse tails treated with 10% Avenue Tar were stained brown in an uneven fashion, which did not appear to be related to any structure in the tail, e.g. the distribution of hair follicles.

(a) HISTOLOGY

There were no differences between groups treated for 2 weeks and those treated for 3 weeks, either in tail thickness or in morphological changes. In all groups, there was no definite sign of granular layer induction - only increases in epidermal thicknesses, which were highly significant, (see Table 4).

(b) BODY WEIGHT : EPIDERMAL THICKNESS

There was no correlation between body weight and tail thickness, (see Graph 1(a-f), pp.96-7, and body weight was not altered as a result of tar treatments.

Comment

The uneven staining noted in the case of Avenue Tar suggested some uneven absorption of the tar rather than an inherent variance in susceptibility of the tail skin to the tar. This together with the fact

that there was still no definite granular layer formation suggested that perhaps higher doses should be used to increase the amount of tar constituents absorbed.

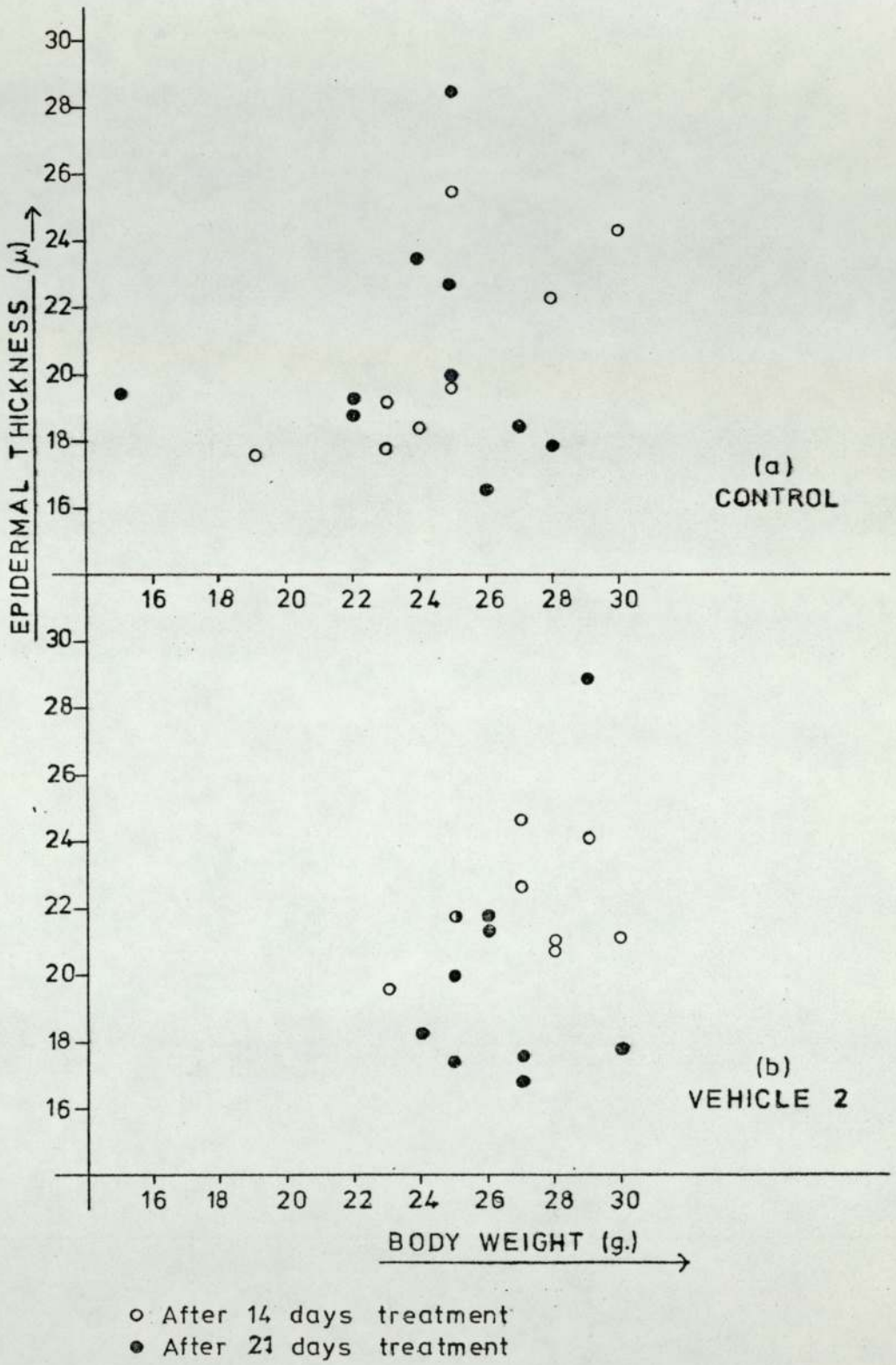
Table 4: Expt. V2 (i)

Initial no. Mice/group = 10; weight range = 28 - 32g.

After 14 days:	E.T.	S.E.	n	t	p
CONTROL	20.6	1.08	8	-	-
10% AVENUE TAR	38.3	1.91	8	8.06	0.001
10% AVENUE OILS	30.8	0.58	7	7.93	0.001
10% COALITE TAR	38.7	2.29	6	7.80	0.001
10% COALITE OILS	31.2	2.98	6	3.75	0.005
VEHICLE II	22.0	0.61	8	1.12	n.s.
After 21 days:					
CONTROL	20.5	1.10	10	0.04	n.s.
10% AVENUE TAR	34.8	0.62	9	11.70	0.001
10% AVENUE OILS	31.8	2.10	9	4.51	0.001
10% COALITE TAR	36.7	1.54	9	8.38	0.001
10% COALITE OILS	28.6	1.40	10	4.33	0.001
VEHICLE II	20.2	1.14	10	0.24	n.s.

There was no significant difference between the same ointments at 14 and 21 days; e.g. Avenue Tar (14) and Avenue Tar (21) 't' = 1.86 (not significant).

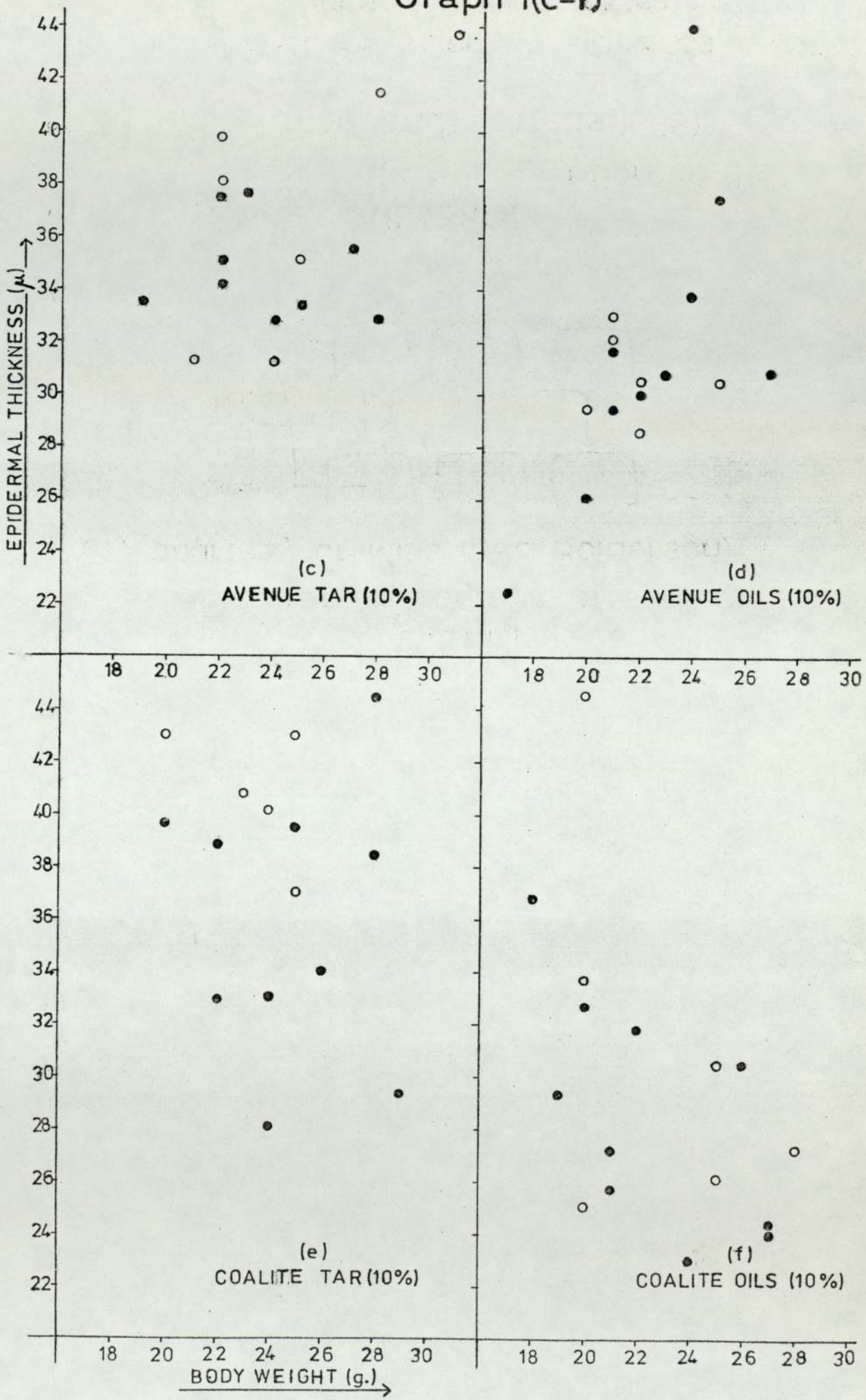
Graph 1(a-b)



EPIDERMAL THICKNESS vs. BODY WEIGHT

CONTROL and VEHICLE 2

Graph 1(c-f)



EPIDERMAL THICKNESS vs. BODY WEIGHT

EXPT. V2(ii)

40% AVENUE TAR
40% AVENUE OILS, OIL ACIDS, OIL BASES + NEUTRALS, OIL NEUTRALS
40% COALITE TAR, TAR ACIDS, TAR BASES + NEUTRALS, TAR NEUTRALS
40% COALITE OILS, OIL ACIDS, OIL BASES + NEUTRALS, OIL NEUTRALS

(See Table 5 for treatment times)

Results

After 4 days of treatment, the mice treated with 40% Avenue Oil Acids became hyperactive and tended to scatter when approached for a treatment, and this group was killed after 9 days, due to the obvious irritant action. The tails were darkly stained in this group after only one treatment; all mice treated with Avenue Oils had very slightly stained tails.

Similar to the effect of Avenue Oil Acids, after 2 applications, Coalite Oil Acid-treated mice became hyperactive; subsequent treatment led to necrosis of the tail below the treated area and so these animals were killed after 11 treatments. In the case of the mice treated with Coalite Oils, towards the end of the treatment period of 28 days, their tails seemed to be showing signs of early necrotic changes. Skin staining by Coalite Oil Acids was seen after one treatment. After two treatments, Coalite Oil-treated mice showed signs of staining, which was not so advanced as in the Acid group. At termination, these mouse tails were fairly patchily stained in all degrees, whereas staining by Coalite Oil Acids was uniform all over the treated area. (Relative degrees of tail skin staining are shown with the body weights in Appendix I).

(a) HISTOLOGY

Avenue Oil Acids induced granular layers of varying thicknesses in only 2 out of 9 mice. In the remainder, the skin was too eroded and in some cases was too badly peeling to determine any such action in these. Sebaceous glands seemed damaged. There was generally increased basophilia of the epidermal cells.

Similarly Coalite Oil Acids produced a great deal of epidermal damage, which was so great that epidermal measurements were not possible and which prevented adequate determination of any granular layer induction.

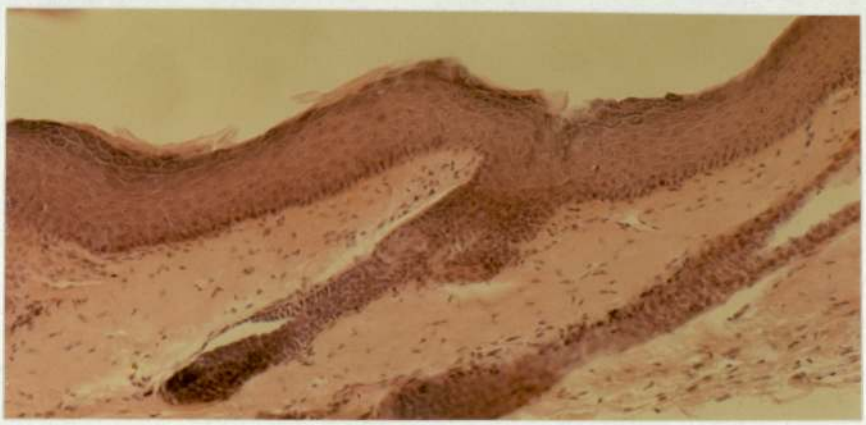
In mice treated with Coalite Tar Acids, granular layers of about 1-3 layers in thickness were induced (see Fig. 14). In parts of the tail, the skin was eroded and peeling. Granular layers were also induced by Coalite Tar, Coalite Oils and in parts in a few Avenue Oil-treated mice. However, in the latter case, most mice suffered erosion of the skin.

In Coalite Tar Oil and the Avenue Oil Neutral and Base + Neutral groups, there was no development of a granular layer. At 40%, all tars, oils and fractions cause highly significant epidermal thickening, (see Table 5).

(b) BODY WEIGHT : EPIDERMAL THICKNESS

Again there was no correlation between body weight and epidermal thickness, (see Graph 2, a - d).

Fig. 14



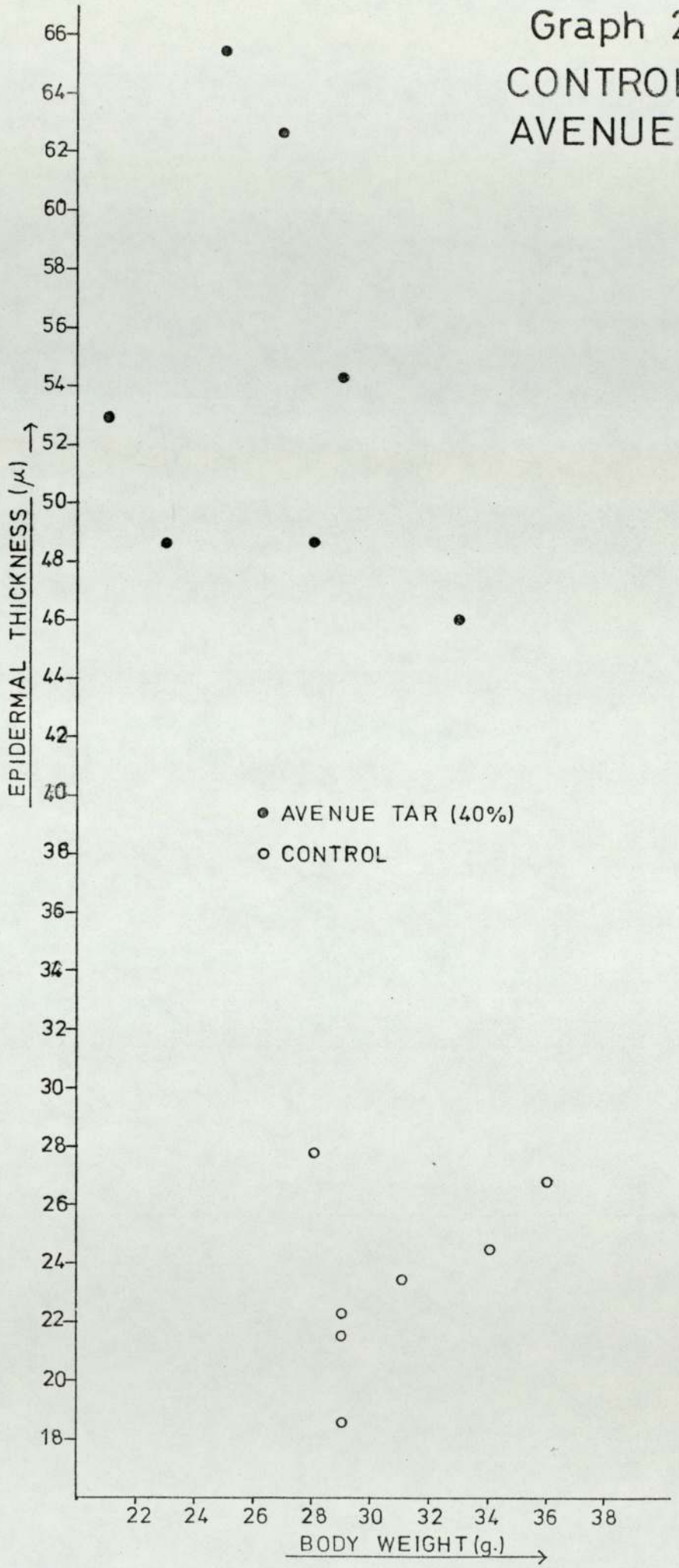
40% COALITE TAR ACIDS

Mouse tail skin after 28 days treatment: the epidermis shows granular layer inducement in the scale region, increased epidermal thickness and increased basophilia.

Table 5 Expt. V2 (ii)

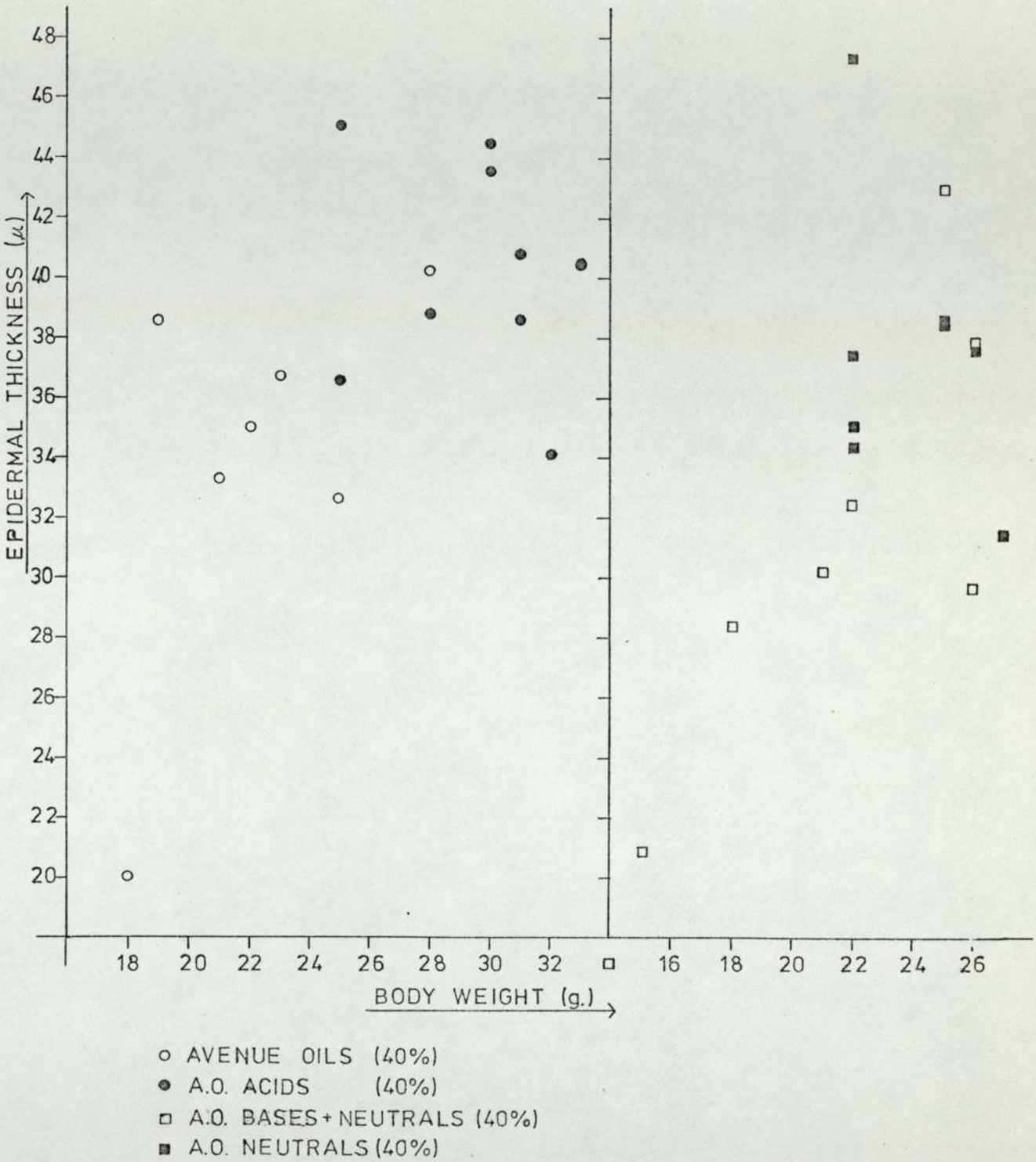
Initial no. mice/group = 10; weight range = 23-27g.

	Days Treated	E.T.	S.E.	n	t	p
CONTROL	(28)	23.6	1.20	7		
40% AVENUE TAR	28	53.2	3.09	7	8.95	0.001
40% AVENUE OILS (A.O.)	28	33.8	2.51	7	3.67	0.005
40% AVENUE OIL ACIDS	9	40.3	1.24	9	9.54	0.001
40% A.O. BASES + NEUTRALS	28	31.8	2.66	7	2.81	0.05
40% A.O. NEUTRALS	28	37.5	1.65	8	6.68	0.001
40% COALITE TAR (C.T.)	28	54.4	3.72	9	7.08	0.001
40% C.T. ACIDS	28	64.0	3.92	6	10.58	0.001
40% C.T. BASES + NEUTRALS	28	42.6	3.61	7	5.02	0.001
40% C.T. NEUTRALS	28	41.6	2.39	10	5.92	0.001
40% COALITE OILS (C.O.)	28	40.1	1.79	10	6.97	0.001
40% C.O. ACIDS	11	N O T M E A S U R A B L E				
40% C.O. BASES + NEUTRALS	28	39.3	0.96	10	10.34	0.001
40% C.O. NEUTRALS	21	35.1	1.40	6	6.27	0.001



EPIDERMAL THICKNESS vs. BODY WEIGHT

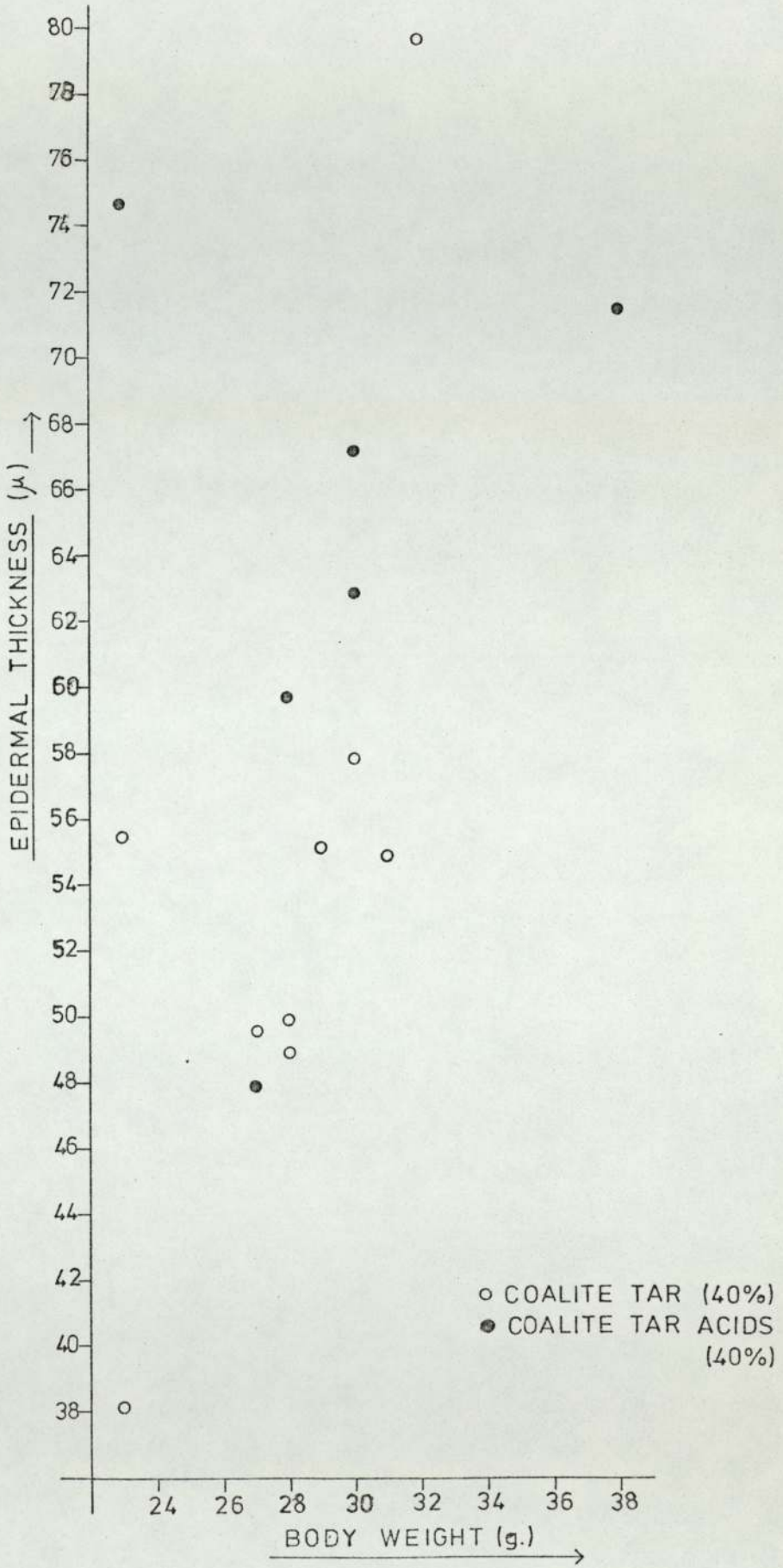
Graph 2(b)



EPIDERMAL THICKNESS vs. BODY WEIGHT

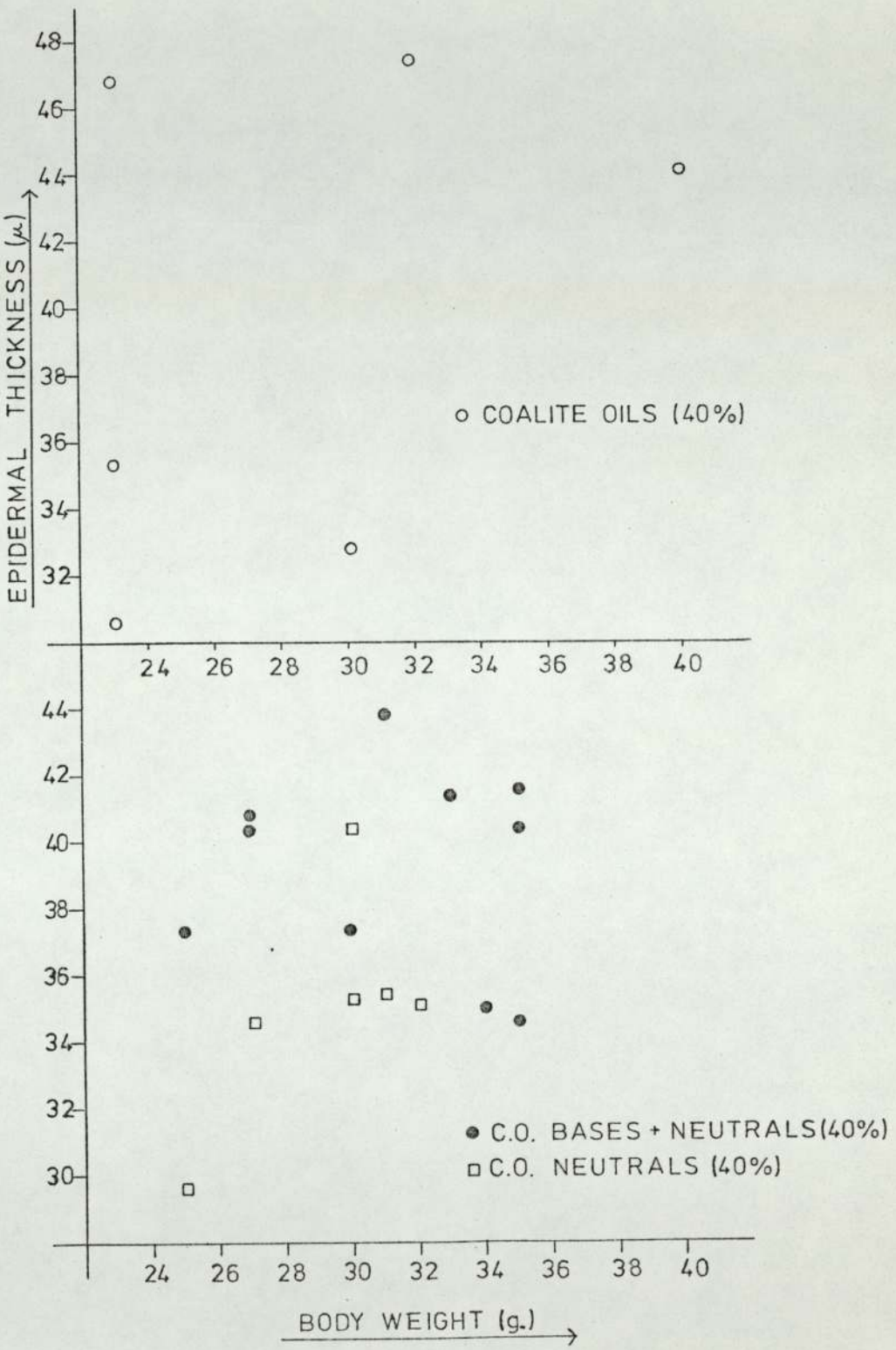
AVENUE OIL FRACTIONS

Graph 2(c)
COALITE TAR and ACIDS



EPIDERMAL THICKNESS vs. BODY WEIGHT

Graph 2(d)
COALITE OIL FRACTIONS



EPIDERMAL THICKNESS vs. BODY WEIGHT

Summary

Granular layer inducement was shown to be a property of the tar and tar oil acids only. Neutral and basic fractions were not studied. The work which follows shows the search to locate a therapeutic boiling range of tar acids.

EXPT. V2 (iii) 5%, 10% and 20% AVENUE OIL ACIDS (A.O.A.)
5%, 10% and 20% A.O.A. '175' (175-185°C)
5%, 10% and 20% A.O.A. '195' (195-210°C)
5%, 10% and 20% A.O.A. '210+' (>210°C)
5%, 10% and 20% COALITE OIL ACIDS

(see Table 6 for treatment times)

Results

The mice after 4 treatments with 20% A.O.A. became very hyperactive and they were killed after a total of 12 treatments. After 3 treatments with 20% A.O.A. '175', the mice were hyperactive and after 5 treatments, 2 had died and 2 were showing signs of necrotic changes in the lower halves of their tails below the treated area, so these animals were killed; the 20% A.O.A. '195' group was also killed at the same time to compare with the '175' group at the same concentration.

After 4 treatments, 20% A.O.A. '210+' - treated tails became stained, and at termination there was some skin peeling.

HISTOLOGY

20% A.O.A. treated tails were impossible to measure: the epidermis was of such varying thickness. Granular layers were induced. In some tails, the epidermis was peeling off. There was some damage to sebaceous glands. In 10% and 5% A.O.A. groups, the tails appeared almost normal, sebaceous glands were intact, and there were only slight increases in basophilia. However, highly significant epidermal thickening was caused by 10% A.O.A. (See Table 6).

In mice treated with 20% A.O.A. '210+', 4 out of 10 mice had granular layers induced all along the length of the tail, (see Fig. 15), and in the remaining 6, there seemed to be slight extensions of the hair

follicle granular layer (H.F.G.L.). This latter observation was also made in the 10% A.O.A. '210+' group, whilst at 5%, there was no effect. All 3 concentrations of acids produced highly significant thickening of the epidermis.

20% A.O.A. '175' caused shedding of the whole epidermis in some cases, with hardly any keratinocyte destruction and nuclei were most obvious although a little faded. In some cases, the peeling epidermis showed induced granular layers (see Fig. 16). Extensive peeling in many cases prevented epidermal measurements. The thickness of the naturally occuring granular layer was increased. The 10% and 5% preparations caused no such effects, but '175' caused highly significant thickening.

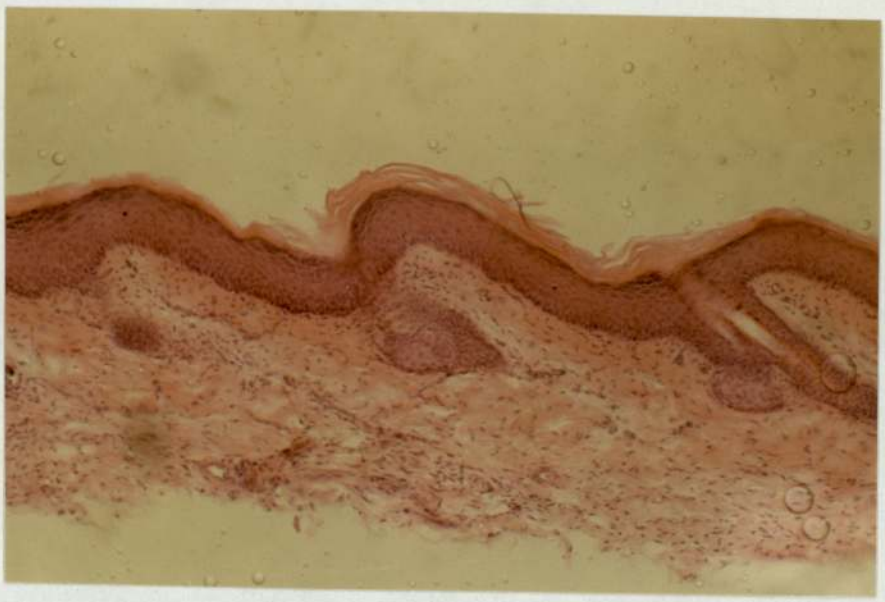
20% A.O.A. '195' produced similar granular layers and peeling in about 6 out of 9 mice. However, the damage to the epidermis was not nearly so extensive as in the '175' group. In the remaining 3, there were H.F.G.L. extensions and increases in thickness of the naturally occuring granular layers. 5% and 10% ointments did not cause thickening.

Very few granular layers were induced in the 20% Coalite Oil Acid group. There were some increases in the depth of the H.F.G.L.'s and some naturally occuring granular layers were increased from about one to two layers. There was an increase in basophilia, which was also found at 10%. 5%, 10% and 20% preparations caused highly significant thickening.

Graph 3, p. 112, illustrates the variation in epidermal thickening produced by various boiling ranges at 5%, 10% and 20%. The graphs

show the gradual increase in epidermal thickness with increasing concentrations of acids used. Generally it seemed that the granular layer induction with minimal epidermal thickening was occuring in the higher boiling fractions and the following experiments were performed to try and locate the boiling range of these compounds. These were initially carried out with acids derived from Coalite Oils.

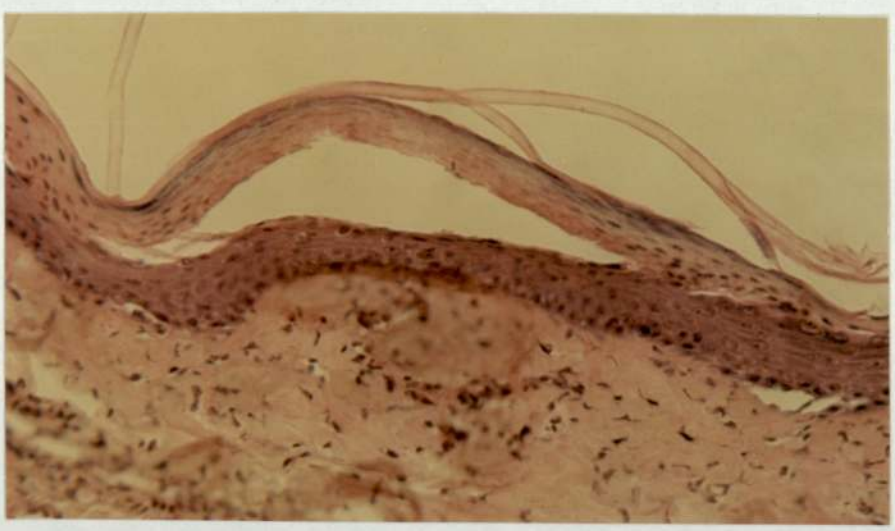
Fig. 15



20% A.O.A. '210+'

4/10 mice showed induced scale granular layers along the length of the treated tail after 21 treatments.

Fig. 16



20% A.O.A. '175'

After 5 treatments, peeling was extensive. This section shows a rapidly sloughing epidermis which previously experienced granular layer induction in the scale region.

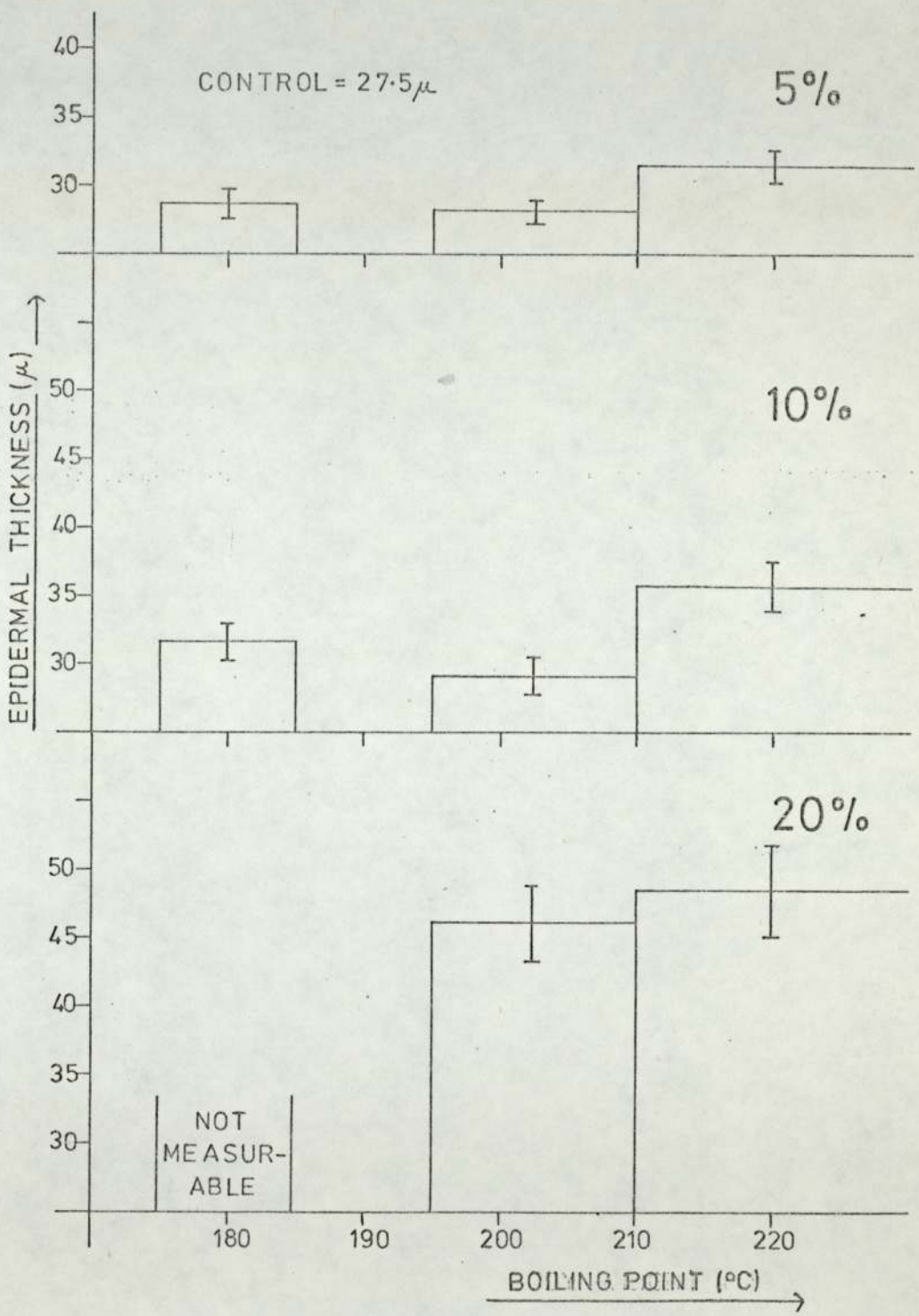
Table 6. Expt. V2 (iii)

Initial no. mice/group = 10; weight range = 23 - 27g.

	Days Treated	E.T.	S.E.	n	t	p
CONTROL	(21)	27.5	0.56	10		
5% A.O.A.	21	28.0	0.80	10	0.47	n.s.
10% A.O.A.	21	34.4	0.72	9	7.66	0.001
20% A.O.A.	12	N O T	M E A S U R A B L E			
5% A.O.A. '175'	21	28.8	1.08	10	1.04	n.s.
10% A.O.A. '175'	21	31.8	1.25	9	3.28	0.005
20% A.O.A. '175'	5	N O T	M E A S U R A B L E			
5% A.O.A. '195'	21	28.2	0.66	10	0.81	n.s.
10% A.O.A. '195'	21	29.2	1.05	10	1.38	n.s.
20% A.O.A. '195'	5	46.1	2.65	9	7.24	0.001
5% A.O.A. '210+'	21	31.7	1.07	9	3.55	0.005
10% A.O.A. '210+'	21	35.8	1.69	10	4.65	0.001
20% A.O.A. '210+'	21	48.5	3.35	10	6.18	0.001
5% COALITE OIL ACIDS	21	31.7	0.99	10	3.69	0.005
10% COALITE OIL ACIDS	21	34.9	0.98	8	6.92	0.001
20% COALITE OIL ACIDS	21	38.6	0.63	9	13.23	0.001

Graph 3

AVENUE OIL ACIDS: Boiling Point vs. Epidermal Thickness



EXPT. V2 (iv) 2% and 5% PHENOL : 14 days
2% and 5% o-CRESOL
2% and 5% m/p-CRESOL
2% and 5% 2,4/2,5-XYLENOL
10% LOW BOILING XYLENOLS (L.B.X.)
10% HIGH BOILING XYLENOLS (H.B.X.)
10% X.L.
10% X.X.L

Results

HISTOLOGY

All individual phenols and xylenols failed to induce granular layers at the concentrations used. There were very slight H.F.G.L. extensions in the 10% X.L. and X.X.L. groups.

Significant thickening occurred in groups treated with 2% and 5% m/p-cresol, 10% L.B.X., H.B.X. and X.X.L. (see Table 7).

Comment

It was decided to see if the granular layer inducing activity lay in the following constituents of X.L. and X.X.L. fractions.

Table 7. Expt. V2 (iv)

Initial no. mice/group = 5; weight range = 20-25g. Duration = 14 days

	E.T.	S.E.	n	t	p
CONTROL	28.7	1.14	7		
2% PHENOL	28.7	2.14	5	0.001	n.s.
5% PHENOL	31.6	1.99	4	1.41	n.s.
2% o-CRESOL	28.4	1.68	5	0.17	n.s.
5% o-CRESOL	29.7	2.17	5	0.43	n.s.
2% m/p-CRESOL	28.2	0.99	5	0.31	n.s.
5% m/p-CRESOL	35.1	2.80	5	2.38	0.05
2% 2,4/2,5-XYLENOL	29.2	1.03	5	0.33	n.s.
5% 2,4/2,5-XYLENOL	34.6	3.66	5	1.78	n.s.
10% L.B.X.	34.9	1.19	5	3.65	0.005
10% H.B.X.	47.6	0.82	5	12.34	0.001
10% X.L.	28.7	1.14	4	0.02	n.s.
10% X.X.L.	34.6	1.99	5	2.77	0.05

EXPY. V2 (v) 5% and 10% L 14/13 (ca. 227 - 232°C)
5% and 10% L 14/19 (ca. 229 - 239°C)
5% and 10% L 14/47 (ca. 241 - 245°C)
5% and 10% L 14/56 (ca. 244 - 248°C)
5% and 10% L 16/38 (ca. 253 - 257°C)
5% and 10% L 16/53 (ca. 256 - 261°C)
5% and 10% L 16/70 (ca. 261 - 264°C)

Results

HISTOLOGY

Scale granular layers were induced by 10% L 14/47, /56, L 16/38, /53 and /70, but in no cases were they continuous over the whole length of the section. These preparations at 5% showed no granular inducements, and there were no such changes caused by 5% and 10% L 14/13 and /19.

The epidermis of mice treated with 10% L 16/38 was not measurable. The thickness of the epidermis was too variable. In most cases both concentrations of these fractions caused significant epidermal thickening, except in the cases of groups treated with 5% L 14/19 and 5% L 14/56.

Graph 4 illustrates the variation of epidermal thickness produced by different boiling ranges.

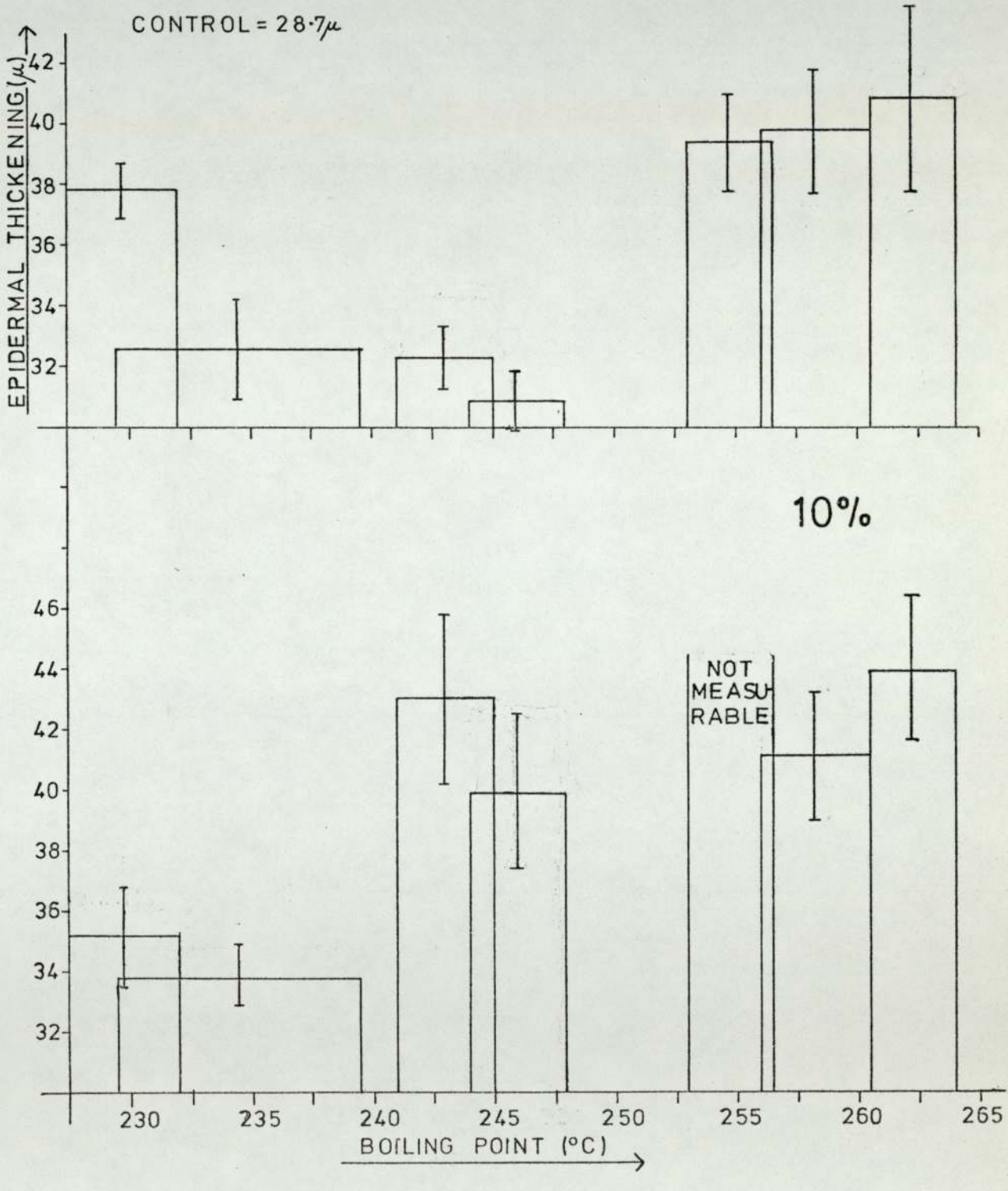
Table 8. Expt. V2 (v)

Initial no. mice/group = 7; weight range = 20-25g. Duration = 14 days

	E.T.	S.E.	n	t	p
CONTROL	28.7	1.14	7		
5% L 14/13	37.8	0.84	7	6.41	0.001
10% L 14/13	35.2	1.70	9	2.99	0.05
5% L 14/19	32.7	1.67	5	2.08	n.s.
10% L 14/19	33.8	0.97	6	3.32	0.001
5% L 14/47	32.3	1.08	6	2.27	0.01
10% L 14/47	43.0	2.85	7	4.69	0.001
5% L 14/56	30.9	1.05	7	1.40	n.s.
10% L 14/56	39.9	2.51	6	4.29	0.005
5% L 16/38	39.5	1.69	7	5.30	0.001
10% L 16/38	N O T		M E A S U R A B L E		
5% L 16/53	39.8	2.02	7	4.80	0.001
10% L 16/53	41.2	2.24	7	4.95	0.001
5% L 16/70	40.9	3.01	7	3.79	0.005
10% L 16/70	44.0	2.37	7	5.80	0.001

Graph 4

COALITE OIL ACIDS: Boiling Point vs. Epidermal Thickness



EXPT. V2 (vi) 5% and 10% A.O.A. '200' (ca. 200 - 230/240°C) : 14 days
5% and 10% A.O.A. '225' (ca. 225 - 300°C)
5% and 10% A.O.A. '290' (ca. 290 - 330°C)
5% and 10% A.O.A. '330+' (ca. 330 - 400°C)

Results

There was slight skin staining in groups 10% A.O.A. '200' and '225'.

HISTOLOGY

5% A.O.A. '200'-treated tails were normal. At 10%, this preparation induced a few scale granular layers in only 3 mice, and these were not continuous along the length of the tail. 10% '225'-treated mice showed similar changes; at 5% there was evidence of a few H.F.G.L. extensions. Similar results were also obtained at 5% and 10% with A.O.A. '330+'.

The outstanding preparation was 10% '290'. At 5% there were a few isolated granular cells just beneath the horny layer, but at 10% granular layers had been induced, which were about on cell thick, (see Fig. 17).

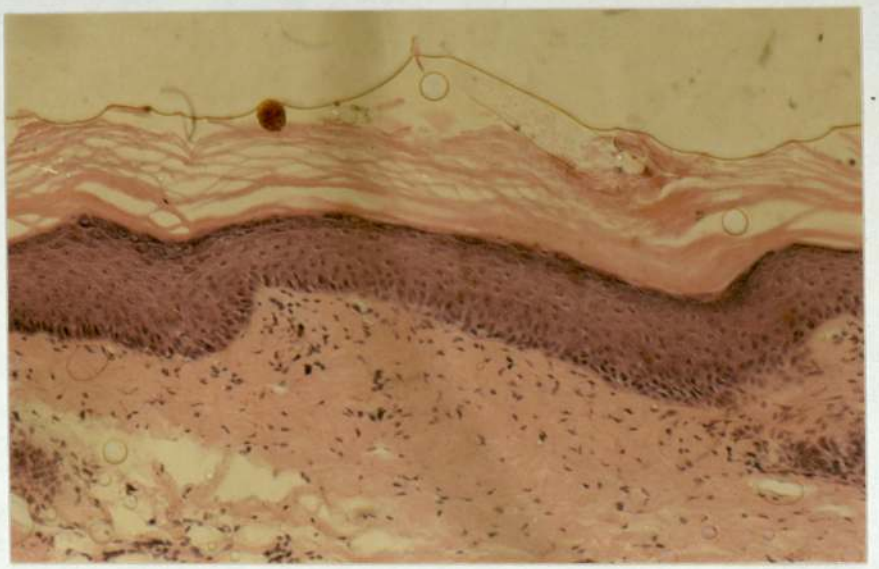
Significant thickening was produced by both concentrations of the four fractions, except in the one case of 5% A.O.A. '330+'(see Table 9).

Graph 5 shows the variation in epidermal thickness produced by the different boiling ranges.

Comment

It was decided that the H.B.T.A. contained especially in the range 290 - 330°C should be further investigated, both by using smaller fractions of the boiling range and by looking at as many individual high-boiling tar acids (H.B.T.A.'s) as were available.

Fig. 17



10% A.O.A. '290'

This fraction has produced scale granular layers and basket-weave keratin.

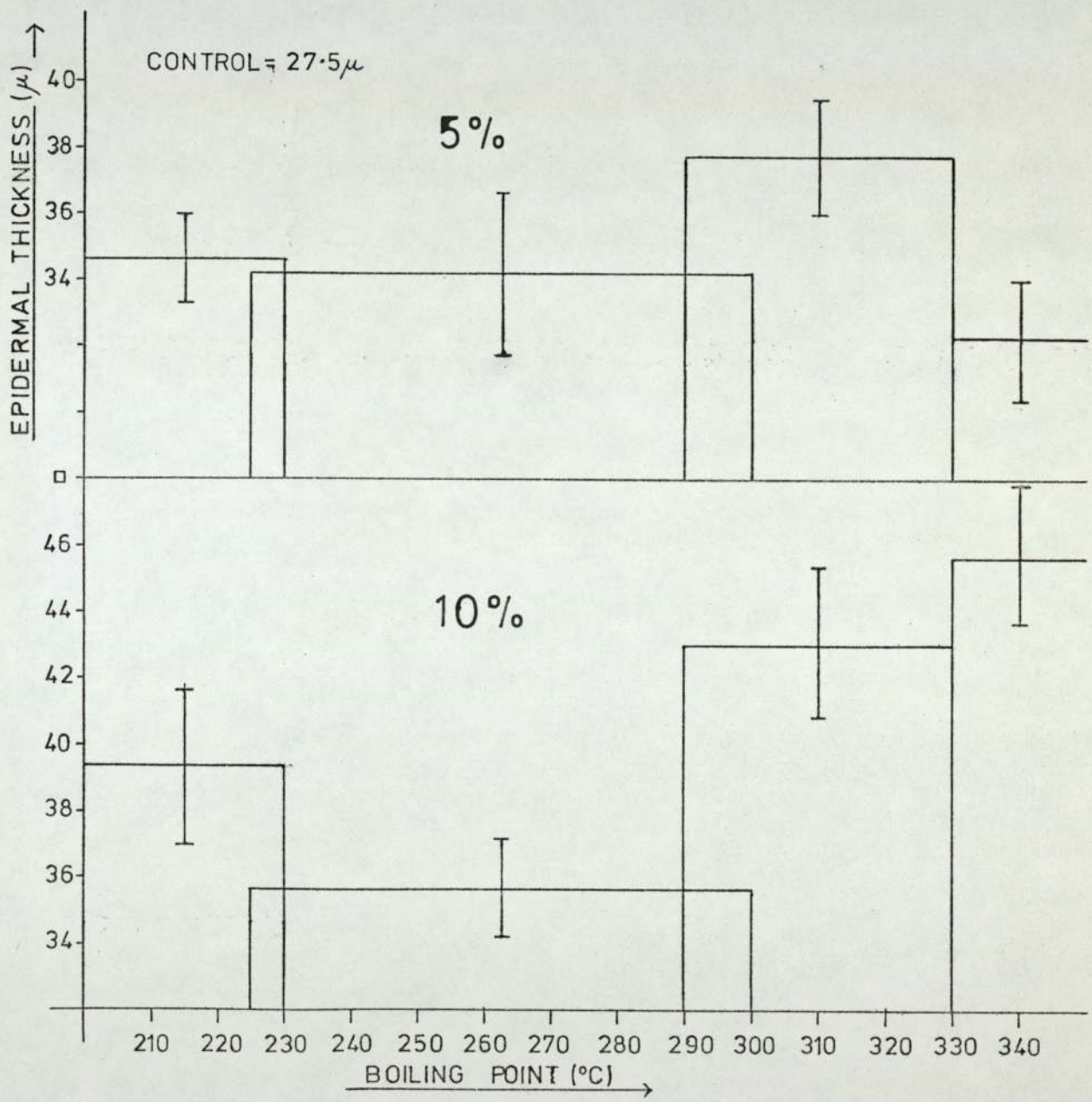
Table 9. Expt. V2 (vi)

Initial no. mice/group = 7; weight range = 20 - 25g.

	E.T.	S.E.	n	t	p
CONTROL	27.5	1.20	5	-	-
5% A.O.A. '200'	34.6	1.33	7	3.79	0.005
10% A.O.A. '200'	39.3	2.30	7	4.06	0.005
5% A.O.A. '225'	34.2	2.40	6	2.35	0.05
10% A.O.A. '225'	35.6	1.49	6	4.14	0.005
5% A.O.A. '290'	37.8	1.74	6	4.68	0.005
10% A.O.A. '290'	43.1	2.25	6	5.78	0.001
5% A.O.A. '330+'	32.3	1.78	7	2.07	n.s.
10% A.O.A. '330+'	45.7	2.03	7	6.96	0.001

Graph 5

AVENUE OIL ACIDS Boiling Point vs. Epidermal Thickness



EXPT. V2 (vii)	2% and 5%	2, 3, 5-trimethyl phenol	: 14 days
	2% and 5%	2, 4, 6-trimethyl phenol	
	2% and 5%	3-methyl-5-ethyl-phenol	
	2% and 5%	4-indanol	
	2% and 5%	5-indanol	
	2% and 5%	6-methyl-4-indanol	
	2% and 5%	1-methyl-5-indanol	
	2% and 5%	7-methyl-5-indanol	
	10%	4-indanol	: 21 days*
	10%	5-indanol	
	10%	7-methyl-5-indanol	

* There were only sufficient quantities of these 3 H.B.T.A.'s to investigate the effects of higher concentrations over longer treatment times.

Results

All concentrations of all individual acids failed to induce a granular layer, and in most cases the skin appeared normal. Significant epidermal thickening was only produced by 2% and 5% 6-methyl-4-indanol, 1-methyl-5-indanol at 5% and 7-methyl-5-indanol at 10% (see Table 10).

Table 10. Expt. V2 (vii)

Initial no. mice/group = 7 (CONTROL = 9); weight range = 20 - 25g.

	Days Treated	E.T.	S.E.	n	t	p
CONTROL	(21)	27.4	0.94	9	—	—
2% 2,3,5-trimethyl phenol	14	29.5	0.98	7	1.57	n.s.
5% 2,3,5-trimethyl phenol	14	30.8	1.65	7	1.97	n.s.
2% 2,4,6-trimethyl phenol	14	27.0	0.77	7	0.27	n.s.
5% 2,4,6-trimethyl phenol	14	29.0	0.66	7	1.35	n.s.
2% 3-methyl-5-ethyl-phenol	14	29.5	1.83	6	1.12	n.s.
5% 3-methyl-5-ethyl-phenol	14	30.0	1.75	7	1.41	n.s.
2% 4-indanol	14	27.8	0.90	5	0.34	n.s.
5% 4-indanol	14	31.8	2.14	7	2.08	n.s.
10% 4-indanol	21	28.1	1.77	5	0.54	n.s.
2% 5-indanol	14	29.7	1.85	5	1.27	n.s.
5% 5-indanol	14	29.8	2.56	5	1.10	n.s.
10% 5-indanol	21	29.7	2.63	5	1.51	n.s.
2% 6-methyl-4-indanol	14	33.9	1.56	6	3.83	0.005
5% 6-methyl-4-indanol	14	32.2	0.61	4	3.26	0.01
5% 1-methyl-5-indanol	14	31.0	1.19	4	2.23	0.05
2% 7-methyl-5-indanol	14	29.9	0.77	5	1.81	n.s.
5% 7-methyl-6-indanol	14	29.7	0.96	6	1.71	n.s.
10% 7-methyl-5-indanol	21	37.9	1.79	6	8.06	0.001

EXPT. V2 (viii) 10% A.O.A., 20% A.O.A.
10% A.O.A. '175', '195', '200', '225', '290' and '330+'
10% L 14/13, /19, /47, /56
10% L 16/38, /53, /70
10% 2, 4, 6- trimethyl phenol
10% 3, 4-dimethyl phenol
10% 2-methyl-resorcinol

(see Table 11 for treatment times)

Results

After 10 treatments the mice in group 20% A.O.A. were so hyperactive and skin peeling was so extensive that they were killed. Similarly after 25 treatments, 10% A.O.A. '330+' mice were also killed. In both groups, there was slight skin staining.

10% A.O.A. '200' and '225' treated-mice also showed slight skin staining at 28 days.

HISTOLOGY

In the group treated with 20% A.O.A. there was extensive peeling and highly significant thickening of the epidermis. There were isolated scale granular layers, but they did not run consecutively over the whole length of the treated tail.

Similar results were seen with 10% A.O.A. '300+' Where there were no granular layers, the H.F.G.L. seemed extended. There was highly significant epidermal thickening (see Table 11).

There was very little change in groups 10% A.O.A. and 10% A.O.A. '175', '195', '200' and '225' except for significant thickening in '175' and '225' Group '290' showed some degree of granular changes, in that a few isolated cells beneath the horny layer contained basophilic granules. The reaction after 28 days was definitely reduced to that

Table 11. Expt. V2 (viii)

Initial no. mice/group = 7; weight range = 23 - 27g.

	Days Treated	E.T.	S.E.	n	t	p
CONTROL	(28)	29.9	1.48	5	-	-
10% A.O.A.	28	37.4	3.12	6	2.05	n.s.
20% A.O.A.	10	57.1	4.99	6	4.82	0.001
10% A.O.A. '175'	28	38.5	1.49	6	4.09	0.005
10% A.O.A. '195'	28	33.7	2.39	5	1.35	n.s.
10% A.O.A. '200'	28	33.8	1.21	5	2.04	n.s.
10% A.O.A. '225'	28	41.3	1.06	7	6.47	0.001
10% A.O.A. '290'	28	51.6	3.48	6	5.36	0.001
10% A.O.A. '330'	25	55.0	4.53	5	5.29	0.001
10% L 14/13	28	45.1	4.68	6	2.85	0.05
10% L 14/19	28	35.4	0.89	6	3.36	0.01
10% L 14/47	28	32.7	1.30	6	1.43	n.s.
10% L 14/56	28	42.6	2.59	5	4.26	0.005
10% L 16/38	28	N O T M E A S U R A B L E				
10% L 16/53	28	48.9	2.91	6	5.46	0.001
10% L 16/70	28	37.9	4.04	7	1.61	n.s.
10% 2,4,6-trimethyl phenol	28	35.4	0.90	7	3.31	0.01
10% 3,4-dimethyl phenol	28	34.9	2.34	5	1.81	n.s.
10% 2-methylresorcinol	28	40.7	2.77	6	3.25	0.01

obtained after 14 days, (see Expt. V2 (vi), p. 118). The epidermis was significantly thickened.

Groups 10% L 14/13, /19, /47, and /56 also showed very little change. However 10% L 16/38-treated tails were impossible to measure and they showed extensions of the hair follicle granular layer and some consecutive scale granular layers, although these still did not cover the whole length of the section. Similar results were obtained from groups L 16/53 and /70; in these groups, the epidermis was significantly thickened, but there was not sufficient epidermal damage to prevent measurement as in L 16/38.

Mice treated with 10% 2,4,6-trimethyl phenol, 2-methylresorcinol and 3,4-dimethyl-phenol showed no change, except significant thickening in the first two groups.

VEHICLE 2 - COMMENT

The lack of granular layer inducing activity in the individual H.B.T.A.'s plus the lack of a continuous granular layer by the most promising fractions, (notably A.O.A. '290' and to a lesser extent, '225'), had suggested that the tars may require a longer time to act. Hence the above experiment was run for a month, but still granular layers which had been induced were in most cases confined to a few scales and were not continuous. It was decided to investigate the vehicle, and Vehicle 3 was found to enhance the granular layer inducing activity, and so was used for the remainder of the experiments.

3. VEHICLE 3

EXPT. V3 (i) 5% and 10% A.O.A. '<230' (below 230°C head temp.)
5% and 10% A.O.A. '230' (230-240°C)
5% and 10% A.O.A. '240' (240-250°C)
5% and 10% A.O.A. '250' (250-260°C)
5% and 10% A.O.A. '260' (260-270°C)
5% and 10% A.O.A. '270' (270-280°C)
5% and 10% A.O.A. '280' (280-290°C)
5% and 10% A.O.A. '290' (290-300°C)
5% and 10% A.O.A. '300' (300-310°C)
5% and 10% A.O.A. '310' (310-320°C)
5% and 10% A.O.A. '320' (320-330°C)
5% and 10% A.O.A. '330' (330-340°C)

(See results for individual treatment times).

Results

After 7 treatments, it was apparent in the groups listed below that these fractions were having an irritant effect and these mice were killed:

10% A.O.A. '<230', '240', '270', '310' and '320'.

A tar preparation was considered irritant when the mice displayed "burrowing" behaviour when approached for treatment, and also after the tar had been applied. The remaining groups did not show this behaviour and so treatment was continued. In groups treated with 10% A.O.A. '<230', '240' and '270', the tail skin was stained very light brown.

After 8 treatments, groups 10% A.O.A. '290' and '300' were also terminated for suspected irritant effects.

10% A.O.A. '330'-treated tails began to peel after about 6 treatments, but there was no sign of distress in the animals. However it was decided to terminate these mice after 14 treatments as the peeling was by then quite extensive. All other groups at 10% were terminated for comparison.

After 16 treatments, 5% '330' was causing peeling; all 5% groups were terminated after 21 treatments. No irritant effects were observed.

HISTOLOGY

Histological examination did not reveal any damage which could account for the apparent irritation in groups treated with 10% A.O.A. '<230', '240' and '270'. Epidermal thickness was significantly increased in '240' and '270', which may have been manifestations of the supposed irritant action. In these two groups, there were a few extensions of the hair follicle granular layer, but otherwise there was no definite granular layer induction.

In contrast, groups 10% A.O.A. '310' and '320' showed peeling of the epidermis. In '310' there were extensions of the hair follicle granular layer and increases in depth of the naturally occuring granular layer. In '320', peeling was so extensive that measurements could not be made. No scale granular layers were observed, but there were hair follicle granular layer extensions in about half of the group.

The above observations were paralleled to a lesser degree in the fractions at 5%, which were treated for 21 days. 5% A.O.A. '<230', '240' and '270' caused no change; there was evidence of peeling in small areas of the sections treated with 5% '310' and '320'. In the latter case, extensions of the hair follicle granular layer were seen and in both cases the epidermal thickness was significantly increased.

Groups treated with 10% A.O.A. '290' and '300' were killed after 8 treatments. In both groups, epidermal thickness was significantly increased and granular layers were induced in parts of the tail.

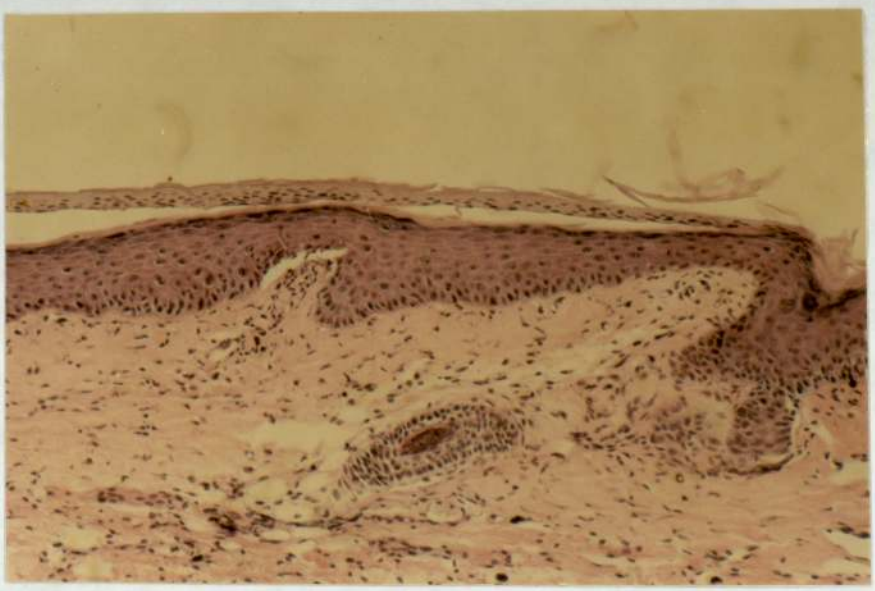
There were also isolated granulated cells just below the horny layer in some scales. Peeling was occuring in some areas of the tail in both groups. At 5%, epidermal thicknesses were significantly increased, with a few extensions of the hair follicle granular layer.

The remainder of the 10% groups were treated for 14 days. Mice treated with 10% A.O.A. '230' and '250' showed significantly increased epidermal thickness, as was also displayed by these preparations at 5%, but there was no granular layer formation. Epidermal thickness was increased by A.O.A. '260' at 5% and 10%, and peeling induced by 10% A.O.A. '280' was so extensive that measurements were impossible to make. At 5% there was no peeling, but significant epidermal thickening.

Measurements were not possible in the case of A.O.A. '330' either at 5% or 10%. At 5% the most obvious effect was peeling; there were a few extensions of the hair follicle granular layer. At 10%, there were some very thick and very thin scales, which must have been due to different rates and degrees of thickening and peeling between individual scales. In some cases the granular layers were up to 3 layers thick, and they were continuous, (see Fig. 18).

The results are summarised in Table 12a.

Fig. 18



10% A.O.A. '330'

After 14 days treatment, granular layers were obvious, epidermal thickness was very variable, and there was extensive peeling.

Table 12. EXPT. V3 (i)

Initial no. mice/group = 7; weight range = 20 - 25g.

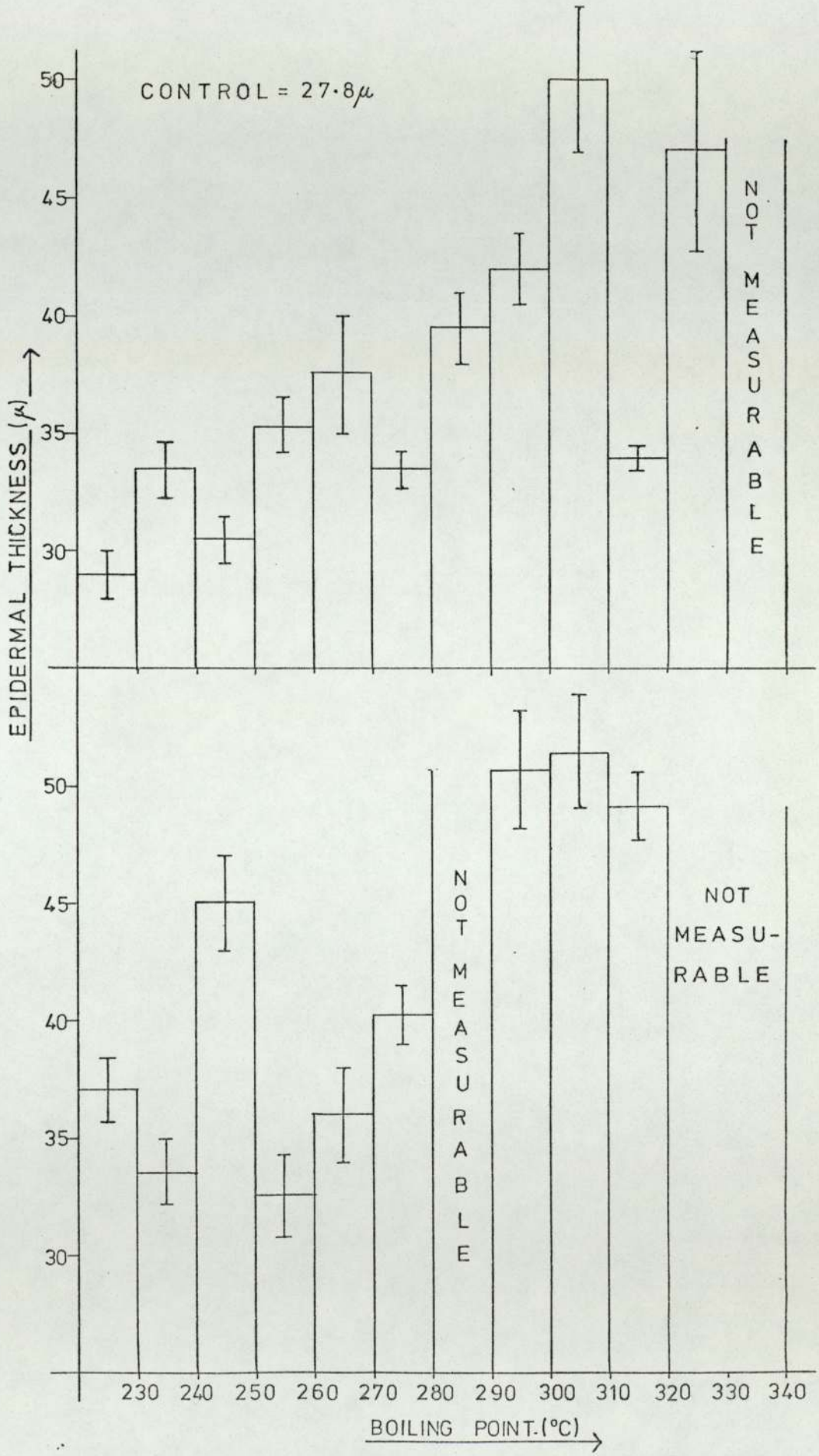
	Days Treated	E.T.	S.E.	n	t	p
CONTROL	(21)	27.8	1.21	7	-	-
5% A.O.A. '<230'	21	28.9	1.11	7	0.66	n.s.
10% A.O.A. '<230'	7	37.0	1.43	7	4.66	0.001
5% A.O.A. '230'	21	33.5	1.21	7	3.33	0.01
10% A.O.A. '230'	14	33.5	1.26	7	3.33	0.01
5% A.O.A. '240'	21	30.5	0.89	7	1.80	n.s.
10% A.O.A. '240'	7	44.8	2.15	7	6.90	0.001
5% A.O.A. '250'	21	35.2	0.91	7	4.87	0.001
10% A.O.A. '250'	14	32.7	1.75	7	2.32	0.05
5% A.O.A. '260'	21	37.7	2.45	7	3.64	0.005
10% A.O.A. '260'	14	35.9	2.16	7	3.26	0.01
5% A.O.A. '270'	21	33.7	0.62	7	4.31	0.005
10% A.O.A. '270'	7	40.2	1.29	7	7.00	0.001
5% A.O.A. '280'	21	39.5	1.44	7	6.21	0.001
10% A.O.A. '280'	14	NOT MEASURABLE				
5% A.O.A. '290'	21	41.9	1.41	7	7.58	0.001
10% A.O.A. '290'	8	50.6	2.50	7	8.23	0.001
5% A.O.A. '300'	21	50.0	3.12	7	6.67	0.001
10% A.O.A. '300'	8	51.4	2.34	7	8.98	0.001
5% A.O.A. '310'	21	33.9	0.45	6	4.43	0.001
10% A.O.A. '310'	7	49.1	1.32	5	11.76	0.001
5% A.O.A. '320'	21	47.1	4.20	6	4.74	0.001
10% A.O.A. '320'	7	NOT MEASURABLE				
5% A.O.A. '330'	21	NOT MEASURABLE				
10% A.O.A. '330'	14	NOT MEASURABLE				

Table 12a: SUMMARY OF RESULTS OF EXPT. V3 (i)

GROUP	Signs of Irritation During Treatment?	Granular cells visible?	Granular layers Induced?	Epidermal Thickening	Peeling
5% '<230'	-	-	-	-	-
230	-	-	-	+	-
240	-	-	-	+	-
250	-	-	-	+	-
260	-	-	-	+	-
270	-	-	-	+	-
280	-	-	-	+	-
290	-	+	-	+	-
300	-	+	-	+	-
310	-	+	-	+	+
320	-	+	-	+	+
330	-	+	-	(+)	+
10% '<230'	+	-	-	+	-
'230'	-	-	-	+	-
'240'	+	-	-	+	-
'250'	-	-	-	+	-
'260'	-	-	-	+	-
'270'	+	-	-	+	-
'280'	-	-	-	(+)	+
'290'	+	+	+	+	+
'300'	+	+	+	+	+
'310'	+	+	-	+	+
'320'	+	+	-	(+)	+
'330'	-	+	+	(+)	+

(+) indicates that extensive peeling and/or too variable epidermal thickness prevented actual measurements being made, but observation showed some parts of the tail skin to be definitely thickened.

Graph 6 ANTHRACENE and CREOSOTE OIL ACIDS



EXPT. V3 (ii) 10% COALITE HIGH-BOILING TAR ACIDS : 7 days

Results

These mice displayed burrowing activity after 7 treatments and so were killed. There was some skin peeling over the treated ares.

HISTOLOGY

There was some evidence of H.F.G.L. extensions in 6 out of the 7 treated mice. The most obvious effect of the tar was highly significant epidermal thickening, (see Table 13).

Table 13. EXPT. V3 (ii)

Initial no. mice/group = 7; weight range = 20-25g. Duration = 7 days.

	E.T.	S.E.	n	t	p
CONTROL	26.6	2.43	7	-	-
10% COALITE H.B.T.A.	57.6	5.23	7	14.25	0.001

EXPT. V3 (iii) A DAILY MONITOR OF AN ACTIVE TAR FRACTION

From previous experiments, it was obvious that the fractions currently under test were still having a complex action on the skin, and it was desirable to investigate this effect in at least one of the very active samples, in the hope that at least in one case, an insight would be gained into these very complex actions - for example did granular layer formation preceed or follow the keratolytic effects? Histological monitoring is of course by definition impossible, since one removes the tissue under test. When large areas are under study, small (ca. 1 mm) punch biopsies representative of the study area can be made. The mouse tail is too small a model to enable this method to be used. The method adopted was to treat a large group of mice with the same ointment daily and to remove and kill 4 each day to get an idea of what was happening daily. This is obviously an imperfect system, and results must be interpreted with this in mind.

10% A.O.A. '330' was chosen for its great granular layer-inducing activity, apparent from previous experiments, (Expt. V3 (i)).

Results

The mice killed after the first three days showed no change, and epidermal thicknesses were normal. However after 4 treatments, changes were observed, but the 4 mice were not all at the same stage of reacting to the fraction. One mouse showed granular layer formation. The other 3 mice remained unchanged.

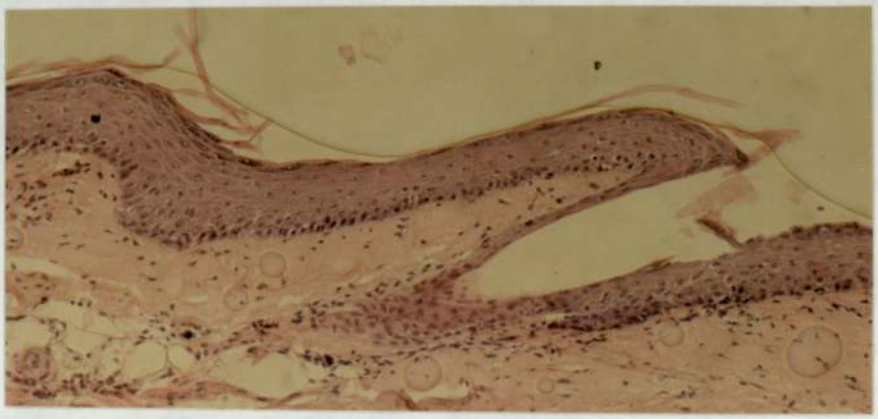
After 5 treatments, epidermal thicknesses were increased and one mouse out of 4 had a continuous granular layer. All mice had

peeling skin and although continuous granular layers were not induced, some isolated granulated cells were visible under the scale keratin. These changes were also seen after 6, 7, 8 and 9 treatments. However after 10 applications, there were no granular layers to be seen, and the epidermis was of very variable thickness due to the extensive peeling. After 11 treatments, peeling was so great that the epidermis was completely eroded in parts.

Fig. 19

(a)

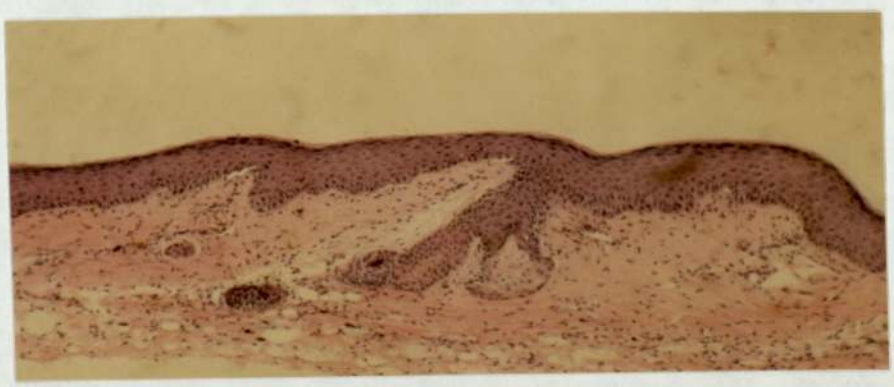
10% A.O.A. '330'



AFTER 6 TREATMENTS

Granular cells are being induced, before maximal development of epidermal thickness.

(b)



AFTER 8 TREATMENTS

Granular layers are present. Epidermal thickness is very variable.

Table 14. V.3. EXPT. (iii)

Weight range = 20 - 25g.

	E.T.	S.E.	n	t	p
CONTROL (DAY 1)	27.1	1.43	4	-	-
DAY 2	27.1	1.02	4	0.04	n.s.
DAY 3	27.5	1.79	4	0.20	n.s.
DAY 4	28.7	1.12	4	0.90	n.s.
CONTROL (DAY 11)	25.4	0.74	4	1.00	n.s.

4. HISTOCHEMICAL EVALUATION OF TARS

Introduction

As previously described, (see p. 69), it has been shown by Jarrett and Spearman (1964) that various enzyme changes accompany the inducement of a granular layer in the scale regions of the mouse tail, paralleling a healing psoriatic skin. Demonstration of such changes as a result of tar treatment would greatly support suggestions from observations of granular layer formation that a certain fraction would be efficacious as an anti-psoriatic drug. However it must be remembered that the tests described below are very difficult to quantitate, and no such attempt has been made. The results are merely to be used as guidelines in the choosing of a promising fraction.

(i) ACID PHOSPHATASE

Jarrett and Spearman (1964) pointed out that this enzyme always seems to be in abundance when cells are losing their nuclei, and it was hoped that the tars would have caused induction of acid phosphatase in the granular layer region as opposed to the normal location in the scale horny layer. This occurred after Vitamin A treatment, (Jarrett and Spearman, 1964).

(ii) NON-SPECIFIC ESTERASE

Normally there is no non-specific esterase (N.S.E.) in the mouse tail scale horny layer, (Riley, 1966b), although it is present in the horny layer of psoriatic skin, (Braun-Falco, 1958). Riley (1966) has shown that the high-level dendritic cells of the mouse tail can be

demonstrated by this technique. It shows the presence of these cells around the area of the hair follicle. It was surmised that there may be an altered distribution of these cells as a result of tar treatments.

(iii) HORNY LAYER FLUORESCENCE

Jarrett and Spearman (1964) described a method of staining the horny layer in mouse tail skin, which under ultra-violet irradiation distinguished between the basket-weave horny layer at the hair follicle and the scale-type keratin. By the technique described below, scale keratin fluoresces blue (as does human parakeratin), and hair follicle keratin fluoresces red (as does human orthokeratin). It was hoped that effective tar treatments would change the fluorescence of the horny layer in the scale regions from blue to red, thus indicating the presence of a more flexible horny layer.

Methods

(a) Sections

For histochemical tests, the use of cryostat sections is useful to ensure maximal enzyme preservation. This quick method for processing several sections was the method of choice. However for tests in which it was hoped to examine the horny layer (for example, for fluorescence changes), the routine wax embedding method was chosen. Although preservation and attachment of the horny layer was greater in cryostat sections, it was often difficult to obtain a flattened section, and a folded-over horny layer greatly confused the section obtained. Thus a large number of wax sections had to be cut in order to gain a representative section with an attached horny layer. Skin was processed as usual, except that in this case it was ensured that the oven temperature did not exceed 58°C (Jarrett and Spearman, 1964). Wax sections were floated out on warm distilled water on pre-washed slides. After flattening, the slides were drained and sections dried by standing in the wax oven at 58°C for 30 minutes.

(b) Histochemical Tests

(i) ACID PHOSPHATASE

Source: GOMORI LEAD NITRATE

(From Pearse (1968), vol. 1 p. 728)

Sections: Cryostat

Substrate:	Sodium β-glycerophosphate	320 mg.
	Lead nitrate	200 mg.
	Acetate buffer, p. H. 5	100 mls.
	(containing 1.15g. sod. acetate, 0.28g. acetic acid in 100 mls.)	

Method

Cryostat sections mounted on clean microscope slides, were air dried for 2-3 minutes, fixed in 70% alcohol for one minute and quickly rinsed in running tap water before being transferred to the substrate at 37°C. After incubation, slides were quickly rinsed in tap water, immersed in freshly-prepared 1% aqueous ammonium sulphide for two minutes, and quickly rinsed again before counterstaining in 2% Methyl Green (chloroform-extracted) for 2 minutes. After brief rinsing, the slides were mounted with cover slips by Glycerine Jelly.

(ii) NON-SPECIFIC ESTERASE

Source: NACHLAS and SELIGMAN (1949) α-NAPHTHYL ACETATE (in Pearse (1968), vol. 2, p. 1303)

Sections: Cryostat

Substrate: 40 mg. α-naphthyl acetate in 1.0 ml. acetone
+ 80 ml. 0.1 M Phosphate Buffer, p.H. 7.4
Shake until cloudiness decreases
+ 400 mg. Fast Red TR
(Reagents added in the above order)

Method

Cryostat sections were mounted, dried, fixed and rinsed as above. Excess water was drained off before the substrate mixture was filtered onto the sections, which were incubated at room temperature. After incubation, the sections were rinsed in tap water, counterstained in Mayer's Haemalum for 4-6 minutes, and rinsed in running tap water for 30 minutes, before mounting in Glycerine Jelly.

(iii) HORNY LAYER FLUORESCENCE

Source: JARRETT and SPEARMAN (1964), p. 32

Sections: Wax (carefully processed)

Staining: (A) 0.01 g. CONGO RED dissolved in 200 mls. distilled water stabilised by

0.1 g. TITAN YELLOW dissolved in 100 mls. distilled water

(B) 0.1% aqueous THIOFLAVINE T.

Method

After bringing the sections to water, the sections were immersed for 40 minutes in solution A. The sections were quickly rinsed in tap water and fluorochromed for 3 minutes in solution B. After rapid rinsing in tap water, sections were dehydrated and mounted in 'FLUOR-LITE'. Sections were visualised using a Leitz S.M. microscope, fitted with a 250 Lamphouse containing a 200 Watt high pressure mercury light. Filters used were (in order from light source):

1. Neutral Diffuser
2. B.G. 38 (Blue) : 4 mm
3. B.G. 12 (Blue) : 5 mm
4. B.G. 12 (Blue) : 3 mm
5. K530 Barrier Filter

A bright field condenser (Leitz 601) was used.

Results

(i) ACID PHOSPHATASE

Untreated control sections were incubated for the following times:

10, 20, 30, 45, 60, 90 and 120 mins.

At 60 minutes, there was good staining of the scale horny layer and in the hair follicle granular layer, (as described by Jarrett and Spearman, 1964). Longer incubation times merely increased the depth of staining. 60 minutes was used for all subsequent incubations.

1) 5% phenol, o-cresol, m/p-cresol, 2,4/2,5-xylenol
10% L.B.X. and H.B.X.
(Expt. V2 (iv) (part), p. 113)

Acid phosphatase distribution was unchanged, except in group 10% H.B.X. Distribution seemed to be shortened (length-wise) along the horny layer; this seemed to correlate with extensions of the H.F.G.L.

2) 10% L 14/13, /19, /47 and /56
10% L 16/38, /53 and /70
(Expt. V2 (v) (part), p. 115)

Distribution was as normal for 10% L 14/13, /19, /47 and /56. In 10% L 16/38, /53 and /70, as in 10% H.B.X., the area of absence of acid phosphatase in the horny layer around the neck of the hair follicle seemed extended.

3) 5% 2,3,5-/ 2,4,6-trimethyl phenols, 3-methyl-5-ethyl phenol, 4-/ 5-indanols, 6-methyl-4-indanol, 1-methyl-/ 7-methyl-5-indanols.
(Expt. V2 (vii) (part), p. 122)

All individual phenols showed normal distribution of acid

phosphatase. Two weeks extra treatment with 2, 4, 6-trimethyl phenol did not change the histochemical picture (Expt. V2 (viii), p. 124).

4) 10% and 20% A.O.A.

10% A.O.A. '175', '195', '200', '225', '290', and '330+'

(Expt. V2 (viii) (part), p. 124)

After treatment with 20% A.O.A., there was hardly any acid phosphatase to be seen in the scale horny layer; where it was still present, the distribution was very sparse, although the reaction product was just as intense as in normal scale keratin. Acid phosphatase had been induced in the granular layer region, all along the length of the tail. However, it was in some cases faint and at best light brown - a little lighter than that found in the hair follicle granular layer after one hour's incubation. (The horny layer normally appears black after this time). 10% A.O.A. skin was almost normal; however there did seem some extensions of absence of acid phosphatase (as described above). Similar results were found with 10% A.O.A. '175', '195', and '200'.

10% A.O.A. '225', '290' and '330+' showed some change. However the effects varied a great deal both within the sections and between mice of the same group. The greatest change was loss of acid phosphatase activity in the horny layer with extremely faint inducement in the granular layer.

5. 10% A.O.A. '330'

(Expt. V3 (iii), p. 135)

The changes in the distribution of acid phosphatase were very variable within the 4 animals killed daily. Generally it was observed

that for haematoxylin and eosin sections displaying scale granular layers there were sections showing acid phosphatase induced in the granular layer region. This experiment attempted to monitor daily the effects of tar, but it was not possible to determine intermediate stages in the change in distribution of acid phosphatase from the scale keratin to the granular layer.

In some cases, where the horny layers had been retained, it was possible to see (Fig.20b) that recently produced basket-weave keratin was produced by a layer containing acid phosphatase (i.e. the granular layer. The following parakeratin overlying the thick epidermis was produced without any such layer.

(ii) NON-SPECIFIC ESTERASE

In normal mouse tail the N.S.E. activity developed in sebaceous glands in 2 minutes of incubation. Within 5 minutes the lighter staining of the high-level dendritic cells was visible in the proximity of the hair follicle, (as described by Riley, 1966b). At 15 minutes, sebaceous gland staining was so great that the borders of the fat cells were not visible, but high-level dendrictic cells staining had not increased. After one hour the whole epidermis was non-specifically stained. 5 minutes was the chosen incubation time.

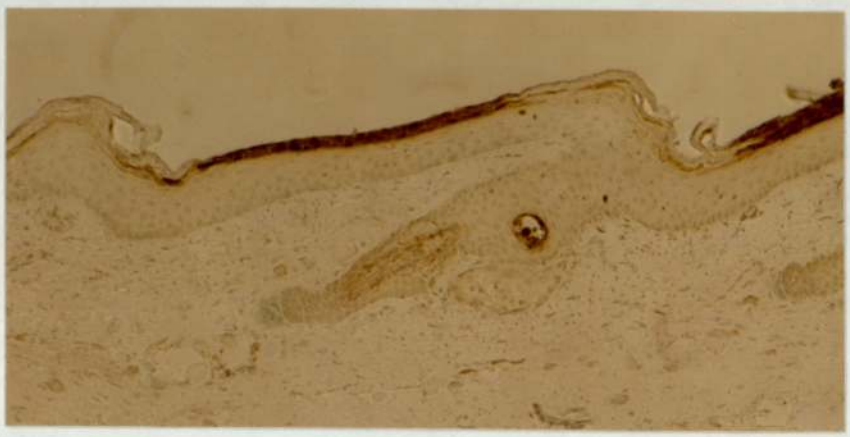
1. 10% A.O.A. '330'
(Expt. V3 (iii), p.135)

In tar-treated mice, N.S.E. activity had been induced in the horny layer. Full development of this new distribution required 10 minutes incubation time.

Fig. 20

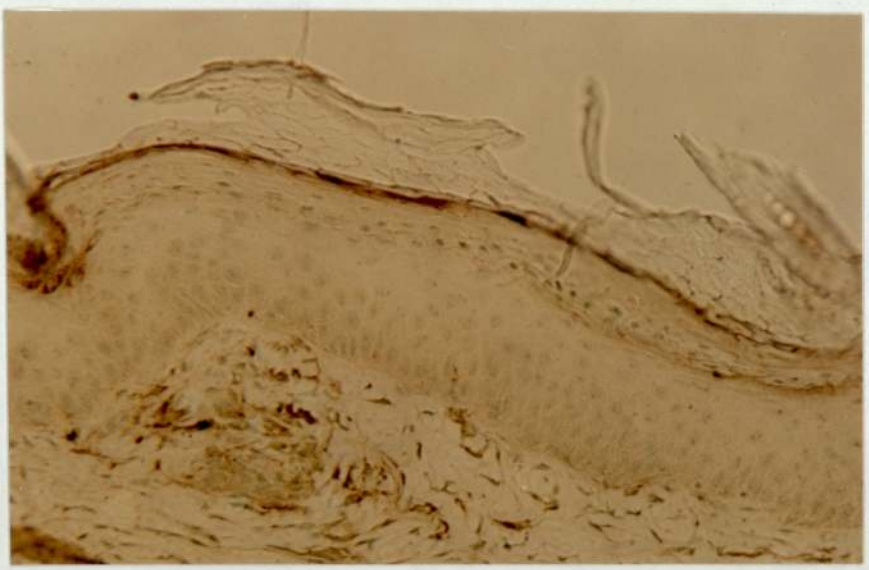
ACID PHOSPHATASE

(a)



UNTREATED SKIN

Dark brown and medium brown distributions of acid phosphatase are evident in the scale keratin and hair follicle granular layer respectively.



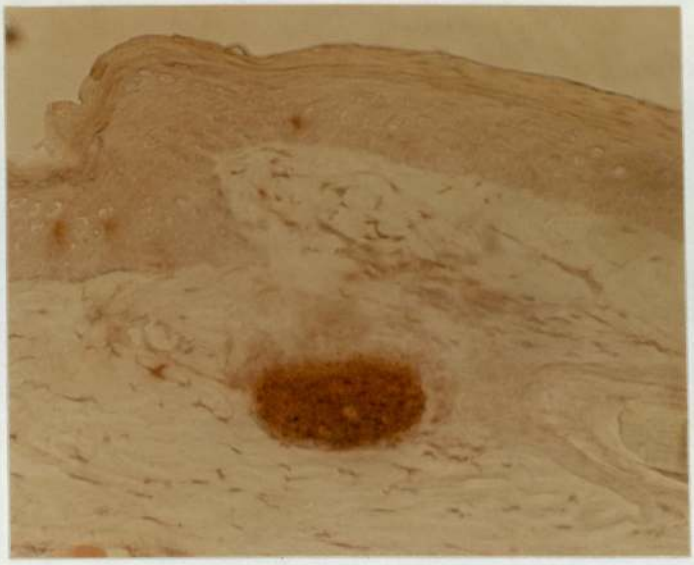
10% A.O.A. '330'

Acid phosphatase is present in the peripheries of orthokeratotic cells of the basket-weave keratin, and in the preceeding granular layer. These cells are being rapidly shed; a parakeratotic horny layer is being formed and a granular layer is absent.

Fig. 21

NON-SPECIFIC ESTERASE

(a)



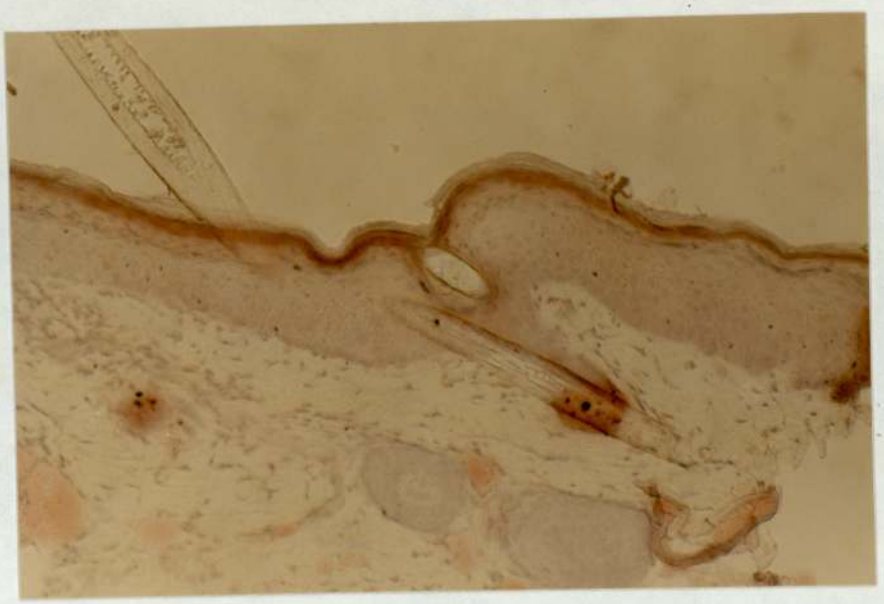
UNTREATED SKIN

N.S.E. activity is present in the dendritic cells around the neck of the hair follicle and in the sebaceous gland.

Fig. 21

NON-SPECIFIC ESTERASE

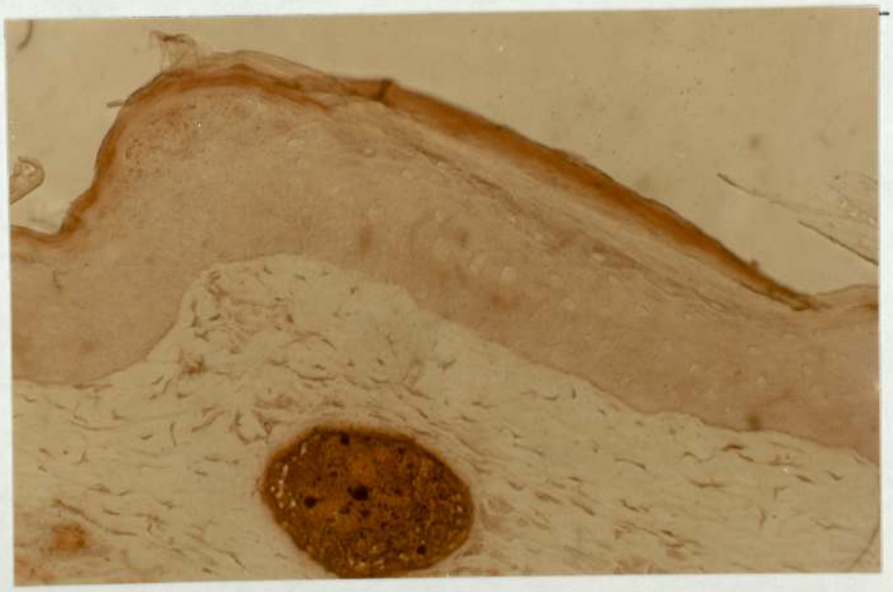
(b)



10% A.O.A. '330'

N.S.E. activity in the horny layer, below which is parakeratin.

(c)



N.S.E. activity in the horny layer; parakeratin is not present. Granular cells are forming underneath in the scale region.

No changes in the distribution of the high-level dendritic cells were observed.

(iii) HORNY LAYER FLUORESCENCE

As described by Jarrett and Spearman (1964) there were distinct differences in fluorescence by the two types of mouse tail horny layer. In these experiments, the tail scale keratin fluoresced green and the flexible horny layer at the neck of the hair follicle fluoresced red.

1. 5% and 10% A.O.A. '<230', '240', '250', '260', '270', '280', '290', '300', '310', '320', '330'
(Expt. V3 (i), p.127)

There was no significant change in mice treated with 5% and 10% A.O.A. '<230', '230', '240', '250', '260', and '270'. The responses in skin treated with acids in the range 280-340°C were very variable. In some scales there was alternating green and red keratin overlying the scale regions. In the higher-boiling acids (310-340°C), the region of the granular layer often fluoresced red, suggesting the beginnings of formation of an orthokeratotic horny layer. In no case did the fluorescence of the horny layer completely change from green to red: the greatest change was in an alternation of green and red scale horny layer.

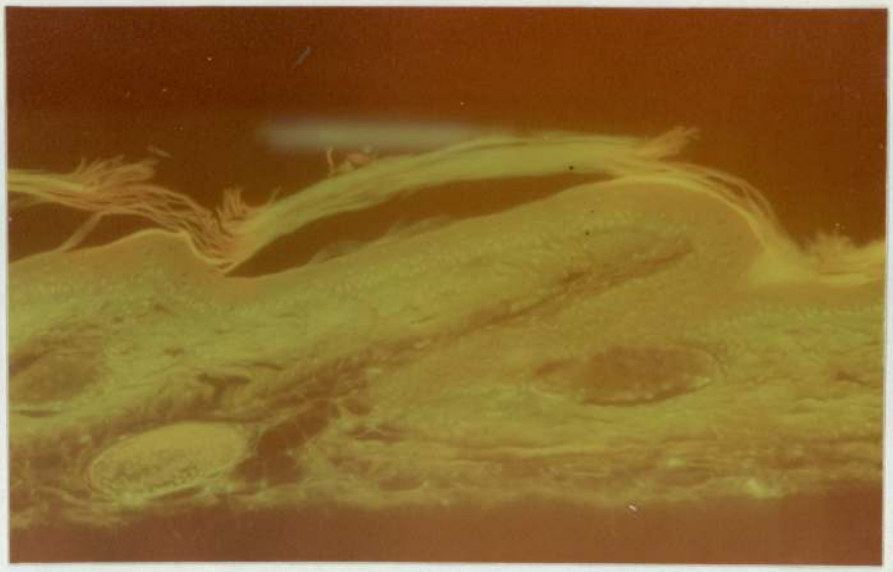
2. 10% A.O.A. '330'
(Expt. V3 (iii), p.135)

Alternating bands of green and red keratin were visible in some mice after at least 4 treatments with 10% A.O.A. '330'. In most cases this could be correlated with the development of a granular layer. In some mice, after 6 and 7 treatments, there was red fluorescence in the region of the granular layer, although the overlying keratin was green.

Fig. 22

HORNY LAYER FLUORESCENCE

(a)

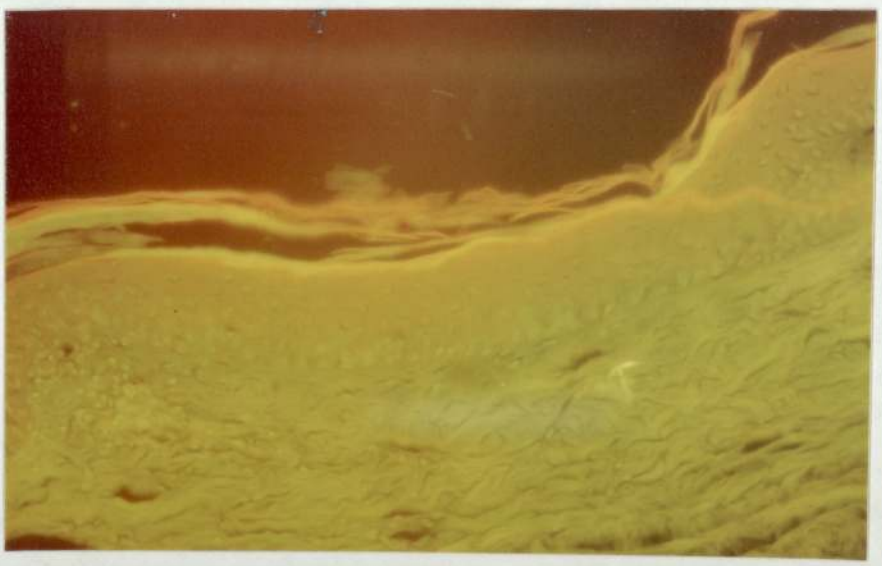


UNTREATED SKIN

Scale keratin fluoresces green.

Basket weave (hair follicle) keratin fluoresces red; there is also some slight red fluorescence in the region of the hair follicle granular layer.

(b)



10% A.O.A. '330'

Phenolic treatments have caused alterations in horny layer fluorescence: there are strands of red keratin in the scale area, and there is slight red fluorescence in the region of the induced granular layer.

Comments on Histochemical Tests

In the cases of acid phosphatase and non-specific esterase demonstrations, controls used were untreated sections of skin displaying enzyme activity in areas previously described by other workers, and also tar-treated tails which were incubated without the substrate. The latter control was not run for each experiment, but only to get accustomed to the small degree of non-specific staining. This in the lead nitrate technique has been extensively discussed by other workers. Newman et al (1950) ascribed this to non-specific staining by lead, but Moretti and Mescon (1956) suggested that this was due to diffusion of the enzyme or product. Grogg and Pearse (1952) stated that only the approximate localization of these enzymes can be achieved with this technique. However on a large scale, i.e. observing the whole skin, the technique consistently demonstrated differences of location in the different types of keratin in the mouse tail, as described by Jarrett and Spearman (1964).

Non-specific staining also occurred in the case of N.S.E., and the use of freshly prepared substrate was essential to minimise the degree of non-specific staining by the azo-dye formed in solution (i.e. not catalysed by the enzyme). Incubation times of about 30 minutes result in general overall staining of the epidermis.

5. OTHER ANTI-PSORIATIC TREATMENTS

A compound widely used in the routine management of psoriasis and which is related to several actual coal tar constituents is dithranol, (see p. 36). It was decided to investigate its effects and also those of a commercial tar preparation, "PSOROX", on mouse tail skin.

Method

Since dithranol is used in man at about one quarter of the concentrations used for coal tar, (e.g. Dithranol Paste, B.P.C., 0.1 - 1.0%; Zinc and Coal Tar Paste, B.P.C. at 6%), it was decided to run dithranol at 10% (at that time 40% tar preparations were being used). Dithranol was run at this concentration in the standard wool fat and yellow soft paraffin ointment being used at that time and also in Lassar's Paste, (which is reputed to be the most efficacious presentation, (Dahl, 1971).

Results

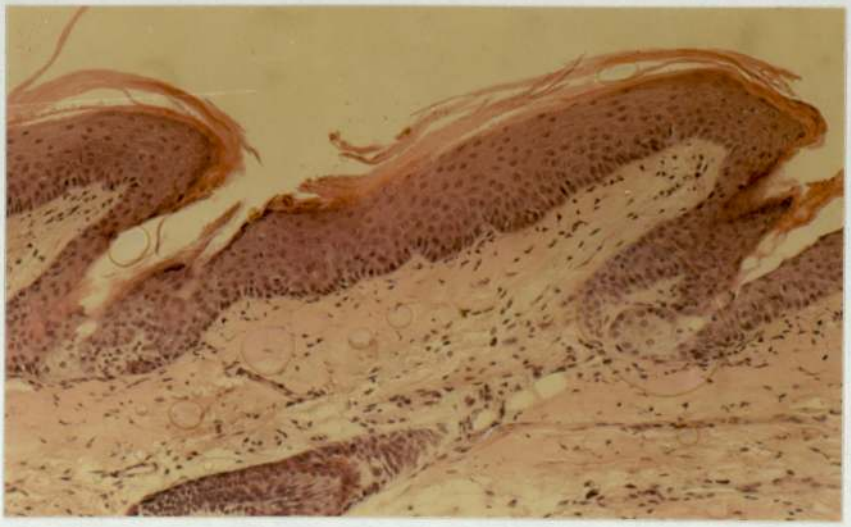
(a) PSOROX

Over 21 days, Psorox did not show any tendency to induce a scale granular layer; it caused highly significant thickening of the epidermis.

(b) DITHRANOL

The ointments were run for 28 days. Dithranol did not stimulate granular layer formation in either vehicle; the epidermis underwent thickening greater than seen after the tar treatments, (see Fig. 23). The degree of skin staining produced by both preparations of dithranol is shown in Appendix I. Graph 7 illustrates the lack of correlation between epidermal thickness and body weight.

Fig. 23



10% DITHRANOL

This section of mouse tail skin treated with Dithranol in Lassar's Paste is typical of the histological picture obtained after 28 days treatment employing both Lassar's Paste and Yellow Soft Paraffin as vehicles. There is no granular layer inducement; the epidermis is thickened and there is increased basophilia.

Table 15. OTHER ANTIPSORIATIC TREATMENTS

(a)

Initial no. mice/group = 10, weight range = 28-32 g. Duration = 21 days

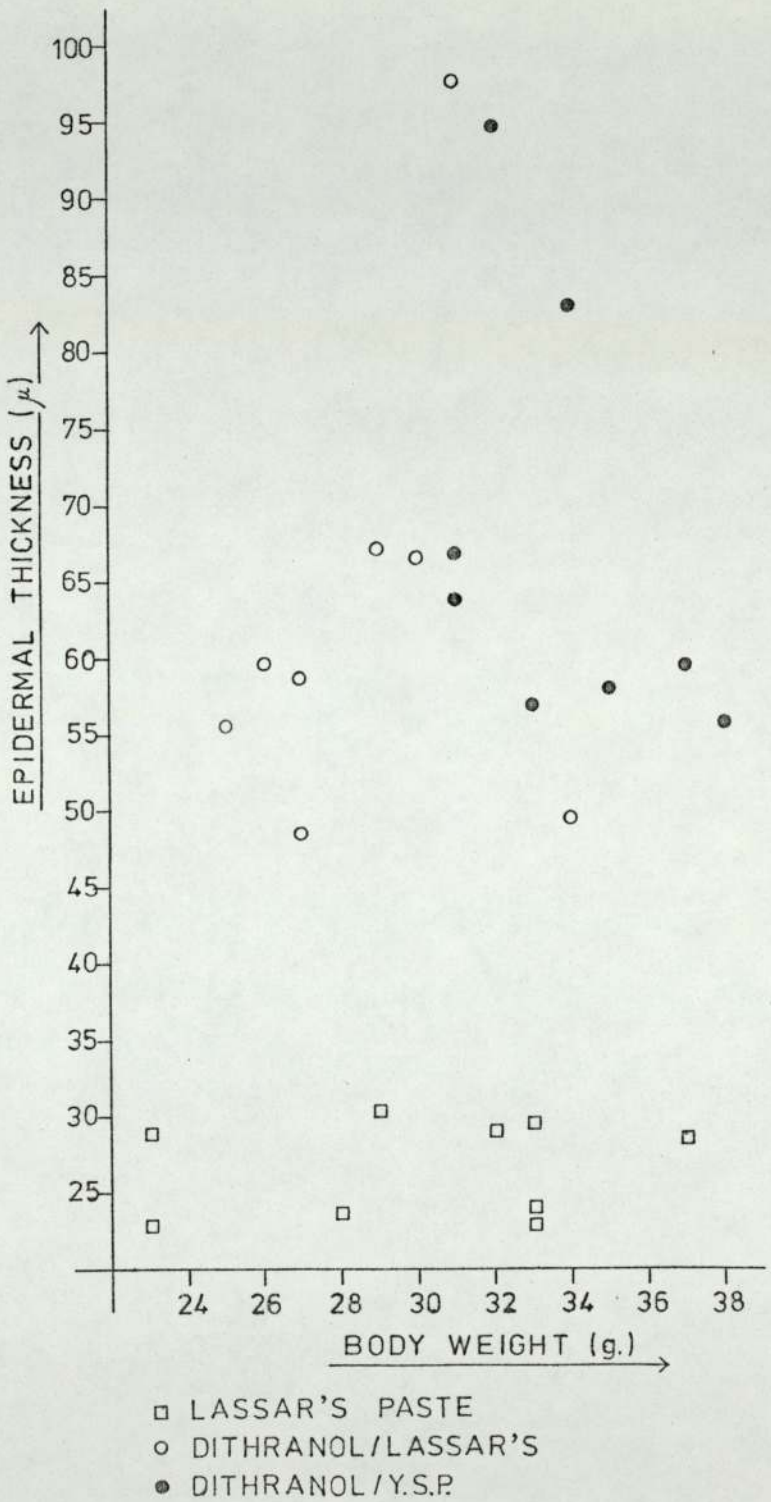
	E.T.	S.E.	n	t	p
CONTROL	35.0	0.90	17	-	-
PSOROX	48.0	2.54	10	5.78	0.001

(b)

Initial no. mice/group = 10; weight range = 23-27 g. Duration = 28 days

	E.T.	S.E.	n	t	p
CONTROL	23.6	1.20	7	-	-
10% DITHRANOL/LASSAR'S	63.0	5.52	8	6.56	0.001
10% DITHRANOL/ Y.S.P.	67.3	4.40	9	12.72	0.001
LASSAR'S PASTE	26.7	1.06	9	1.92	n.s.

Graph 7
DITHRANOL and LASSAR'S PASTE



EPIDERMAL THICKNESS vs. BODY WEIGHT

6. EXAMINATION OF YOUNG MOUSE TAILS

It had been noted that the tails of newly-born and of young mice were very smooth, unlike the rough scaly appearance and feel of tails of mature mice, and it was decided to investigate the histological structure which was producing this smooth skin, as from all premises underlying the work so far described, a granular layer should be associated with such smooth skin.

Methods

Routine histological sections were taken from about 5 mice of each age; these were male 't.o.' mice chosen randomly, the collection of sections being added to over a period of time. No attempt was made to take examples of mice from the same breeding colony.

Results

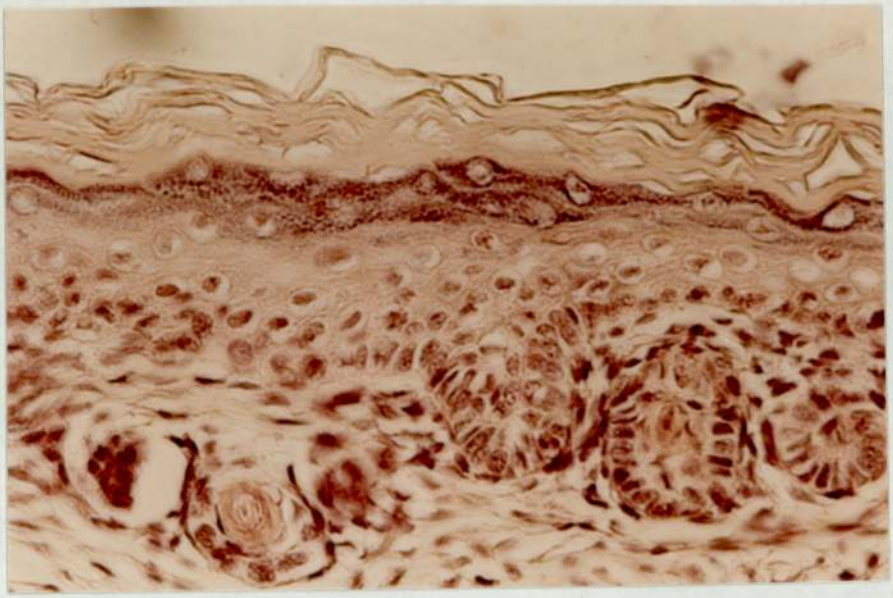
As expected, underlying the smooth skin was a well-developed granular layer in mice aged 1-7 days. This seemed to be regressing between 7 and 10 days, (see Figs. 24a-d).

However, sections along the length of the tail showed that the rapid growth of the mouse tail from about 1-2 cms. in length to 3-4 cms. from day 1 to day 10 is due to growth of the interpapillary, i.e. scale, regions. The granular layers observed are the granular layers associated with the hair follicles, which at these young ages are in very close proximity to each other. At about 10 days, the scales are formed, and epidermal thicknesses are comparable to those found in adult mice.

Fig. 24

YOUNG MOUSE TAIL SKIN

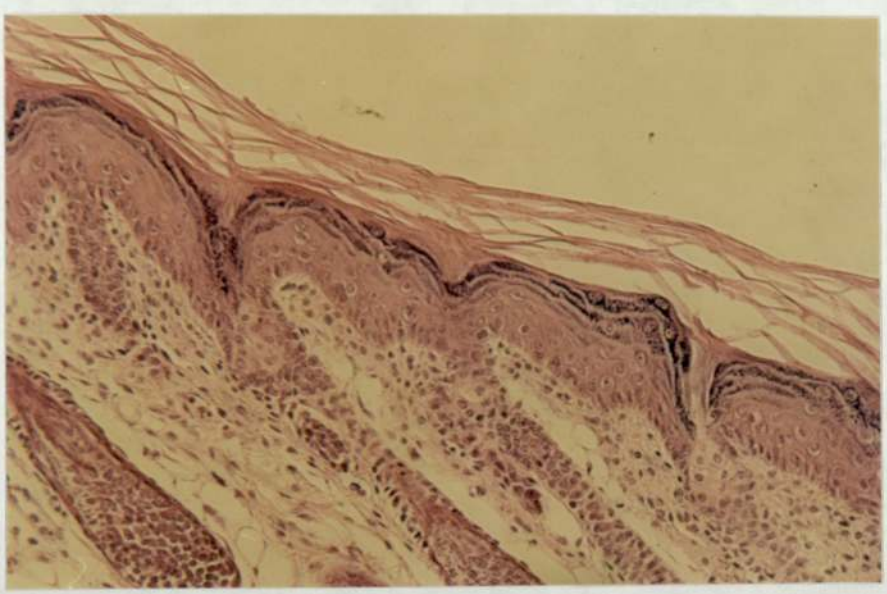
(a)



2 DAYS OLD

This transverse dorso-ventral section illustrates the presence of granular layers and the overlying parakeratin.

(b)

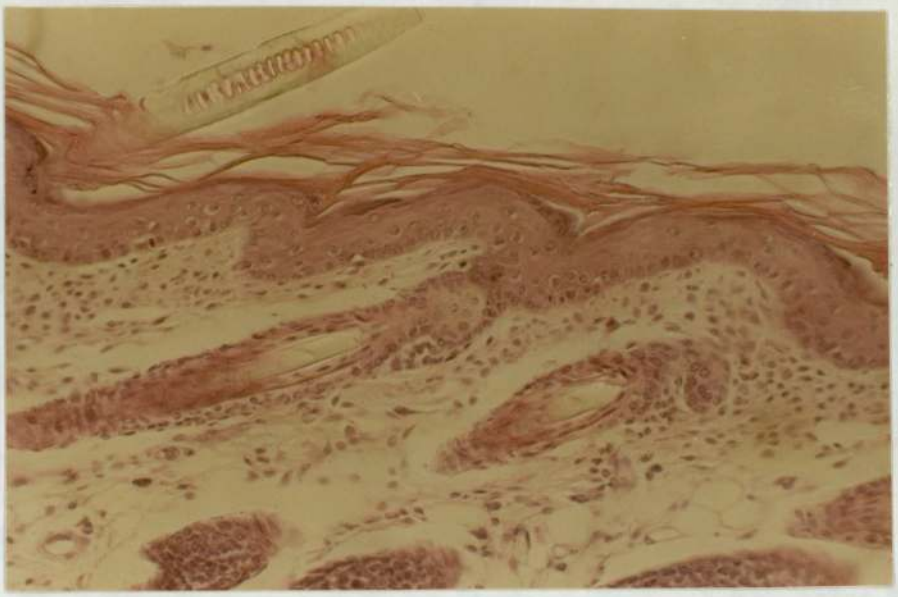


5 DAYS OLD

A vertical proximo-distal section reveals that the granular layers are in fact associated with the closely-packed hair follicles.

Fig. 24

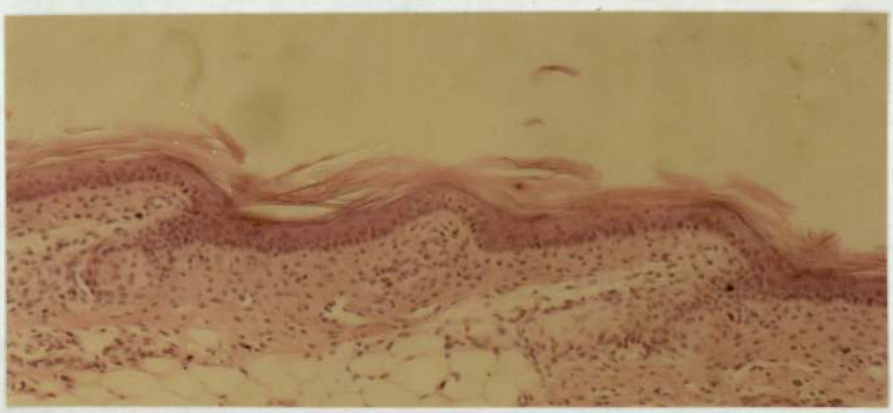
(c)



7 DAYS OLD

The granular cells are now "disappearing" as the scale regions grow. Scaley keratin is forming.

(d)



11 DAYS OLD

The tail skin now has the appearance of mature skin; scale regions are fully developed, and there is very little remaining basket-weave keratin over scale regions.

III

CHARACTERISTICS OF TAR FRACTIONS

III

1. CHARACTERISATION OF FRACTIONS

(i) Avenue Oil Acids

G.L.C. ANALYSIS

Full characterisation of acid fractions used was not possible due to the inability to obtain pure samples of individual H.B.T.A.'s. for calibration purposes. G.L.C. methods were employed for qualitative comparisons between fractions. This technique was especially useful in comparing the following fractions supplied by the N.C.B., namely:

A.O.A. '175', '185', '190', '195' and '210+'

Method

Chromatograms were run on a Perkin Elmer F11 gas chromatograph fitted with a Flame Ionization Detector using an Apiezon L/Carbowax column. These samples were run as 1% solutions in acetone.

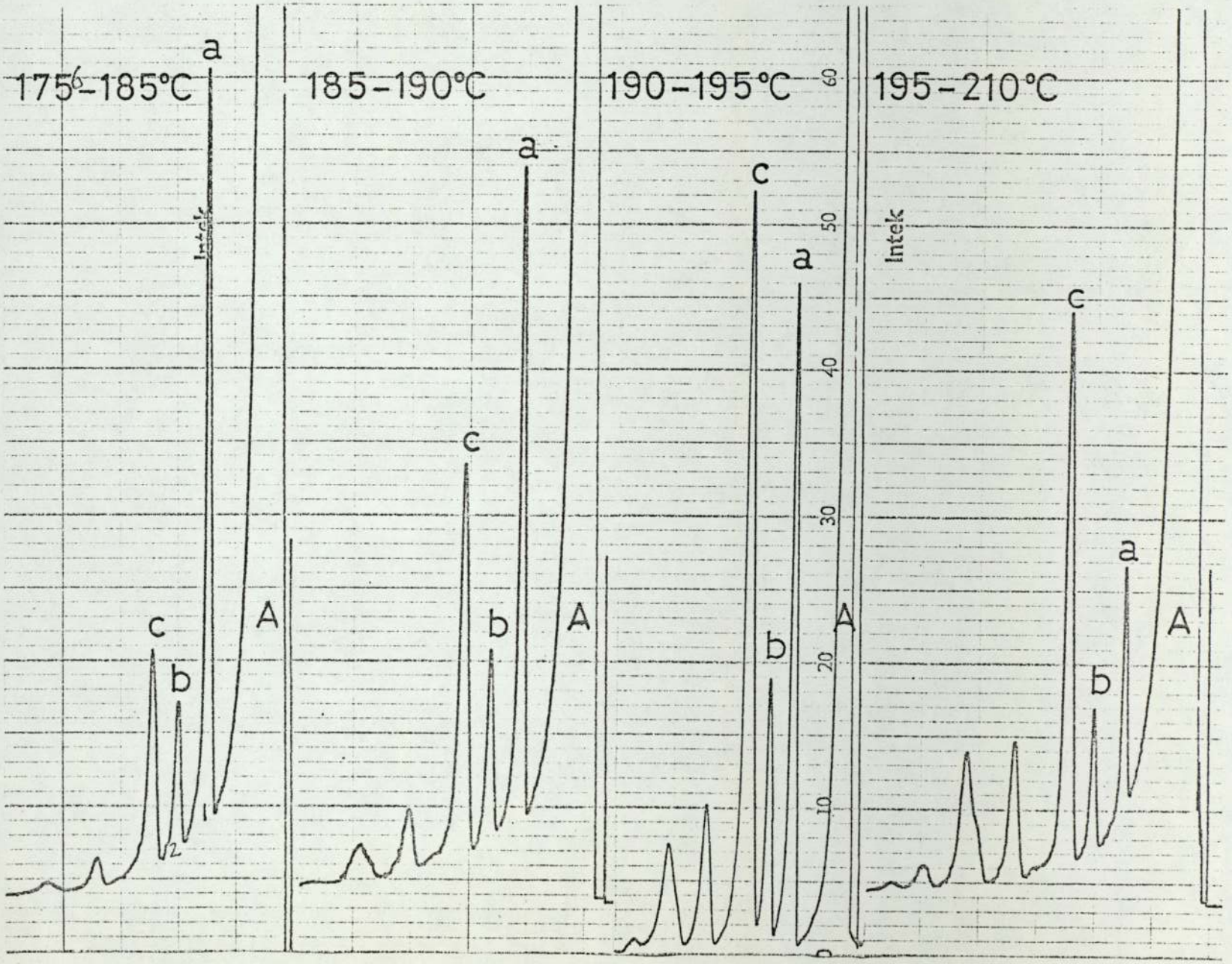
Gas pressures: (lbs./sq. in.)	H_2	19
	AIR	20
	N_2	14
Temperature:	$125^{\circ}C$	
Sensitivity:	$10^2 \times 20$	

Results

Chromatograms, (Fig. 25), showed the first four fractions to be similar in that they contained mainly phenol and the three isomers of cresol in differing proportions, according to the boiling range. A.O.A. '210+' contained fewer of these compounds and consisted chiefly of higher boiling phenols (meta- and para- cresols were not resolvable, since this column separates on the basis of boiling points, which in this differ only by $0.2^{\circ}C$.)

Fig. 25 G.L.C.
Avenue Oil Acids

a : PHENOL
b : o-CRESOL
c : m/p-CRESOL
A : Acetone



A.O.A. '185' and '190' were shown to be of very similar composition in that they both had similar relative quantities of phenol and m/p-cresol. It was decided not to test these biologically, and only to test the extremes of the boiling range, namely 175-185°C and 195-210°C, these respectively containing relatively more phenol and relatively more m/p-cresol.

INFRA-RED ANALYSIS

I.R. spectra were run of the whole oil acids to determine whether there were any carboxyl components in the acid mixtures. Failure to locate carboxyl groups would indicate that the acids were phenolic only.

Method

Infra-Red spectra were run on a Pye Unicam Infra red Spectrometer, (S.P. 200).

Results

Infra-red analysis of Avenue Oil whole Acids showed the absence of carboxyl groups, and therefore the acids were assumed to be all phenolic, (see Fig. 26).

(ii) Coalite Oil Acids

G.L.C. analysis was not carried out for Coalite Oil Acids, since the Coal Tar Research Association provided the data shown in Table 16 for fractions L 14/13, /19, /47, /56 and L16/38, /53 and /70. This data was obtained by capillary column G.L.C.

INFRA-RED ANALYSIS

Method

As for Avenue Oil Acids.

Results

Infra-red analysis of Coalite Oil Acids showed the lack of any C=O grouping, and similarly it is thought that Coalite Oil Acids are phenols. Similar results have been found by Fair and Friedrich (1955), Irvine and Mitchell (1958) and Karr et al (1960).

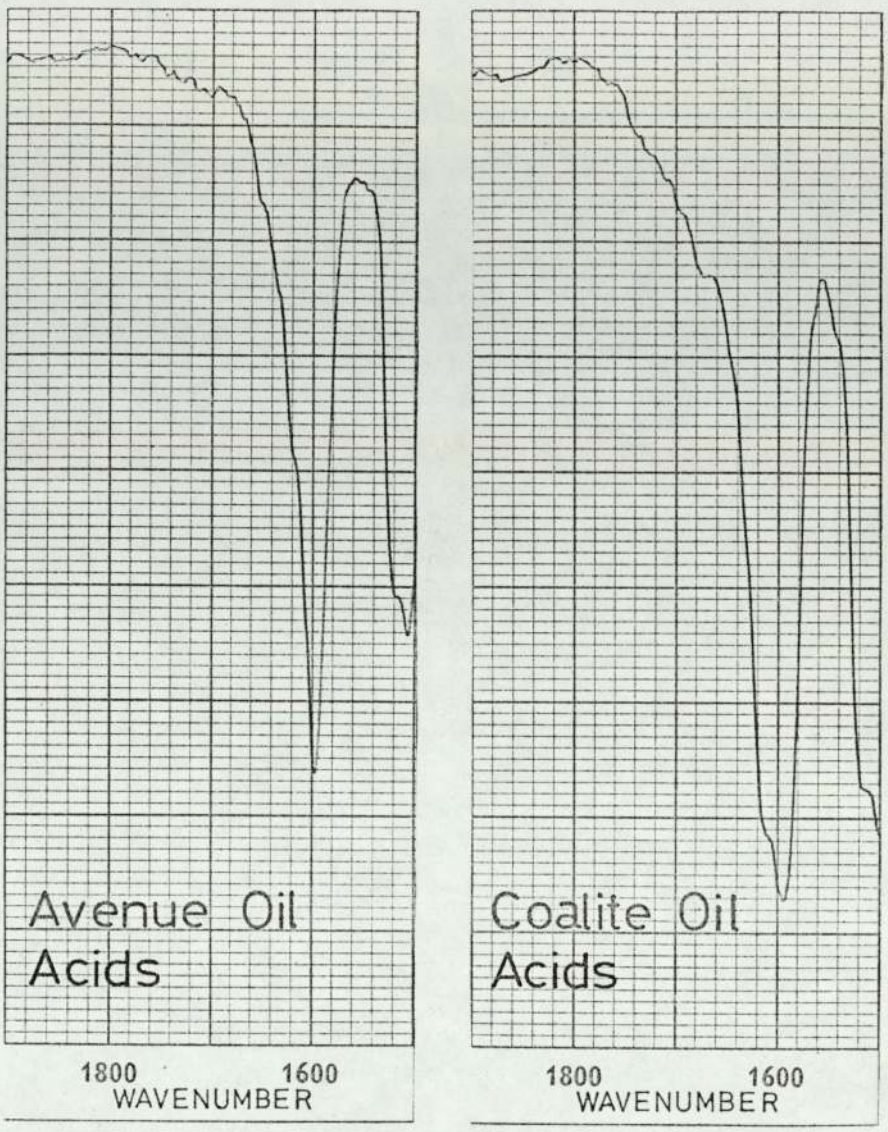


Fig. 26

INFRA-RED SPECTRA OF TAR OIL ACIDS

Table 16

COMPOSITION OF COALITE OIL H.B.T.A. FRACTIONS

Component	L14 13	L14 19	L14 47	L14 56	L16 38	L16 53	L16 70
o-cresol	0.4	-					
m-cresol	0.4	-					
p-cresol	0.2	-					
3,4-xylenol	9.7	5.2					
3-methyl-5-methyl phenol	17.9	28.6	10.1	2.2			
2,3,5-trimethyl phenol	11.5	8.8	-	-			
2,4,5-trimethyl phenol	9.0	9.5	5.3	4.0			
4-indanol	3.6	5.6	21.4	17.1	2.9	1.6	
5-indanol	1.1	0.6	7.7	19.7	13.8	4.1	0.6
1-methyl-4-indanol					4.0	0.7	-
3-methyl-5-indanol					12.2	11.4	5.4
1-methyl-5-indanol					12.8	11.4	4.5
6-methyl-4-indanol					10.2	11.1	7.5
5-methyl-4-indanol			1.0	2.7	7.0	8.2	7.4
7-methyl-4-indanol					5.9	11.6	17.5
7-methyl-5-indanol					0.8	2.7	9.3
4-methyl-5-indanol					1.1	3.7	5.7
2-methylresorcinol					7.5	8.1	9.3
Unknown A					2.1	0.5	-
" B					2.1	0.6	-
" C					1.1	0.3	-
" D					-	0.2	-
" E					-	-	3.1
" F					3.1	4.6	4.0
" G					2.9	3.8	6.5
" H					5.8	8.6	10.2
" I					0.7	2.0	3.8
" J					0.1	2.1	2.3
" K					-	0.8	2.0
Boiling Range °C							
5%	227.0	229.5	241.0	244.0	253.0	256.0	260.5
95%	232.0	239.5	245.0	247.5	256.5	260.5	264.0

(Data supplied by Coal Tar Research Association)

2. HIGH-BOILING TAR ACIDS

(i) Introduction

Mouse tail experiments indicated that H.B.T.A.'s may be of value in the treatment of psoriaform diseases. Martindale (1972) states that the low-boiling fraction of tar acids covers the boiling range 188-205°C, and forms 'Cresol, B.P.', from which "LYSOL" is made. This will contain phenol and the three cresol isomers. The middle fraction (205-230°C) contains mixed cresols and xylenols - a mixture known as "cresylic acids". The H.B.T.A.'s are considered to distil from 230°C and contain a very wide range of phenols, the general structures being listed in Table 17.

It can be seen that H.B.T.A.'s cover a very wide range of substituted phenolic compounds. Many analyses have been carried out on these mixtures, (Karr 1963; Pichler et al, 1970) mostly by G.L.C. and I.R. methods.

Most H.B.T.A.'s are produced from low-temperature tars, since they are more abundant in these tars. They are also present in the ammoniacal liquor (see p. 5) of low-temperature tars, (Bristow, 1947). Bristow states that high-temperature tar liquor does not contain dihydric phenols and so would not be a good source of H.B.T.A. mixtures.

There have been many methods described for improving efficiency in springing tar acids, (Milner, 1947; Horne et al, 1950, and Papkov and Pats, 1969). However most work has been carried out on low-temperature tars, as high-temperature tar oils are notorious for their emulsifying actions when combined with NaOH, (see p. 85). In the work

Table 17

Mono- and poly- Substituted:	Boiling Range (°C)
Phenol	182
Methyl phenols	190-267
Ethyl phenols	207-248
Ethyl-methyl-phenols	212-250
Propyl-phenols	220-256
Isopopyl-phenols	214-229
Methyl-propyl-phenols	233-241
Methyl-isopropyl-phenols	228-241
Cyclopenten-2-yl phenols	270-293
Cyclopenten-1-yl phenols	272-293
Phenylphenols	275-325
Cyclohexylphenols	283-293
4-indanol	245
5-indanol	255
Methyl-indanols	250-260
Catechol	245
Methyl-catechols	248-258
1-naphthol	288
2-naphthol	295
Methyl-naphthols	295-315
7-ethyl-4-methyl-1-naphthols	320
Tetrahydronaphthols	265-276
Methyl-tetrahydronaphthols	280-305
Ethyl-resorcinols	265-276
5-acenaphthol	332
4-acenaphthol	338
1-fluorenol	245
2-fluorenols	340-350
3-fluorenol	350
8-methyl-2-fluorenol	355

(Source: Coal Tar Data Book)

herein described, addition of and agitation with NaOH caused formation of resinous compounds, which had to be discarded; it is not known whether any H.B.T.A!s were lost due to this. Consequently most attempts to extract these on a large scale from high-temperature tars have been abandoned.

(ii) Current Uses

At present H.B.T.A.'s are mainly used in disinfectants (as bactericidal agents), for wood impregnation (as anti-bacterial and fungal compounds, e.g. "creosote", (Mayfield, 1951), and substances such as catechol are used in the chemical industry, (Karr, 1963).

"IZAL GERMICIDE" (Izal Ltd.) contains H.B.T.A's but the boiling ranges are not stated. Creosote Oil Acids seem to be present in products such as "CREOLIN" (William Pearson, Ltd; boiling range = 230-270°C, (communication)) and "CRESOLOX" (Compass Chemicals, Ltd.; boiling range = 230-260°C, (communication)). Jeyes (U.K.) Ltd. use the X.X.L. fraction described on p. 86 in their product "JEYES FLUID".

The anti-bacterial activity is often measured in "Rideal-Walker coefficients", which increase with an increase in boiling point. Anti-bacterial activity is also increased in chlorinated phenols, (Martindale, 1972).

(iii) Future Work

For future evaluation of the therapeutic activity of H.B.T.A.'s further separation of them is required. This has been in the past attempted by sophisticated G.L.C. methods.

The anthracene oil acids in these experiments (Expts. V3 (i and III)) were separated without the use of a fractionating column. Future work will require the provision of "cleaner" cuts in boiling point. Preparative G.L.C. methods have been suggested for the separation of tar acids, (C.T.R.A., 1971). Grant (1960) recommended the use of capillary columns for the separation of tar components.

It is also suggested that tar bases are investigated biologically and if they show activity, preparative and analytical techniques will also be required for these fractions. Karr and Chang (1958) have described such analyses for a low-temperature tar.

If preparative techniques such as those outlined above do not supply sufficient of the H.B.T.A.'s synthetic methods will have to be investigated after structural determinations, such as those described by Yarboro and Karr (1959).

Kusy (1970) stated that a combination of analytical methods is required to analyse these complex phenolic mixtures; identification becomes more difficult with increasing molecular weight and the concomitant number of isomers.

IV

TAR FORMULATION PROBLEMS

IV

TAR FORMULATION PROBLEMS

1. INTRODUCTION

It was essential in this project to hold constant the one factor which could probably most influence the results and conclusions derived from this work and that was the choice of vehicle. The vehicle dilutes the pharmaco-dynamic agents and promotes their absorption into the skin. Because of the many influences of the vehicle on the tar and the skin, in such an experimental set-up, it was necessary to restrict vehicle effects to a minimum at which one could only hope that by using the same vehicle for each fraction tested, the effects caused by the vehicle and tar would be similar in each trial, so that fractions could be compared in as well-defined a system as possible.

In the first instance, it was decided to use an oil-in-water emulsion base (Vehicle 1) which, it was thought, would accomodate both hydrophilic and hydrophobic substances in tar. The composition of this base is shown on p. 88. It soon became obvious that the vehicle itself was causing thickening of the epidermis, (see Expt. (a) below). A yellow soft paraffin and wool fat ointment was then adopted, (Vehicle 2). Most of the fractions were tested in this vehicle, but in the later experiments, in search for a greater therapeutic effect, further vehicle experimentation was required, which is described below, (Expt. (b)).

Finally a few individual H.B.T.A.'s were run in a vehicle comprising dimethyl sulphoxide (DMSO) and ethanol, (Vehicle 4).

2. EXPERIMENTS

(a) Vehicles I and II

Method

A trial was run in which the vehicles were tested along with a group of mice which had their tails rubbed daily with cotton wool to simulate the application of the vehicle or tar. The duration of treatments was 21 days.

Results

Rubbing the skin did not alter tail thickness, and so was not the cause of thickening by Vehicle I, (Table 18).

Comment

The thickening was then due to the consituents of the vehicle. In the tar experiments this effect provided an extra variable. This together with the fact that thickening by tar preparations was hoped to be reduced made the thickening effect an undesirable feature of the vehicle.

Vehicle 2 had no effect on the skin, (Expt. V2 (i)).

(b) Vehicle III

Two vehicles recently used with success for steroids in the treatment of psoriasis are "PLASTIBASE" and "F.A.P.G." (Fatty Acid/Propylene Glycol), and it was decided to compare these with the yellow soft paraffin.

Method

Avenue Oil Acid fractions '235' and '290' were chosen as models,

with which to compare the vehicles. The following groups were run:-

10% A.O.A. '225' in YELLOW SOFT PARAFFIN/WOOL FAT ('225Y')
10% A.O.A. '225' in PLASTIBASE ('225P')
10% A.O.A. '225' in F.A.P.G. ('225F')
10% A.O.A. '290' in YELLOW SOFT PARAFFIN/WOOL FAT ('290Y')
10% A.O.A. '290' in PLASTIBASE ('290P')
10% A.O.A. '290' in F.A.P.G. ('290F')
PLASTIBASE
F.A.P.G.

Results

After 6 treatments, '290P' was causing skin peeling in about half of the group. After 9 treatments these mice were killed as there was extensive skin peeling. After 10 treatments, '225P' seemed to be causing scaling, and mouse tails became progressively more scaly as mice were treated for a further week.

HISTOLOGY

There was no change in mice treated with both A.O.A. '225' and '290' in F.A.P.G. In A.O.A. '225Y' few isolated granular cells were found, but no complete granular layers were induced. These changes were more advanced in '290Y'. However in groups treated with acids in Plastibase, there was peeling of the skin and induction of scale granular layers. However these were not continuous in either group. Significant epidermal thickening was produced by both groups when in yellow soft paraffin and Plastibase vehicles, (see Graph 8).

Table 18 EXPT (a)

Initial no. mice/group = 10; weight range = 28-32g. Duration = 21 days

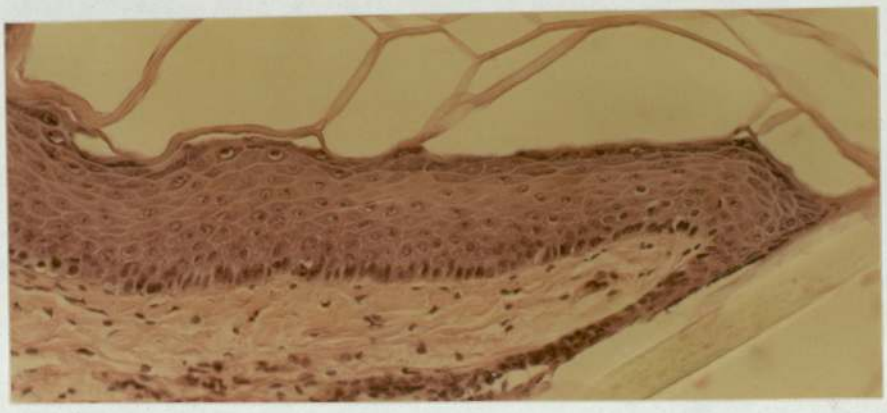
	E.T.	S.E.	n	t	p
CONTROL	35.0	0.90	17	-	-
COTTON WOOL ONLY	34.5	1.18	8	0.32	-
VEHICLE I	40.4	0.77	18	4.61	0.001

Table 19 EXPT (b)

Initial no. mice/group = 7; weight range = 20-25g.

	Days Treated	E.T.	S.E.	n	t	p
CONTROL	(21)	28.1	0.79	4	-	-
10% A.O.A. '225Y'	21	43.7	2.42	7	4.68	0.005
10% A.O.A. '225P'	21	46.7	1.98	4	8.69	0.001
10% A.O.A. '225F'	21	30.9	1.58	6	1.32	n.s.
10% A.O.A. '290Y'	21	39.7	2.95	6	3.10	0.05
10% A.O.A. '290P'	9	45.9	2.40	4	7.04	0.001
10% A.O.A. '290F'	21	32.4	1.68	7	1.81	n.s.
PLASTIBASE	21	27.7	1.09	6	0.29	n.s.
F.A.P.G	21	28.7	1.27	7	0.29	n.s.

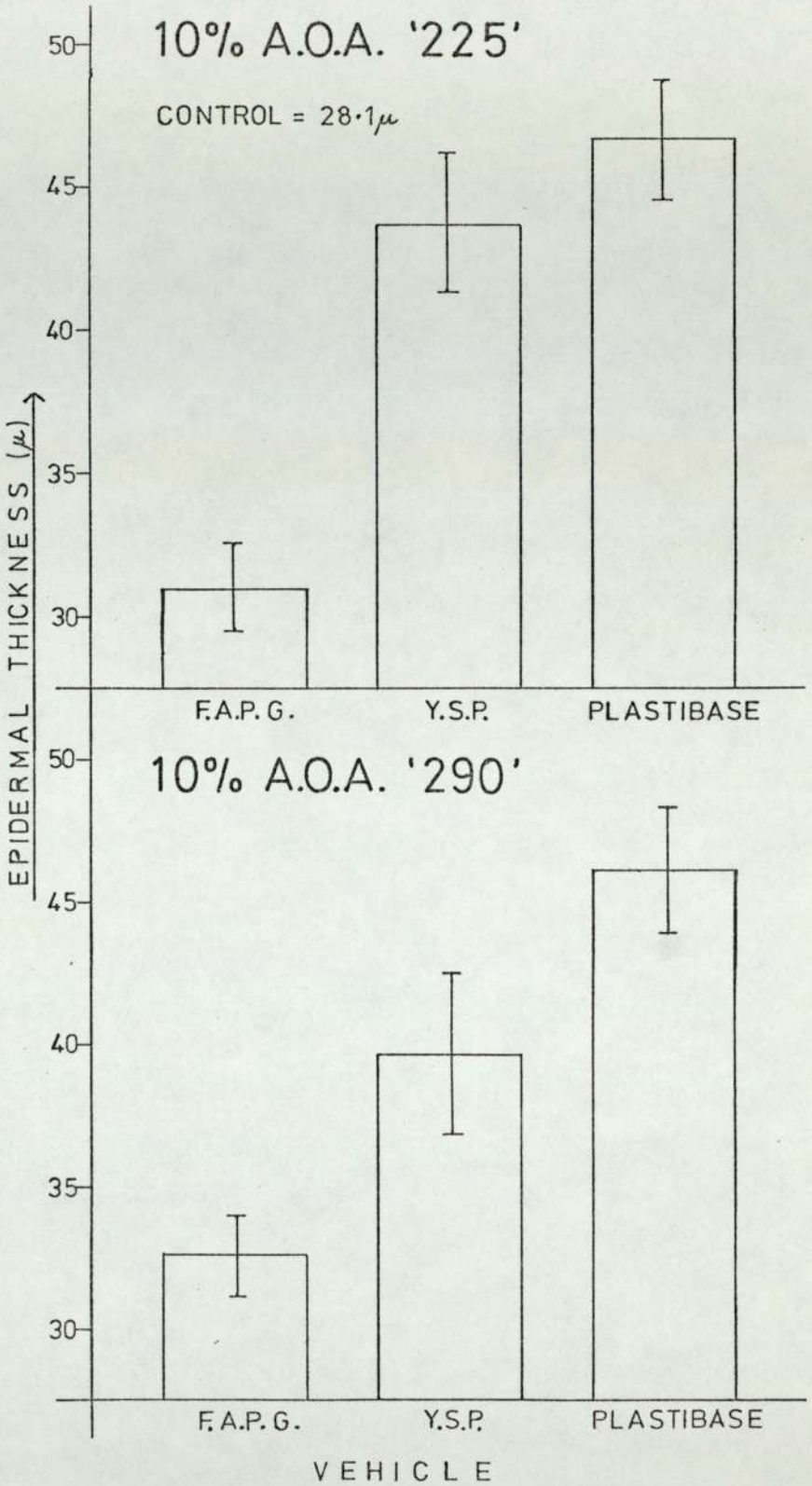
Fig. 27



10 A.O.A. '330'
(PLASTIBASE)

This section shows the induced scale granular layer and increased epidermal thickness; both effects were maximised when 'Plastibase' was used as a vehicle for these acids.

Graph 8



VEHICLE EFFECTS

(c) Vehicle IV

In view of the high activity of some H.B.T.A. mixtures, it was extremely surprising to find that individual H.B.T.A.'s, even in concentrations equalling those of potent H.B.T.A. preparations (i.e. 10%) caused very little change in the skin. It was decided that this was probably due to vehicle effects, i.e. that the drug was not getting into the skin. Good percutaneous absorption of steroids had occurred when formulated in varying concentrations of ethanol, (Garnier, 1971), and it was decided to run the tar acids in ethanol with dimethylsulphoxide (DMSO) as a penetration promoter.

Method

The following lotions were made up:

	Acid (g.)	DMSO (mls.)	Ethanol (mls.)	Final concentration of acid
3,4-dimethyl phenol	2	2	16(Absol.)	10%
2,3,5-trimethyl phenol	2	2	16(70%)	10%
2,4,6-trimethyl phenol	2	2	16(70%)	10%
3-methyl-5-ethyl-phenol	2	2	16(70%)	10%
5-indanol	2	2	16(70%)	10%
VEHICLE 4	-	2	18(70%)	-

Results

All acids and the control vehicle had no effect on the skin. Tail thicknesses are shown in Table 19, and display no variation from normal.

Table 19. EXPT. (c)

Initial no. mice/group = 8; weight range = 20-25g. Duration = 21 days

	E.T.	S.E.	n	t	p
CONTROL	30.9	1.12	6	-	-
3,4-dimethyl phenol	31.9	1.37	7	0.55	n.s.
2,3,5-trimethyl phenol	32.3	1.32	7	0.80	n.s.
2,4,6-trimethyl phenol	30.7	1.01	7	0.10	n.s.
3-methyl-5-ethyl-phenol	31.5	1.70	7	0.28	n.s.
5-indanol	29.2	1.04	8	1.10	n.s.
VEHICLE 4	31.4	1.11	7	0.35	n.s.

COMMENT

The all important role of the vehicle employed in the screening of pharmacodynamic agents has been demonstrated in these few experiments. The greater effects from using yellow soft paraffin and Plastibase ointments may be in part due to their occlusive effects, occlusive formulations being well-known to enhance percutaneous absorption, (Katz and Poulsen, 1972). The complete lack of effect by H.B.T.A.'s in F.A.P.G. may have been due to hydrogen-bonding between the fatty acids and the phenols preventing adequate release of phenols into the skin.

The absence of effects by individual H.B.T.A.'s in DMSO/ethanol is discussed later.

3. THE STABILITY OF TAR PREPARATIONS

All creams and ointments when made up were instantly transferred to a refrigerator at 4°C, and were only removed to treat the animals. This helped prevent breakdown of emulsions and mixtures, particularly prevalent when very non-viscous fractions were made up at high percentages, e.g. 20%, 40%. Tars are often reported as being easily oxidised, an effect which would be reduced under refrigeration. Obermayer and Becker (1935) reported that freshly distilled tar darkened on standing in the presence of air. They attributed this to the presence of reducing substances such as tri-hydroxybenzene, aminophenols and phenol. Indeed, some of the preparations containing high-boiling tar acids darkened even under refrigeration; the most notable one was the ointment of the high-boiling tar acid mixture (Grade 108) at 10% in

Plastibase, which after formulation was a very light grey colour; within 4 days the ointment was red.

Ointments made up with Lassar's paste were kept at room temperature, since refrigeration rendered them too stiff for manipulation.

GENERAL DISCUSSION

GENERAL CONCLUSIONS

All tars and tar fractions which had effects on the skin such as granular layer inducement, peeling and general epidermal damage also caused epidermal thickening. This was first observed using 5% tars and 10% tar oils in the oil-in-water vehicle. The vehicle itself was shown to thicken the skin, and so was substituted by an innocous yellow soft paraffin base.

There was no sign of specific granular layer-inducing activity until the tar and oil acids were applied at 40%. Although at these high concentrations there was associated extensive skin damage, granular layer induction was definitely being produced by the acid fractions of Avenue Oil and Coalite Tar and Oil, (Avenue Tar acids not being available to test). This tendency was perhaps greater in the oil acid fractions. Neutral and basic constituents and neutral constituents on their own seemed to only cause an increase in epidermal thickness with cell hypertrophy and, possibly associated increased basophilia. The results indicated that the epidermal thickening effects of tar could be reduced by ommitting the neutral substances; (the question of the bases could not be settled without independent 'base' tests).

In two of the above experiments, it had been attempted to correlate body weight to tail thickness, but they were quite independent of each other. Generally age and weight seemed to be associated with the tail thickness of untreated mice (as observed from 'CONTROL' thicknesses and the weight ranges for experiments). However no such correlation can be demonstrated on an individual basis. Since the acid fractions were, amongst their many other actions, stimulating epidermal

growth, it was too early to postulate whether the granular layer inducing properties of tar could be separated from any thickening activity.

Further experiments with Avenue Oil Acid fractions notably the phenol and cresol mixtures (175° - 185°, 195 - 210°C) and the higher-boiling acids (>210°C), suggested that relatively more granular layer induction was occuring with relatively less skin thickening fractions, although these differences were very small. However in tests with 20% preparations rich in phenol, it was obvious that the lower-boiling fraction was very much more irritant. This was the first suggestion that the higher-boiling acids may be more beneficial.

The second indication came from work on a Coalite Oil Acid fractions, where the individual low-boiling phenols, cresols and xylenols were without effect, except to produce significant thickening in the cases of the meta- and para-cresol mixtures and also by high-boiling xylenols and high-boiling tar acid mixtures. The slight extensions of the hair follicle granular layers by the latter two fractions also suggested that higher-boiling fractions should be examined.

Thirdly the use of smaller cuts of these high-boiling acid mixtures again indicated that granular layer induction was prevalent in the higher-boiling fractions.

The runs of Avenue Oil acids in the range 200 - 400°C seemed to highlight greater activity particularly in the range 290 - 330°C, with lesser activity by phenols boiling over 330°C and between 225-300°C, and virtually none by the acids boiling below 225°C. However experiments with individual high-boiling tar acids were disappointing in that no granular layers were induced. The failure also of these acids to

produce significant epidermal thickening except in the cases of meta- and para-cresols, and the methyl-indanols suggested that these individual constituents were not penetrating into the skin as were their parent mixtures. It was probable that these crystalline acids were not sufficiently dispersed in the vehicle - i.e. the particle size, even after grinding, was still too large. This would have been overcome by dissolution in a liquid vehicle. However formulation at 10% into lotions containing 10% DMSO in ethanol did not result in granular layer formation. In this case it was probable that soon after application to the skin the ethanol evaporated, leaving insufficient DMSO to aid penetration. Despite evidence to the contrary, it still does seem extremely unlikely that these individual high-boiling acids chosen at random for testing are without effect when their parent mixtures are so active. It is felt that the lack of effect so far observed is due to vehicle failures.

However with the use of more refined acid mixtures and lower doses, the effects on mouse tail skin were less drastic than when the first acid fractions were run at 40%. With the decrease in irritation came a decrease in the number of granular cells forming, thus making it very difficult to differentiate between fractions. Several fractions representative of the whole boiling range of tar oil acids were repeated for longer periods which had been used at the beginning of the tar experiments, but these did not produce the desired well defined granular layer. In fact, in the case of the phenols boiling between 290-340^{o}C, the granular layer inducement after one month's treatment was reduced to that found after two weeks. Also, in the mice treated with the phenols in the ranges 200 - 240^{o}C, epidermal thickening which was highly

significant after two weeks treatment had not remained after 28 days.

The use of more refined fractions suggested that a more specific and efficient vehicle could enhance penetration and maybe so enhance granular layer-inducing ability. It was decided to use penetration enhancing vehicles which are not usually employed in conventional tar formulation. Using the Avenue Oil Acid boiling ranges of 225 - 300°C and 290 - 340°C as model fractions, two new preparations were tested against the ointment of yellow soft paraffin. As a result, a liquid paraffin vehicle "PLASTIBASE" was adopted for further experiments on high-boiling tar acids.

The 10° fractions of a combination of the ranges 225-300°C and 290 - 340°C were run in Plastibase. In this experiment, the overall picture seemed at first to be confused by the range of treatment times used. However, several fractions appeared not to induce any granular layer and some of these were also found to be irritant to the mouse tail skin. These were acids boiling below 280°C. In fractions boiling above 290°C, there was increasing evidence of granular layer induction however concomitant with this was also increased epidermal thickening and peeling. Both of these two changes were produced by 10% preparations of the range 280 - 290°C and thickening was produced by this range at 5%. Granular layer induction was at a maximum in the highest boiling fraction (330 - 340°C) when run at 10%. However thickening and peeling were also maximised. It was not possible to grade granular layer induction for these higher-boiling acids, but it did seem that very active constituents were contained in the higher boiling acids of Anthracene and Creosote Oils.

It was concluded that the acids in boiling range 280-340^{0}C had a more specific action in inducing granular layers than did the parent tars, oils or whole acids at similar concentrations. These acids were still causing thickening and in some cases extensive peeling of the skin. However it is thought that these effects only highlight even more its specific granular layer inducing activity and that they may be able to be controlled by use of a suitable vehicle.

The results from histochemical demonstrations of tar-induced changes reinforced conclusions from granular layer observations, as was predictable from the work of Jarrett and Spearman (1964). Acid phosphatase was induced in the granular layer region by Avenue Oil whole acids and also by the higher-boiling fractions of Antracene Oil acids. The highest boiling fraction (330-340^{o}C) induced acid phosphatase in the granular layer and in the peripheries of cells in the newly-formed basket-weave keratin. Jarrett and Spearman have described these distributions. In no case did the fluorescence of the horny layer change from green to completely red (as described by Jarrett and Spearman after Vitamin A treatments). Strands of red horny layer interspersed with green orthokeratin was produced by the higher-boiling acids of Cresote and Anthracene Oils.

These results illustrated the very complex variety of actions on the skin by these acid mixtures, partly due to the large number of compounds still in the fractions, and partly due to the variability in response which can be expected from the action of only one such chemical in the skin.

This is evidenced in the variable structure seen within individual specimens and the different rates of response of individual scales. Some were found to have full granular layers and some 'normal' scales producing scale keratin. This was demonstrated especially by the fluorescence studies. It could be seen that there had been different types of keratinization, in many cases producing a "sandwich" effect, where the orthokeratin alternated with scale keratin. Spearman and Garretts (1966) described such an effect after saline injections into the mouse tail skin, which also caused granular layer induction and mitotic stimulation. They suggested that these effects could be explained by the daily periodicity of treatments, the parakeratin being partly the result of mitotic stimulation and orthokeratin being the product of a process which includes the formation of a granular layer. They suggested that the two types of keratin are the manifestations of the two effects: i.e. granular layer induction and mitotic stimulation. This also explains the similar effect found in the tar experiments. Lewin et al (1972) similarly related the areas of ortho- and para-keratin in psoriatic fingernails to the presence or absence of a granular layer.

Non-specific esterase activity was induced in the horny layer after treatments with the highest-boiling fraction of Anthracene Oil acids (330-340^{o}C). It was not known whether this activity was immediately induced in the horny layer or whether it was originally induced in the lower levels and moved upwards with the keratinocytes. This highlights the need for more detailed experiments to monitor the effects of the acids with time. However it was noted from experiments with the above fraction in which mice were treated, removed and killed daily,

that maximum granular layer induction did preceed maximum development of epidermal thickness. This contention was supported by the observation that in a rapidly sloughing epidermis (e.g. by 20% preparations of predominantly phenol and cresol mixtures), often a granular layer could be seen in the peeling tissue. Granular layer induction does seem to be one of the primary effects of tar acids and epidermal thickening is secondary to this and/or a result of the granular induction.

At the present stage it seems that the granular layer inducing character and the epidermal thickening effects cannot be separated. Keratinization can be altered by rubbing, which stimulates mitosis, (Spearman and Garretts, 1966). However experiments involving rubbing the skin with cotton wool showed that mere application of the preparations for about 30 seconds each day was not sufficient to cause the epidermal thickening and this was thus attributed to the effects of the tar. However epidermal thickening relative to granular layer inducing ability has been reduced in the higher-boiling acids to what it was in the whole acid fractions, and the same applies to skin staining propensities. Skin staining seems to be caused by tar and tar oil acid fractions, and although this has not been completely eliminated, it has been reduced relative to possible therapeutic effects. It is well-known that the phenolic anti -psoriatic drug, dithranol, stains the skin. Loss of the phenolic character inthe compound triacetoxyanthracene is reported to reduce staining, but there is a great loss in efficacy.

It is not known why both dithranol and 'Psorox' failed to induce granular layers in mouse tail scale regions - it could show a lack of

specificity on the part of the drugs as anti-psoriatic treatments and/or on the part of the mouse tail test as a model for demonstrating anti-psoriatic specificity. It is suggested that the former is the case as at the high concentration used, it could be that mitotic stimulatory actions of dithranol predominated over any other effects; or perhaps dithranol does not stimulate granular layer formation anyway. Its ability to restore a granular layer in psoriasis (Fry and McMinn 1968) may be a result of its property to also inhibit mitosis, (Swanbeck and Liden, 1966), giving more time for the granular layer to re-form.

A lack of specificity is also suggested in the case of 'Psorox', which contains the ingredients described by Kinmont (1957), (see p.31). In his clinical trial of this admixture of tar constituents, there was no histological evaluation and so a specific effect of inducing granular layers is still in question. However the preparation is certainly not known as a highly effective anti-psoriatic treatment. Young (1970) recently showed this synthetic tar to have no beneficial effect in a clinical trial.

During treatment with dithranol, it was noted that it caused staining, which at first was more marked in the Lassar's paste preparation; however the degree of staining in the two groups as a whole was more or less equal by the end of the trials. In all other cases of mouse tail treatment except the Dithranol/Lassar's group, all traces of the previous application had dissapeared in 24 hours. In this case, Lassar's paste could still be seen on the skin when the new treatment was due. Perhaps this persistent adherence on such scaley skin

illustrates how dithranol may have a more prolonged effect in psoriasis when in Lassar's paste, thereby being a more potent preparation. Maclennon and Hellier (1961) state that perhaps the most important feature of the 'Ingram regimen' is the formulation of dithranol into Lassar's paste.

Criticisms of Experiments

(a) THE EXPERIMENTAL MODEL

The use of an animal model to simulate a human pathological condition is of course fraught with disadvantages. These require that results obtained must be interpreted only as 'possible guidelines' as to the effects which may be observed from use of the drug in the disease. Criticisms that the mouse tail scale keratin is "normal" for the mouse and that psoriasis is an abnormal condition must be levelled with the question of, 'if the psoriatic is genetically predisposed to the disease, which is the "normal" state for the psoriatic patient?' - i.e. is parakeratin "more normal" than the flexible horny layer production? A point in the favour of the mouse tail as a psoriatic model is that if the orthokeratotic state of mouse tail skin is "further removed" from the scale state, than the psoriatic condition is from the orthokeratotic condition in humans, then maybe the mouse tail test is even more specific - i.e. it will take a more potent granular layer inducer to effect a change. Obviously the mouse tail test is not the ideal anti-psoriatic model. There are no dermal distrubances such as abnormally dilated and tortuous blood vessels, leucocyte invasion, abcess formation or oedema; i.e. there is no general inflammatory reaction in the mouse tail skin.

Jarrett and Spearman described the similarities of mouse tail skin and psoriatic keratinization processes. Some experiments demonstrating altered acid phosphatase distributions after tar treatments follow predictions from their work. In terms of the pathological keratinization process in psoriasis, the events are definitely closely

paralleled in mouse tail skin.

Up to date, this is probably the most realistic model for psoriasis and so was used for the tar screening.

(b) VEHICLE EFFECTS

Only 3 vehicles were used for the screening of which one was soon eliminated because of its unwanted thickening effects. Hence the experiments described only evaluated tar constituents which were well dispersed in and released from these two vehicles. From these experiments, high-boiling tar acids were shown to be superior to other consitituents tested, and it is these other compounds that may have had their effects masked due to vehicle incompatibilities. Effects could be due to the following:

(a) Strong interactions between the drug and the vehicle such that the vehicle/drug complex does not release the drug into the skin

(b) Other possibly active compounds may have been screened out due to their inability to dissolve in the vehicle.

(c) The vehicle may not release the drug at the optimum time or place.

(d) The conformation of the drug in the vehicle may result in the blocking of active groups on the drug molecule.

Drug/vehicle interactions are very intricate as they also are between the complex and the skin. These interactions have not been studied. Ideally all the constituents of coal tar should have been

screened in a large number of vehicles in order to determine the individual properties of the compounds.

The conclusion then is that in using two greasy and occlusive ointments, high-boiling tar acids have emerged as possible valuable anti-psoriatic agents. However it is unlikely that a large number of compounds have been "missed" due to the use of poorly-penetrating vehicles. This view is prompted by observations that the acids seem to induce a granular layer first, which suggests that the acids do not have far to travel in order to exert their primary effects - i.e. deep penetration is not required.

(c) DURATION OF TREATMENTS

It became apparent that tars and fractions, which did not induce granular layers in the scales but only seemed to cause epidermal thickening, did not cause variable effects over the treatment times. Avenue and Coalite Tars and Oils were compared over 14 and 21 days, and in all cases, responses were similar. There was no difference in epidermal thickening caused by the same preparation run for different times.

However the variance of effect of especially the highest boiling tar acid fraction (330-340^{o}C) with time, shows that the duration of treatment is an important parameter when comparing very active fractions. For example, some animals treated with the afore-mentioned acids had granular layers induced prior to the preparations causing peeling. Over long treatment times this peeling effect may have appeared to be the major effect of the tar. Peeling often results in parakeratosis, which effect would normally screen out a fraction -

however the sequence of events leading to these effects must be appreciated and in comparing such preparations, it is obviously necessary to investigate all effects with respect to time. This has not been done in these experiments, except in a preliminary manner for the highest boiling Anthracene Oil acid fraction.

2. HIGH-BOILING TAR ACIDS

(i) Previous Reports of Therapeutic Effects

Phenol itself has long been a constituent of many commercial "medicated" shampoos and scalp lotions. Sulzberger and Obadia (1956) reported the use of a solution containing under 1%, phenol, NaCl and liquid petroleum for the treatment of scalp dermatoses, especially psoriasis and seborrheic dermatitis. This preparation was also highly recommended by Wechsler in 1962. However the above experiments do not support the contention that low - boiling phenols are specific for treating psoriaform diseases. Thorne (1963) found that a preparation containing tar acids and bases in equivalent proportions produced greater improvements in patients with psoriasis than did crude coal tar.

Hellier and Whitefield(1967) suggested from their work with dithranol that polyhydric phenols in tar may constitute the therapeutic fraction. They suggested that these may be removed in "purification" to obtain more cosmetically acceptable tar preparations and their loss could explain the decrease in efficicacy so often seen with these preparations.

As early as 1928, Jaffrey found that the most useful coal tar was that containing the greatest amount of acids and that the most important boiling ranges were 170 - 300^{o}C, a range which includes low - and high - boiling tar acids. Rothman and Shapiro (1949) stated that the therapeutic fraction of coal tar was the anthracene oil fraction, but they made no special mention of the acid fraction. Specific mention of high-boiling acids was made in 1935 by Obermayer and Becker who suggested that substances such as 8-hydroxyquinoline and catechol should be further

investigated.

(ii) Previous Reports of Toxic Effects

The irritant actions of phenols are well-known. Martindale (1972) states that the toxicity of higher - boiling tar acids is similar to that of phenol, but occurs to a far lesser extent. Systemic effects on ingestion of phenol itself are:

a) extensive local corrosion with pain, nausea and vomiting.

b) depression of the central nervous system with respiratory failure (often the cause of death).

c) pulmonary oedema.

d) liver and kidney damage and possible failure

Ingestion of 1 - 15 g. has caused fatalities.

Severe and fatal poisoning can occur from percuataneous absorption especially where the skin is damaged. In general, tar acids, are very irritant and corrosive to the skin, even in dilutions used for disinfectants, (Finch, 1953). In fact phenol has been used in chemosurgical techniques to induce peeling of the skin, (Mohs, 1956; Epstein, 1962). However, the higher boiling acids are less corrosive to the skin, (Martindale, 1972).

As regards the Anthracene Oils as a whole, many workers have attributed carcinogenic properties to these fractions, (Kennaway, 1924; Bloch and Widner, 1926 and Combes, 1954a), but again the acid fractions have not been specifically implicated.

However subjection of rabbits to an aerosol fog of 'STERICOL' disinfectant (Izal Ltd.), which contains mostly dimethyl phenols, did not cause any injury to the eyes and lungs of the animals, (Scott-Wilson, 1968).

3. SUGGESTED MODES OF ACTION

(i) General

Any proposed mode of action must be superficial, taking into account the complex nature of the mixture of phenols contained in boiling range 280-340°C. and also because no sufficiently detailed experiments have been performed to ascribe specific actions. It could be postulated that the induction of a granular layer in mouse tail is an abnormal effect and that the skin reacts with a general inflammatory reaction, thereby causing mitotic stimulation. However this is too non-specific and the following specific actions are proposed.

DIRECT EPIDERMAL THICKENING

It is not known whether the epidermal thickening observed was caused by an increase in cell size (hypertrophy) and/or an increase in the cell number (hyperplasia). Cell hypertrophy was observed in most tar-treated animals, and obviously contributed to the increased epidermal thickness. Mitotic counts need to be carried out to determine just how much mitotic stimulation did occur.

If there had been direct mitotic stimulation, parakeratosis would have been expected, without the formation of a granular layer. It is also possible that peeling caused mitotic stimulation. The suggestion that peeling stimulates mitosis is well-known from observations in skin-stripping experiments, in which the loss of keratinized cells is thought to be the primary stimulus of epidermal cell proliferation, (Pinkus, 1952; Allenby et al, 1966). Pinkus (1970) also stated that the epidermal thickening found after skin stripping experiments was due to

oedema and hypertrophy of the living cells.

DIRECT GRANULAR LAYER INDUCTION

(a) Past work involving granular layer induction in both animals (Bern et al, 1955) and people (Fry and McMinn, 1968), suggests from the speed with which a granular layer forms (or re-forms) that there must be a direct and primary action of granular layer induction. Bern et al did not obtain granular layers and epidermal thickness increases with subcutaneous injections of Vitamin A; all granular layer inductions occurred with topical application only. Preliminary experiments with active tar acids suggest that one of their first actions is to induce granular layers.

It is possible that in the region of the granular layer the acids cause induction, but when they (or their metabolites) penetrate deeper into the epidermis they are potent mitotic stimulators and/or mediators of cell hypertrophy.

In the formation of a more flexible horny layer, the transport of high-boiling tar acids across this horny layer may be increased, with day by day increasing amounts of the acids reaching the basal layer, and the mitotic stimulatory effects may predominate, as was suggested for dithranol at high concentrations.

The following mechanism encompasses both mitotic stimulatory and granular layer-inducing characteristics.

(b) It has been pointed out that there is not a simple relationship between mitotic rate and the presence or extent of a granular layer, (Jarrett and Spearman, 1970). However these workers offer an

explanation for the seemingly contradictory effects of Vitamin A to induce a granular layer but to also stimulate mitosis. It would have been thought that mitotic stimulation would result in parakeratosis. However granular layer induction could have been a result of lysosome rupture in this region, liberating hydrolytic enzymes and thereby initiating orthokeratinization. Such large enzyme release in the basal layer, i.e. amongst very active and therefore highly sensitive cells, would consititute an inflammatory stimulus, one of the many inflammatory reactions being mitotic stimulation.

Such a mechanism is proposed for the high-boiling tar acids. This is the most attractive idea, since a common action, i.e. rupturing of lysosomes, is the basis of both granular layer induction and mitotic stimulation. This does not infer any intrinsic mitotic stimulatory properties of the phenols themselves, which would be a most undersirable side-effect in proposing the use of high-boiling phenols in anti-psoriatic therapy. Similarly skin peeling could be explained by lysosome rupture. Too extensive hydrolysis by the released enzymes could lead to rapid and extensive cell death. The dead cells would rapidly be sloughed, and the conservation of several layers of peeling epidermal sheets illustrates this possibility.

If the above events do take place, it would perhaps be theoretically possible to control mitotic stimulation by employing an inefficient vehicle which only released the acids into the horny layer, (where lysosome rupture could be initiated), but which did not aid further percutaneous absorption to the basal layer, where such effects would be injurous.

Although control of absorption could possibly be effected by restricting entry via the horny layer, the deep penetration is readily available via the pilo-sebaceous route. Higuchi (1960) stated that it is now thought that percutaneous absorption occurs via the horny layer and the pilosebaceous glands, the majority of the routes taken depending on the nature of the drug.

Suntzeff et al (1955) found that tars caused degeneration of sub-aceous glands, which was also caused by high concentrations of tar and oil acids in these experiments. This damage could have been part of the general extensive epidermal damage, but could possibly illustrate a preferential concentration of acids in the sebaceous glands. This is also supported by the fact that they were readily taken up and released by the greasy base, Plastibase. However, a vehicle could be designed such that partition between vehicle-drug and drug-sebum was preferentially in the former system.

(ii) A Special Action of High-Boiling Tar Acids

(a) The Dendritic Cell Theory of Keratinization

The following ideas from other workers are presented as the necessary background in which a role of the high-boiling tar acids is then proposed.

About one cell in ten in the basal layer of the human epidermis is a melanocyte, (Cochran, 1970). These cells synthesise melanin and pass it in granules to keratinocytes, where it protects cells from the harmful effects of ultra-violet light (Jarrett, 1967). The enzyme tyrosinase catalyses the oxidation of tyrosine to dihydroxy-phenylalanine

(DOPA) which is gradually oxidised to the final product, the pigment melanin. These cells can be demonstrated histochemically from their ability to oxidise DOPA, and hence are known as "DOPA-+ve" cells, although the enyme which is demonstrated has been shown to be tyrosinase, (Fitzpatrick et al, 1950). The shape of the cells is also characteristic in that they possess processes known as "dendrites", by which it is thought that the cytocrine transfer of melanin granules is effected.

There is another group of dendritic cells in the higher levels of the epidermis, called high-level dendritic cells or Langerhans cells. At one time they were thought to be "effete" melanocytes, which could no longer oxidise DOPA and so were being shed along with other high-level keratinocytes. However these high-level cells have been shown to possess substantial enzyme activity (Jarrett and Spearman, 1964; Riley, 1966, 1966b), and are believed to be involved in the organisation of the different keratinization processes.

Some workers believe that melanocytes and high-level dendritic cells are derived from two distinct cell populations, (Prunieras, 1969). Some think that they come from a common stem cell, (Quevedo and Montagna, 1962; Breathnach, 1963 and Chase and Lyne, 1966). Jarrett and Spearman (1964) and Riley (1966b) suggest that after melanin synthesis, these cells move up through the epidermis and somehow influence the type of keratin produced by influencing the high energy reactions taking place in the region of the granular layer. That is the high-level dendritic cells have a definite function in the keratization process, and the suggestion that they move towards this level was prompted by the

finding of ATPase in these cells which is known to be involved in cell motility, (Jarrett and Spearman, 1964). Chase and Lyne (1966) found that in the merino sheep epidermis, the regular pattern of the distribution of these cells and their close proximity to all epidermal cells suggested that they may have a trophic function.

Supporting evidence that melanocytes move up to become Langerhans cells came from the observation by Mishima (1966) in tape-stripping experiments. It was shown that melanocytes after discharging their melanosomes to keratinocytes, moved upwards and aquired the ability to synthesise Langerhans granules, the bodies shown by electron microscopy to be present in Langerhans cells, (Zelickson, 1966). The nature of these granules is as yet unknown.

Jarrett and Spearman (1964) suggest that these cells may augment cytolytic activity in the granular layer by providing lysosomes, perhaps by cytocrine transfer. Indeed in psoriatic skin they are only weakly ATPase + ve and are abnormal in structure. In plantar and palmar skin where there is relatively less cytolysis of the keratinocytes these cells are very difficult to detect, (Jarrett and Spearman, 1964). The suggestion that a number of sufficiently active Langerhans cells is a necessary part of orthokeratotic processes was also supported by a study of these cells in seborrheic warts, (Molokhia and Portnoy, 1971).

HIGH-LEVEL DENDRITIC CELLS IN THE MOUSE

In the mouse these cells do not possess ATPase activity, but are demonstrable by the technique for high-level dendritic cells, (Jarrett and Riley, 1963). Riley (1966a) showed that they were present

around the region of the hair follicle, and were absent in scale epidermis and Riley correlated the occurence of orthokeratin with the presence of underlying high-level dendritic cells and their absence with scale keratin. It does seem that the presence of these cells is an important pre-requisite for orthokeratinization.

(b) Suggested Roles of High-Boiling Tar Acids in the Dendritic Cell Theory

1. It is thought that initiators of orthokeratotic processes, such as the high-boiling tar acids, could exert their effects at least in part by making available either an increased number of and possibly an increased activity of dendritic cells. In this way the acids may initiate orthokeratotic processes in the scale region of the mouse tail. However preliminary studies failed to show invasion of the scale region by these cells after treatment with high-boiling phenols, as shown by the technique for N.S.E. N.S.E. was shown to be present in the horny layer after application of the acids, but from this work it could not be stated whether this activity was due to release of pre-existing enzyme, stimulated synthesis of N.S.E. or whether the activity was in any way connected with the dendritic cell population.

2. A highly-speculative mechanism is put forward for a role of high-boiling phenols in skin known to possess substantial DOPA-oxidase activity (i.e. as opposed to albino mouse tail skin).

Riley (1966b) cites evidence for the suggestion that the Melanocyte/Langerhans cell transformation takes place by inhibition of tyrosinase, thus terminating, or attenuating, the melanocyte phase of

the cell. This means that there would be relatively more ATPase activity in the dendritic cells. Reid and Jarrett (1967) suggested the concept of dendritic cells having greater or lesser DOPA +ve and ATPase activity depending on conditions.

McGuire and Hendee (1971) found that certain germicides containing high-boiling tar acids caused de-pigmentation in human skin. From biochemical tests, they found that the alkyl phenols, e.g. p-tertiary butyl phenol, inhibited tyrosinase activity, and suggested this as a reason for the de-pigmentation seen. They compared the phenolic structures to the natural substrates for tyrosinase, i.e. tyrosine and DOPA, and in finding similarities suggested there had been competitive inhibition of tyrosinase.

It is possible that the Anthracene Oil Acids could inhibit tyrosinase in the dendritic cells leading to a relative increase in the activity which results in a greater degree of ortho-keratinization. Such a proposition requires detailed investigations into the activity, numbers and locations of high-level dendritic cells (such as those described above) as a possible result of treatment by high-boiling phenols.

4. FUTURE WORK REQUIRED

(a) Further screening of smaller fractions of Avenue Oil acids boiling in the ranges 280-340°C is suggested. Whilst the observations for development of granular layers in the mouse scale regions should continue to be the main parameter for comparison, it is felt that quantisation of histochemical testing would be of great value when comparing similar fractions. Jarrett and Please (1970) outlined the many problems involved in such a venture; however a very useful diagnostic tool would be the quantisation of the changes in horny layer fluorescence. This has been described for foetal keratins by Jarrett and Van Wyk (1972).

(b) An important point to clarify is whether epidermal thickening is due in any way to direct mitotic stimulation by the phenols. Mitotic counts are necessary for any such determination. It is known that part of the thickening was due to increases in cell volume. The increased basophilia may have been due to increased levels of RNA (Montagna, 1962), which would cause hypertrophy. This can be determined by removal of the RNA in normal and treated cells by RNase (Jarrett and Hardy, 1957).

If there proves to be mitotic stimulation by the acids, this may not be so great in human skin, since smaller doses are more likely to be used, and Twort and Twort (1935) pointed out that in many cases the mouse skin is more susceptible to carcinogens than is human skin, due to the closer proximity of the applied substances to the basal layer cells.

(c) Preliminary experiments have demonstrated the wide variation in effects caused by different formulations of tar constituents. Further experimentation with a much wider range of vehicles is required to get a good idea of the total range of dermatological actions of coal tar and its fractions.

PART 3

I
INTRODUCTION

I. INTRODUCTION

1. The Work So Far

The experimental work has suggested that the anthracene oil acids of the N. C. B. 's Avenue Tar may provide a valuable treatment for psoriasis. These acids are predominantly contained in the anthracene oil acid fraction, but it is thought that the boiling range, 280-340°C should be further investigated, and this then covers the upper boiling ranges of creosote oil acids, (see p. 86). These conclusions are based on the ability of the acids to induce granular layers in the scale region of the mouse tail, (p. 127).

At similar concentrations these fractions show a great deal more specificity as possible anti-psoriatic drugs than does crude coal tar. However, these acids are still causing epidermal thickening and skin peeling, but it has been pointed out (p. 196) that these effects may be a manifestation of a specific activity, i. e. to induce granular layers. It is suggested that these problems are not insurmountable, and that they could in part be controlled by special formulation.

Although they still have an unpleasant odour, this will be considerably reduced, in that it will be required in far smaller concentrations than those used for conventional tar preparations, because of its far greater specificity. At present, the fraction is still coloured (dark brown), but this causes only minimal staining in mouse tail skin.

Anthracene oil acids are readily incorporated into "PLASTIBASE", a pleasant and innocuous paraffin/polyethylene vehicle. For these H. B. T. A. 's, this vehicle was found to be more effective than the conventional yellow soft paraffin and wool fat base and also F. A. P. G., (a Fatty Acid/

Propylene Glycol base).

2. Further Work Required

It is suggested that the whole anthracene oil acid fraction is used for further testing for therapeutic acceptibility, (i. e. effectiveness, safety, etc.), and also that further fractionation is carried out to identify and test individual constituents of this fraction. This is suggested for two reasons:

i) From previous work, it is likely that anti-psoriatic specificity would be increased and that less effective compounds and possibly compounds with adverse side-effects could be eliminated.

ii) Identification of an individual compound with highly specific anti-psoriatic activity, and subsequent study of its structure and properties would be a major advance in discovering the etiology of this distressing disease.

Adoption or rejection of such general proposals requires evaluation of the markets for anti-psoriatic drugs in conjunction with estimates of the cost of the necessary research and development. Salient aspects of these considerations are discussed in the following sections.

II

MARKET AND DEVELOPMENTAL CONSIDERATIONS

II

MARKET AND DEVELOPMENTAL CONSIDERATIONS

1. The Incidence of Psoriaform Diseases

The incidence of psoriasis in Great Britain is frequently quoted as 2%; however this figure has no substantive basis, (O.H.E., 1973). There has never been a comprehensive nationwide survey of the incidence of psoriaform diseases and the incidence survey below illustrates the wide range of values produced by individual surveys.

A major difficulty arising when undertaking any such study is the almost impossible task of classifying different degrees of the disease; for example, below a certain level the lesions would not be included in the survey. Many types of psoriasis have been described and dermatologists differ amongst themselves over the question of classification, (Grant, 1958; Ratzer, 1969). Perhaps more confusing than this is the fact that psoriatic plaques look very different at different stages in the disease. Also, although in its more extensive and severe forms psoriasis is very debilitating, some milder forms are often treated by the patient himself and therefore are never reported; indeed skin disease treatment has a long tradition of self-medication, (O.H.E., 1973). Peterkin (1959) points out that many psoriatics do not present for treatment as they have heard there is little that can be done for this incurable disease.

A review of the recorded occurrences of psoriasis follows, showing the variation between individual estimates. The incidence actually measured in these reports is the percentage of people suffering from psoriasis at that time, and since psoriatic exacerbations are sporadic,

the reports do not give the total number of people who could be suffering. Even fairly regularly attending psoriatic patients do not usually have lesions requiring treatment continually throughout their lives - they have periods of remission, and also times when the lesions are not sufficiently widespread to merit treatment, and hence would be omitted in such surveys, particularly those covering only a short time period.

Psoriasis is a disease which, once the skin has reacted in this way, is likely to recur throughout life, (if there is an inherited tendency). Although agreement on one figure of incidence is impossible, the incidence of psoriasis has remained fairly constant over the years for which figures exist.

The figures are classified under (a) Population Incidence and (b) Relative Incidence; the latter is the percentage of psoriasis amongst other skin diseases, e.g. the percentage of all new patients presenting with psoriasis during a given time interval.

PSORIASIS

E U R O P E

1. GREAT BRITAIN

(a) Population Incidence

REFERENCE		DETAILS OF SAMPLE	COMMENTS	%
Logan & Cushion	1958	General practices		0.33
Kidd and Meenan	1961	1,700 long-stay mentally ill patients	At least one year since last admission	2.0
Ingram	1964	'Estimated'		2

(b) Relative Incidence

Crocker	1903		Cited by Goodman (1929)	7
Bettley	1952	St. John's Hospital for Diseases of the Skin, LONDON, 1951	6th most prevalent skin disease	4.5
Ingram	1954	Private patients		6
Calnan and Meara	1957	St. John's Hospital for Diseases of the Skin		6
Ingram and Brain	1957			5-6
Bettley	1963	Middlesex Hospital, LONDON, 1961		6
Warin	1965	BRISTOL, 1963		7
Wells and Barr	1965	READING, 1962	Reading is considered a "most average" town as regards social class, birth rate, etc. and is often used for market and social research	4.3
Neves	1966	St. John's Hospital for Diseases of the Skin, 1952-1965	5th most prevalent skin disease	6
Shuster and Comaish	1966	NEWCASTLE		8-9

SCOTLAND

Ratzer	1969	Outpatients from two hospitals, 1955-1964		4.8

NORTHERN IRELAND

Hall and Burrows	1968	157,381 patients, 1954-1966		4.23 (1954) to 6.59 (1964)

2. AUSTRIA

REFERENCE		DETAILS OF SAMPLE	COMMENTS	%
(a) Population Incidence: not available (n.a.)				
(b) Relative Incidence				
Nobl	1928			3-5

3. DENMARK

(a) Population Incidence				
Heinild	1942	270 patients with arthrosis		0.0
Wassmann	1949	10,000 patients with different other diseases		0.43
Schwartz	1952	3,815 relatives of asthmatics, COPENHAGEN		1.65
(b) Relative Incidence				
Frandson and Hamptoft	1961	Psoriatics In-patients as % all In-patients	Cited by Lomholt (1963)	3.55 (1950) 2.56 (1959)
Lomholt	1963	Dermatology Clinic, Finsen Institute and Dermatology Centre, University Hospital, COPENHAGEN, 1959	Warts, hair loss and corns subtracted first, due to their large number	5.2 5.0

4. FAROE ISLANDS

(a) Population Incidence				
Lomholt	1963	10,984: whole population examined for a skin disease	More common in isolated villages of Northern Isles. Maybe a slight male predominance, but $\gtrless$ 10%	2.84
(b) Relative Incidence: n.a.				

5. GERMANY

(a) Population Incidence				
Holst	1944	Estimated in 5 rural districts in Unterfränken, served by clinic in WÜRZBURG	Frequency nearest hospital Frequency in remoter districts	0.62 0.26
(b) Relative Incidence				
Hirsh	1905	BERLIN		6.6
Hoede	1927	1,515 in WÜRZBURG		7.4
Hellier	1940	KIEL,	Cited by Hellgren (1967)	3.37
		BRESLAU		4.96
Tiedmann	1950			6.68

6. GREENLAND

REFERENCE		DETAILS OF SAMPLE	COMMENTS	%
(a) Population Incidence				
Bertilsen	1940	GREENLAND: 1 case of psoriasis		
Lomholt	1958	346 Greenlanders	Cited by Hellgren (1967)	1.4
(b) Relative Incidence: n.a.				

7. HUNGARY

(a) Population Incidence				
Szanto	1935	'Estimated' for Budapest		0.26
(b) Relative Incidence: n.a.				

8. ICELAND

(a) Population Incidence: n.a.				
(b) Relative Incidence				
Finsen	1874			8.0 8.2
Hellier	1940		Cited by Hellgren (1967)	8.1

9. NETHERLANDS

(a) Population Incidence: n.a.				
(b) Relative Incidence				
Simons	1949			4.7

10. NORWAY

(a) Population Incidence: n.a.				
(b) Relative Incidence				
Hiort	1887	1,594 patients	Cited by Hellgren (1967)	4.5
Schwartz	1952			1.65
Lomholt	1958	3,002 patients	Cited by Hellgren (1967)	5.0

11. PORTUGAL

(a) Population Incidence: n.a.				
(b) Relative Incidence				
Esteves	1960		Cited by Marshall (1964)	7.3

12. SWEDEN

(a) Population Incidence				
Romanus	1945	132,672 mentally ill patients	Cited by Hellgren (1967)	0.1
		1,987 conscripts	Cited by Lomholt (1963)	0.1

SWEDEN

REFERENCE		DETAILS OF SAMPLE	COMMENTS	%
Forssman	1947	693 mentally ill patients		1.44
Nilsen	1950	633 recruits		0.63
Forssman	1967	13,000 mentally ill patients	Communication to Hellgren (1967)	

(b) Relative Incidence

Bafverstedt	1958			5.5
Hellerstrom	1962			5.5
Hellgren	1964	GOTHENBURG		6.0

13. U.S.S.R.

(a) Population Incidence: n.a.

(b) Relative Incidence

Jordan	1922	11,127, MOSCOW		2.4
EUROPEANS				
Clarke	1962	LAGOS		2.5

A M E R I C A

1. NORTH AMERICA

(a) Population Incidence

Lane and Crawford	1937	No. of psoriatic Out-patients admitted to all departments		0.5
Dawson and Tyson	1938	1000 patients with osteo-arthritis		0.3
Bauer et al	1941	300; BOSTON		0.7
Bereston and Ceccolini	1943	20,000 soldiers, aged 18 - 45 years	Second only to acne	
Gahan	1943	'Estimation' for New York		1.0
Forssmann	1947			1.4
Sutton	1948	4,732 soldiers		0.2
Bereston	1950	20,000 army inductees		0.27
Beerman and Bastras	1961			
Farber and Peterson	1961		Maybe 3-4% if include all mild cases	1-2
Cullen	1963	a "good cross-section" of 26,655 men of military age; SOUTH TEXAS		0.02
Voorhees	1971	Quote 5 x 10^6 sufferers in U.S.A.		3
Lynfield et al	1972	A minimal estimation		1

NORTH AMERICA

REFERENCE		DETAILS OF SAMPLE	COMMENTS	%
(b) Relative Incidence				
Fox	1908			4.2
Bulkley	1898	10,000 patients	Cited by Goodman (1929)	3
Pollitzer	1914	1898-1911		2.8
Lane and Crawford	1937			6
Sutton and Sutton	1939			2-3
Freeman	1954	Private dermato-syphillogic patients		4
Ormsby and Montgomery	1954			4
Corson et al	1959	1930	7th most prevalent skin disease	3.39
Curtis	1959	4,833 patients	9th most prevalent skin disease	4.4

2. CENTRAL AMERICA

(a) Population Incidence: n.a.

(b) Relative Incidence

Pardo-Costello	1956	CUBA; Whit and coloured	5th most prevalent skin disease	6.33
Canizares	1960	Other data from colleagues		0.0
		MEXICO CITY	8th most prevalent skin disease	3.0
		GUATEMALA CITY	10th most prevalent skin disease	2.0
		TEGUCIGALPA, HONDURAS	One of 7 most prevalent skin disease	
		SAN JOSE, COSTA RICA		

3. SOUTH AMERICA

(a) Population Incidence: n.a.

(b) Relative Incidence

Ramose e Silva	1964	BRAZIL	Cited by Marshall (1964) 13th most prevalent skin disease	1.65

A U S T R A L I S I A

AUSTRALIA

(a) Population Incidence: n.a.

AUSTRALIA

REFERENCE		DETAILS OF SAMPLE	COMMENTS	%
(b) Relative Incidence				
Summons	1955	Private patients		2.57

A S I A

(a) Population Incidence: n.a.

(b) Relative Incidence

INDIA				
Thambiah	1938	An annual range, MADRAS		5.55 to 7
Ghosh	1948			1.192
Desai	1960	Hospital patients	9th most prevalent skin disease	0.9
		Private patients	6th most prevalent skin disease	3.3
Okhandiar and	1963		Accord. to location	0.44 to 2.2
			Overall incidence	1.02
IRAN				
Pettit	1962		8th most prevalent skin disease	2.3
Mehregan	1964			2
JAVA				
Simons	1940	Whites		3.7
		Indo-Europeans and Chinese		1.1
		Indian born natives		0.015
		Mariana Islanders		0.05
KOREA				
Arimoto	1941	Koreans only		0.68
PALESTINE				
Tas	1947	Jews, 1920-1946	7x more prevalent in Ashkenazin (European) than in Sephardic (Oriental) Jews	0.56
SUMMATRA				
Genner	1924			0.0
SYRIA				
Hellier	1940		Cited by Hellgren (1967)	0.52

A F R I C A

REFERENCE		DETAILS OF SAMPLE	COMMENTS	%
(a) Population Incidence: n.a.				
(b) Relative Incidence				
Rufz	1859	Negroes; MARTINIQUE	Cited by Lewis(1943)	0.0
Morison	1888	Negro		1
		White		5
Corlett	1906	Negro	Cited by Lewis(1943)	0.0
Dade	1909	Negro: 3 cases	Cited by Lewis(1943)	
Wright	1924	Negro: 1 case	Cited by Lewis(1943)	
Parounagian	1926	Negro: 1 case	Cited by Lewis(1943)	
Simons	1949	556 Negroes hospitalised for skin diseases		0.4
Findlay	1960	White South Africans:		
		i) hospital		3.2
		ii) private		4.6
Schaller	1962	ETHOPIA	15th most prevalent skin disease	1.25
Schulz et al	1962	2000 patients Pretoria General Hospital, 1959-1961		
		Bantu	18th most prevalent skin disease	1.45
		White		3.2
Clarke	1962	LAGOS: Negro		0.0
		White		2.5
Marshall		1962-1963		
		1000 White private patients	5th most prevalent skin disease	4.9
		1500 White hospital	5th most prevalent skin disease	5.0
		1500 Coloured hospital patients	7th most prevalent skin disease	3.3

SEBORRHEIC DERMATITIS

E U R O P E

GREAT BRITAIN

REFERENCE		DETAILS OF SAMPLE	COMMENTS	%
(a) Relative Incidence				
Crocker	1905	LONDON	Cited by Marshall (1964)	1
Bettley	1952	St. John's Hospital for Diseases of the Skin, (1951)	2nd most prevalent skin disease	7.1
Wells and Barr	1965	READING		2
EUROPEANS				
Clarke	1962	LAGOS	5th most prevalent skin disease	5.1

A M E R I C A

1. NORTH AMERICA

(a) Population Incidence				
Greenberg	1961			70
(b) Relative Incidence				
Freeman	1954	Dermato-syphillogic patients		9
Corson et al	1959	1955	4th most prevalent skin disease	6.4
Curtis	1959	Cleveland Clinic, 1955	4th most prevalent skin disease	6.5

2. CENTRAL AMERICA

(b) Relative Incidence				
Pardo-Costello	1956		13th most prevalent skin disease	2.14

A S I A

INDIA

(b) Relative Incidence				
Desai	1960	Private patients	10th most prevalent skin disease	0.5

A F R I C A

EGYPT

(b) Relative Incidence				
El-Zawahry	1963	2,000 patients		3

Discussion of Survey

The first point to note from this survey is the extremely wide variation in figures quoted for incidence. Rook and Wilkinson (1968) pointed out that it is almost impossible to determine accurately the incidence of diseases which are not invariably fatal.

Two surveys which are not quoted above involve questioning the potential patients themselves over whether they have suffered any of a list of symptoms over the last fortnight, (Dunnell and Cartwright, 1972; Wadsworth et al., 1971). However, in this type of survey and in the area of psoriasis, many cases are likely to be missed due to the sporadic nature of the disease, and also many lesions may not have been recognised by the patient himself as psoriatic lesions, (O.H.E., 1973).

Generally it can be seen that psoriasis is certainly more prevalent in the Northern hemsiphere amongst Caucasians. Occurrence is at its minimum in full-blooded Negroes. Farber et al (1965) reviewed the racial incidence and found psoriasis to be common in all Europeans, less frequent in Asiatics, rare in Arabs and Indonesians and very rare in the American and Latin Indians.

Relatively few figures have been recorded for seborrheic dermatitis; only one is shown here for population incidence, and this is 70% for the U.S.A., Greenberg (1961). Estimates for relative incidence show it to be a very common disease, most figures being greater and a few less than those quoted for psoriasis. The actual incidence is probably higher than the figures quoted as this too is a disease which often

goes unreported in its mild forms.

It is not possible to combine data from the many surveys above to obtain meaningful figures, since the surveys are nearly all based on different criteria. Firstly, reports differ in the number and type of patients studied, i.e. hospital practice or general practice. Taking a hospital study as the most precise source of information and which usually has a large sample for analysis, the following factors greatly influence the figures obtained:

i) Social customs - i.e. whether or not to report the disease at all.

ii) Regional referral rates and customs, including factors such as diagnostic acumen of the general practitioner, (Rook and Wilkinson, 1968).

iii) Time to reach the hospital, (Carmichael, et al., 1963). (Psoriasis may go through one of its spontaneous remissions).

iv) Location of the hospital as a deterrent to travelling for treatment.

v) Diagnosis by consultant, and diagnostic facilities available, (Rook and Wilkinson, 1968).

vi) If the survey is only over a period of one year, some psoriatics may be missed, since chronic psoriatics do not present every year.

vii) In determining relative incidence, i.e. the percentage of psoriasis amongst other skin diseases, psoriasis and some other diseases may appear to be more prevalent, due to a lower number of other skin disorders reaching the hospitals. For example, since the introduction of readily available steroids and antibiotics in the 1950's, fewer trivial inflammatory dermatoses and infections are being referred by the G.P., who can often successfully treat the diseases with these new drugs.

However, despite these many influencing factors, it seems that

the most significant results come from the studies of relative incidence, i.e. the percentage of all new Out and/or In* - patients presenting with psoriaform diseases. In Great Britain, this incidence has been around the 5% level for many years.

2. The Search for an Accurate Market Size

To obtain ideas on the size of a potential market, pharmaceutical companies usually directly approach the future distributors of their products, the doctors themselves. One method is to approach one or two of the large skin clinics to obtain up-to-date information on the occurrence of psoriaform diseases, (Mitchell, communication). Another approach is through prescription analyses. A representative sample of G.P.'s is asked to record diagnoses and prescribed drugs during a certain period of time. This gives a market size in terms of the number of prescriptions issued. From the cost of the items, an actual monetary market size can be calculated, (Williamson, communication). (The data on p. 34 was collected in this way by Intercontinental Medical Statistics Ltd.

In calculating the overseas market for any pharmaceutical product, a company first attempts to determine the incidence; factors are calculated to give the 'level of health care' for that country and the incidence is multiplied by these factors to give a market size. Take, for the sake of argument, the level of incidence in the U.K. to be 2%, giving a market size of 1.3 million. In another country of similar population size and of similar ethnic groups, if the level of health care is half that of the U.K., the market size would be only 0.5 x 1.3 million,

* In-patients obviously tend to have the more serious and rarer forms.

(Williamson, communication).

However, in the case of psoriasis, prescription analysis is not such a good method because it does not include hospital treatments, which form a large part of the patients treatment. Figures for hosptial useage are not readily available though, (Vestric Ltd., communication). Over-the-counter sales data is produced by private market researchers, but this sort of information is confidential for their clients, (Nielson, Ltd., communication). These latter figures would be very valuable, since self-medication seems very important in psoriasis. A survey of two London areas by Wadsworth et al. (1971) showed that self-medication far exceeded medical prescription for skin complaints (experience in the previous two weeks) although the one case of psoriasis in their study had been treated by a doctor. Dunnell and Cartwright (1972) found dermatological drugs hoarded in far greater proportions than any other type of medicine.

This lack of precise information makes it almost impossible to quote a market size for a drug specific for psoriasis. A figure of 1.3 million has been quoted *; it is most likely that this is based on that ubiquitous "incidence" of 2%.

In the present research, the experimental work so far suggests that the anthracene oil acid fraction would be a more specific anti-psoriatic treatment than present drugs on the market. However, the figures obtained by pharmaceutical companies by the above methods usually cover a large diagnostic group. Psoriasis is not usually considered alone, but also with 'eczema' and 'dermatitis', (Mitchell, comm-

*Source: market research department of a large pharmaceutical firm known to be interested in dermatologic therapy.

unication), although recent text-books consider psoriasis in a separate classification, (Lipman Cohen and Pegum 1970). (This approach by pharmaceutical firms in the field again highlights how non-specific many anti-psoriatic treatments are). So even if the above methods could give accurate figures, they would still be of no use for the purposes of this particular project.

This brings us back to examining the individual reports of prevalence, (pp. 209-216). To obtain an accurate figure of prevalence, a very detailed analysis of the criteria employed in each survey would have to be undertaken. However, any attempt to render comparable the many different surveys would be virtually impossible, since they differ in so many crucial variables such as the number and type of patients included, and the duration of the study. It is thought that the time required could be better spent in performing a very carefully designed nationwide survey of all sufferers, both actual and potential. Here again this would only be viable if the anthracene oil acids proved to be both highly active and specific; i.e. in this event, the total market size for psoriasis alone would be extremely useful for product development predictions. In the case of fairly non-specific anti-psoriatic drugs, pharmaceutical firms in this field obviously find the present state of market knowledge adequate for new product prediction purposes, (Mitchell, communication).

SUMMARY

It has been shown that accurate figures of market size for psoriasis are not readily calculable. However, pharmaceutical firms

already in this field consider the market size large enough to stand development costs, (Mitchell, communication).

3. Research and Development Requirements

(i) Activities

The suggestion that the anthracene oil acids of Avenue Tar would have a more specific anti-psoriatic effect than crude coal tar is based on the results from simple screening for granular layer induction.

These acids have not been screened for possible systemic toxicity through percutaneous absorption of the constituent acids, and must obviously be put through the toxicological tests, normally employed in dermatological product development.

The development of a chemical, or in this case, still a crude mixture of several compounds is a very costly and time-consuming project. In developing both the anthracene oil acid fraction and the constituents of the fraction, the activities necessary are outlined below.

A promising fraction or mixture is put through a general screening procedure for about 3 - 6 months, during which time effects of very high and very low doses, and doses expected to be used in people, are monitored for therapeutic and toxicological effects in rats and mice. The blood and urine are examined for metabolites and changes in their normal composition brought about by the drug. About 32 organs are removed from each animal at the end of a run, and are examined histologically for signs of altered morphology, (Mitchell, communication). It would probably be at this stage that many individual constituents would be discarded, (Mitchell, communication).

After reviewing the data from this initial screen, the fraction and/or compounds would undergo longer-term tests from which full pharmacological, toxicological and biochemical data would be collected. Subject to the approval of the Committee on Safety of Medicines, clinical trials would be arranged to determine dose schedules and also the side-effects of the drugs. At this stage, it would be possible to predict quantities of raw materials (tar fractions) required annually.

The longer-term animal tests would monitor pharmacological and biochemical effects. Pharmacological screening would involve, for example, alterations in muscle tone, gut motility cardio-vascular responses, etc. Biochemical tests would trace the metabolic path (s) of the drug through the body. Evaluation of the possible toxic effects of metabolites and ensurance of adequate clearance in excretion is necessary. For example, it is vital to determine whether there is a build-up of the drug and/or its metabolites in tissues. These experiments are performed in a large number of mammals, such as rabbits, guinea pigs and dogs. Pigs also are used, because of the many similarities to human skin, (Weinstein, 1965). In the case of tar compounds, there would have to be long-term studies to investigate possible carcinogenic effects, which in the case of anthracene oils have been previously reported, (see p. 194). However, the acids themselves have not previously been implicated in carcinogenesis.

Meanwhile critical work is taking place on the production side, notably in production engineering, in analytical specifications and in formulation. The product must be consistently produced at the specified level of purity and must be stable in the optimum vehicle, (Lees,

1972). It has yet to be decided whether the N. C. B. would be involved in the production of these H. B. T. A. 's, and at what level of purity they would be provided.

(ii) General Discussion

Development of the existing fraction and the isolation, production and development of individual constituents of Anthracene Oil Acids would involve a very large allocation of research resources for a possibly very active product, but also one which may prove after several years of further research to be unsuitable on therapeutic, toxicological or economic grounds. Lees (1972) showed that it takes about 3 years to complete toxicological tests and clinical trials followed by another year of product development including licensing, packing and marketing. A figure of about £200, 000 has been quoted as the sum required for the development of one product, (Mitchell, communication). Dixon, (1973) states that 7 years work and £2-3 million expenditure are now thought to be average estimates before a newly registered product reaches the market in Britain.

Getting a new product onto the market in any manufacturing industry involves very careful forecasting, the predictions of time and cost being the most important factors. The pharmaceutical industry has particular problems here. Drug testing takes a great deal of time and money, (Business Week, 1966), and risk and uncertainty characterise the development of a new drug, (Mund, 1969), even after launching it as a new product, (Bogue, 1965; Dunning, 1965; Clymer, 1969). Research expenditure can only be assessed by looking at the data on past products - it is impossible to project into the future, (Lee, 1965).

This is mainly because pharmaceutical research and development involves experimentation in biological systems, which are notoriously unpredictable.

Pharmaceutical developments present a special case in their high market and research costs. As in other chemical production industries, a successful research development must be accompanied by successful marketing or else the innovation is rendered useless, (Teeling-Smith, 1965). In Great Britain pharmaceutical advertising costs rival spending on research, (Dixon, 1973).

Research spending will in the long-term be linked to price, (Fryers and Lee, 1967), but it is not possible to predict a selling price for a new tar preparation at this stage. It is generally considered (NEDO, 1973) that a major breakthrough in therapy could be priced above previous inadequate treatments. A minor variation on existing products may have to be priced below to give it some promotional advantage, (NEDO, 1973). It is not known into which category the new tar product would fall. From this experimental study, it is thought that it would be a significant advance in treatment, and indeed is unlikely to be adopted for development unless it shows this kind of promise. However, this may illustrate a promotional problem; for instance, over the last 50 years references to many "promising tar fractions" have appeared only once in the literature, never to be recommended again. The anthracene acid preparation may be regarded as "yet another tar preparation."

Again it is impossible to state with any accuracy what proportion of the market the new product would occupy. It is not yet known which types and which stages of the disease will best respond to the preparation.

If the new preparation was shown to be as effective as say, steroids and dithranol, and was completely safe, in theory the new product could replace all existing anti-psoriatic drugs, but this is a rare occurrence in any field of therapy, (Williamson, personal communication). A new product does not usually reach its full potential as a market leader until at least 5 or 6 years after its launching, (NEDO, 1973), and it is unlikely that even a successful compound would draw a larger market size through previously untreated patients coming forward. Rook and Wilkinson (1968) state that advances in treatment only temporarily affect the figures.

A rather pessimistic picture has emerged, but even in a very costly project, work may still go ahead. This was the case in the development of the steroid, triamcinolone, (see p. 43), which had high production costs. Early clinical tests suggested that it constituted a real advance and the effectiveness could never have been assessed unless sufficient of the drug had been produced to enable full clinical trials to be carried out, (Parker, 1962).

Although very detailed data on this fraction and its predicted future would have to be furnished, decisions to go ahead with its development would involve a great deal of personal judgement based on acquired experience within the pharmaceutical firm, (Lees, communication).

SUMMARY

The further biological and pharmaceutical work required obviously needs the immense resources, both in terms of equipment and personnel, of a large pharmaceutical firm. It would obviously not be viable for the N.C.B. to enter into this type of work.

It has been pointed out that such development takes a long time and is very costly. It is not possible at this stage to predict a product selling price, and hence one for which the N. C. B. could sell the tar fraction. Predictions of the effectiveness and consequently of the market share of the new product are also not possible until at least preliminary clinical trials have been carried out.

III

SUGGESTED COURSES OF ACTION FOR THE N.C.B.

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1. Provision of Tar Fractions

The experimental work has shown that anthracene oil acids in the boiling ranges 280 - 340°C may provide valuable anti-psoriatic treatments. Further fractionation and development of these acid mixtures will require the provision of samples of anthracene oil acids, and if found efficacious at the end of toxicological and clinical trials, large scale production may be required. At present in the Avenue plant, extracted anthracene oils are blended back with a lighter oil, e.g. wash oil or drained naphthalene oil (i.e. which has had the naphthalene removed) and "whizzed" to obtain the anthracene - rich "anthracene oake". Anthracene oils are then available for sale for use in dyes and road tars in combination with a light oil after removal of the "cake". Facilities are available at the Avenue plant for batch-wise preparation of anthracene oil acids, (Fere, communication), although at present this is not done.

As described on p. 168, further fractionation requires sophisticated chromatographic methods of analysis and for preparation of individual acids. Experience of these techniques in conjunction with H.B.T.A. is probably most abundant in a body such as the Coal Tar Research Association or Coalite and Chemical Products Ltd., who have carried out similar work with H.B.T.A.'s from low temperature tars. The acids of anthracene oils are mostly phenolic type high-boiling tar acids (H.B.T.A.). These compounds have for many years been marketed as

industrial and domestic disinfectants especially by low temperature tar distillers, because of the far greater concentrations of H.B.T.A. in low temperature tars. Indeed considering this greater abundance in low-temperature tars, (Bristow, 1947) it would probably be more worthwhile to investigate these sources. If not, the problem of who would perform the acid extractions arises; the N.C.B. would obviously have more experience, but a well-equipped large pharmaceutical firm may have more of the equipment at their disposal.

(i) Quantities Required for Development

It is not possible to give a definite figure for amounts of acids required for product development, (Lees, communication), but Mitchell (communication) suggested that about 2 Kg. of a pure chemical would cover biological and pharmaceutical development. In this case, 200 Kg. of anthracene oils would be required to prepare the acids.

(ii) Quantities Required Annually for a New Product

Here again it is not possible to quote amounts of anti-psoriatic drugs prescribed annually, (Miller, communication). Anti-psoriatic drugs fall within the Department of Health and Social Security Therapeutic Group 42 - 'Vehicles, sedatives, antiseptics and other preparations acting on the skin and mucocutaneous junctions'. Figures are available for this very broad group of drugs, as they are for topical corticosteroids, but in the latter case they are used for such a wide range of skin diseases that these figures would alos be useless. As in attempting to determine market size, one needs to have inside information of a pharmaceutical firm in the field of dermatology. Pharma-

ceutical project managers rely on a feedback from representatives, sales figures of existing products and their market shares to give a good "guestimate" of the quantities of drug, and hence raw materials required annually both here and abroad, (Lees, communication). It can be appreciated that this data takes some time to compile.

When a clear idea of efficacy has been established by clinical trials, the advances in treatment must obviously be weighed against the costs of further development and particularly of quantities required and the cost of anthracene oil acids.

On the question of planning for such an outlet for coal tar, figures cannot be provided at this stage, but Bailey (communication) considered that from past experience, the pharmaceutical useage of coal tar was so low compared with total output that there was little need to budget for supplies.

2. Considerations for Action

General suggestions are outlined below:

(a) Firstly, it is thought on the basis of the experiments described that Anthracene Oil acids boiling in the range 280 - 340^{o}C are worthy of further development. This conclusion is based solely on the possible advance in treatment which may come about from its development, taking into account the lack of adequate and safe long-term treatment for psoriasis. There is also the possibility of medically less important, but probably economically viable "spin-offs" into dandruff treatments.

(b) It would certainly not be feasible for the N.C.B. to enter into the

pharmaceutical development of this fraction itself, such development requiring the massive resources of a pharmaceutical company, from the point of view of manpower and physical assets. For further development, the N.C.B. needs to 'hand over' the fraction to a pharmaceutical concern in the near future, or perhaps when further work has been carried out on individual constituents of anthracene oil acids.

(c) It is not yet possible to assess the economics of entering into this further development. Amounts of anthracene acids required are unknown until the safety and efficacy has been evaluated in animal tests and clinical trials. In the event of individual H.B.T.A.'s being shown to be superior to the fraction it is likely the pharmaceutical firm would very soon begin to investigate synthetic pathways to individual constituents, since this will probably be cheaper than 'large-scale' preparative methods to obtain the raw materials. Hence the role of the N.C.B. in supplying raw materials may be short-lived in the event of individual acids showing greater activity than the whole anthracene oil acid fraction. If economic gain was intended, the N.C.B. would have to patent the idea to use H.B.T.A.'s in the treatment of psoriasis on the basis of this work.

(d) As the fraction contains H.B.T.A.'s, the N.C.B. may feel that it is more worthwhile to pass this idea over to bodies actively concerned with their production, i.e. low-temperature tar distillers, since H.B.T.A. yields would be greater, and the firms concerned have a great deal of experience in their production, and would be well-

equipped to advise on their properties.

(e) In the case of these acids, further work which revealed toxic effects would not necessarily be of no use. Information on the hazardous effects of H.B.T.A. would be of great value to the manufacturers of disinfectants which contain these compounds.

CONCLUSION

The results from this investigation have revealed that there is a marked difference in the therapeutic efficacy of coal tar fractions. Creosote and Anthracene Oil acids boiling in the range 280-340^{o}C hold promise for specific use in psoriaform diseases. Other dermatological conditions have not been considered. These results are considered to be sufficient to warrant further investigation both in terms of secondary toxicological screening and also in a further characterisation of the chemical constituents of this boiling range.

Normally the N.C.B. is not directly concerned with the final uses of their tar fractions. However in this case, it is suggested that the N.C.B. approaches a large pharmaceutical firm, already in the field of dermatology, for assessment of the work so far carried out, with a view to further development.

It would be premature to state at this stage whether development and production of a preparation based on this fraction would be economically viable. Even if the whole product development proved to be costly, the N.C.B. would surely gain some considerable prestige from innovating a safe and effective treatment for a well-known and extremely distressing skin disease.

APPENDICES

APPENDIX I

INDIVIDUAL MOUSE TAIL SCALE EPIDERMAL THICKNESSES

FOR TABLES 2-15 and 18-20

E.T.(μ)

TABLE 2

CONTROL	5% COALITE TAR
31.595	44.392
37.561	48.078
41.296	47.507
36.783	52.439
33.670	46.936
34.137	46.416
30.402	44.703
37.146	32.294
35.745	40.394
32.321	46.313
34.241	
43.579	Mean
34.085	44.947
33.670	
37.509	
30.454	
30.713	
Mean	
34.994	

5% AVENUE TAR
45.361
42.766
36.641
47.665
40.539
35.655
37.576
41.104
47.333
Mean
41.626

TABLES 2 and 3

VEHICLE 1	10%COALITE OILS
38.502	46.972
40.523	48.167
44.824	56.801
44.565	41.152
37.932	49.414
44.565	45.205
41.560	48.063
44.410	
40.109	Mean
41.871	47.967
36.896	
40.627	
39.798	
38.658	VEHICLE 1
31.766	39.438
38.450	49.258
42.181	40.113
40.212	39.697
	40.841
Mean	43.802
40.413	48.790
	42.218
TABLE 2	
CONTROL	Mean
	43.019
38.191	
36.372	
38.710	
31.644	
29.773	
35.385	
Mean	
35.012	

10% AVENUE OILS
53.257
47.832
56.543
55.252
58.002
53.010
45.072
Mean
52.709

TABLE 4

INDIVIDUAL EPIDERMAL THICKNESSES and BODY WEIGHTS

After 14 days:-

CONTROL

Mouse	Weight(g.)	E.T.(μ)
1	23	17.815
2	23	19.261
3	25	19.617
4	24	18.486
5	19	17.609
6	28	22.308
7	30	24.373
8	25	25.528
		Mean 20.624

10% COALITE TAR

Mouse	Weight	E.T.(μ)
1	23	40.846
2	24	40.174
3	24	28.194
4	25	37.128
5	25	43.019
6	20	43.010
		Mean 38.728

10% AVENUE TAR

1	31	43.686
2	28	41.452
3	22	38.057
4	21	31.368
5	24	31.241
6	25	35.114
7	22	39.813
8	22	45.648
		Mean 38.297

10% COALITE OILS

1	20	44.667
2	20	33.720
3	25	26.129
4	20	25.148
5	25	30.466
6	28	27.317
		Mean 31.241

10% AVENUE OILS

1	22	28.556
2	21	33.152
3	25	30.446
4	21	32.067
5	27	30.983
6	22	30.570
7	20	29.537
		Mean 30.758

VEHICLE 2

1	27	24.735
2	28	20.862
3	23	19.622
4	29	24.063
5	30	21.127
6	28	21.172
7	25	21.843
8	27	22.669
		Mean 22.011

Table 4 (cont/d.)

After 21 days:-

CONTROL

Mouse	Weight	E.T.
1	26	16.627
2	22	18.848
3	27	18.470
4	24	23.547
5	25	28.504
6	25	20.063
7	15	19.468
8	22	19.313
9	28	17.970
10	25	22.775
		Mean 20.558

10% AVENUE TAR

1	27	35.527
2	25	33.410
3	19	33.565
4	22	35.114
5	22	37.501
6	24	32.842
7	28	32.945
8	22	34.236
9	23	37.747
		Mean 34.765

10% AVENUE OILS

1	20	26.129
2	17	22.514
3	24	44.099
4	23	30.983
5	21	29.485
6	21	31.706
7	22	30.002
8	25	37.541
9	24	33.875
		Mean 31.814

10% COALITE TAR

Mouse	Weight	E.T.
1	22	32.997
2	28	44.408
3	24	33.049
4	29	29.434
5	20	39.710
6	26	34.184
7	26	39.503
8	28	38.470
9	22	38.987
		Mean 36.749

10% COALITE OILS

1	27	24.115
2	21	25.871
3	26	30.466
4	19	29.330
5	21	27.265
6	22	31.809
7	27	24.425
8	24	23.134
9	18	36.870
10	20	32.738
		Mean 28.602

VEHICLE 1

1	27	16.937
2	30	17.815
3	24	18.331
4	25	17.454
5	27	17.660
6	25	21.998
7	29	28.917
8	26	21.899
9	26	21.326
10	25	20.036
		Mean 20.237

TABLE 5

INDIVIDUAL EPIDERMAL THICKNESSES, BODY WEIGHTS and TAIL STAINING

Mouse	Wt.	E.T.	Staining
CONTROL			
1	31	23.560	-
2	34	24.588	-
3	29	18.567	-
4	28	27.828	-
5	29	21.577	-
6	36	26.846	-
7	29	22.330	-
		Mean 23.613	
40% AVENUE TAR			
1	29	54.293	+
2	23	45.682	-
3	21	52.987	-
4	27	62.598	+
5	33	46.002	++
6	28	45.663	-
7	25	65.324	-
		Mean 53.221	
40% AVENUE OILS			
1	23	36.765	+
2	21	33.250	+
3	18	20.080	++
4	22	35.014	+
5	25	32.763	+
6	28	40.266	+
7	19	38.515	+
		Mean 33.807	
40% A.O. ACIDS + NEUTRALS			
1	32	34.127	+++
2	25	36.618	+++
3	28	38.860	+++
4	31	40.750	+++
5	30	44.589	+++
6	33	40.449	+++
7	25	45.199	+++
8	30	43.593	+++
9	31	38.659	+++
		Mean 40.282	

Mouse	Wt.	E.T.	Staining
40% A.O. BASES + NEUTRALS			
1	21	30.202	-
2	22	32.448	-
3	26	29.702	-
4	26	37.939	-
5	15	20.966	-
6	18	28.454	-
7	25	42.931	-
		Mean 31.806	
40% A.O. NEUTRALS			
1	26	37.644	-
2	27	31.412	-
3	25	38.392	-
4	22	35.151	-
5	22	47.367	-
6	22	34.403	-
7	25	38.642	-
8	22	37.440	-
		Mean 37.556	
40% COALITE TAR			
1	32	79.786	-
2	28	48.926	-
3	23	55.449	-
4	23	38.137	+
5	29	55.198	++
6	31	54.947	-
7	28	49.929	+
8	27	49.678	-
9	30	57.958	-
		Mean 54.445	
40% C.T. ACIDS			
1	38	71.507	++
2	30	62.976	++
3	23	74.768	+
4	30	67.241	+
5	28	59.714	+
6	27	47.922	+
		Mean 64.021	

KEY:

- : no staining of tail skin
+ : patchy medium-brown staining
++ : patchy dark-brown staining
+++ : dark brown staining all over the treated area.

TABLE 5 (cont/d.)

Mouse	Wt.	E.T.	Staining
40% C.T. BASES + NEUTRALS			
1	32	45.413	+
2	22	26.094	-
3	40	38.137	-
4	29	37.886	+
5	29	55.700	+
6	38	46.667	-
7	35	48.675	-
		Mean 42.653	
40% C.T. NEUTRALS			
1	37	34.122	-
2	35	40.646	-
3	35	41.649	-
4	38	46.667	-
5	25	38.137	-
6	32	46.918	-
7	34	41.399	-
8	35	54.194	-
9	24	45.413	-
10	24	26.846	-
		Mean 41.599	
40% COALITE OILS			
1	32	47.420	++
2	23	46.918	-
3	30	32.868	+
4	23	35.377	++
5	23	30.610	++
6	40	44.125	-
7	29	39.391	-
8	26	42.402	-
9	26	40.897	+
10	24	41.399	+
		Mean 40.140	

Mouse	Wt.	E.T.	Staining
40% C.O. ACIDS			
1	27		+++
2	21		+++
3	27		+++
4	30	Not	+++
5	26	Measur-	+++
6	17	able	+++
7	21		+++
8	26		+++
9	27		+++
10	26		+++
40% C.O. BASES + NEUTRALS			
1	35	40.395	-
2	30	37.384	-
3	35	34.624	-
4	27	40.395	-
5	35	41.649	-
6	34	35.126	-
7	25	37.384	-
8	27	40.897	-
9	33	41.399	-
10	31	43.908	-
		Mean 39.316	
40% C.O. NEUTRALS			
1	30	35.314	-
2	25	29.606	-
3	31	35.377	-
4	32	35.126	-
5	27	34.624	-
6	30	40.395	-
		Mean 35.073	

TABLE 6/7

(Epidermal Thicknesses)

CONTROL	10% A.O.A.'175'	5% A.O.A.'210+'	10% C.O. ACIDS
26.921	27.710	26.973	37.826
24.866	30.444	36.297	32.874
28.132	37.141	29.291	37.878
27.282	30.397	30.608	31.240
26.604	30.608	33.347	32.926
31.609	28.185	32.505	38.510
28.659	32.136	27.658	34.560
26.446	38.773	33.664	33.664
27.553	31.240	34.507	
27.078			Mean
	Mean	Mean	34.934
Mean	31.848	31.650	
27.515			
	5% A.O.A. '195'	10% A.O.A.'210+'	20% C.O. ACIDS
5% A.O.A.			
	31.978	25.287	42.145
25.287	30.661	34.507	36.614
28.449	26.025	34.243	38.142
25.287	28.185	46.096	40.512
27.658	28.659	36.877	37.299
28.975	28.869	35.560	37.615
27.131	25.445	38.721	37.141
25.024	26.868	32.926	37.775
28.185	29.028	33.979	40.302
30.819	26.394	39.511	
32.926			Mean
	Mean	Mean	38.616
Mean	28.211	35.770	
27.974			
	10% A.O.A. '195'	20% A.O.A.'210+'	TABLE 7
10% A.O.A.			
	27.025	37.667	CONTROL
32.926	31.556	43.463	
34.770	28.659	50.575	31.241
32.243	28.922	34.770	27.388
37.141	23.338	41.355	32.282
33.189	36.139	48.994	31.137
36.877	28.275	66.379	24.784
36.787	30.819	64.009	28.950
34.507	27.869	43.199	25.201
31.346	28.922	54.262	
			Mean
Mean	Mean	Mean	28.711
34.420	29.152	48.467	
			2% PHENOL
5% A.O.A.'175'	20% A.O.A.'195'	5% C.O. ACIDS	
			26.359
26.446	43.673	32.136	28.646
26.816	44.200	35.560	27.005
28.237	55.105	33.664	31.562
32.799	34.770	35.666	29.996
27.973	36.192	26.499	
27.184	47.730	28.870	Mean
25.709	54.842	28.185	28.713
25.919	56.053	30.397	
36.245	42.725	33.716	
30.502		32.505	
	Mean		
Mean	46.143	Mean	
28.783		31.719	

TABLE 7 (cont/d.)/8

5% PHENOL

27.827
30.336
31.356
37.230

Mean
31.687

2% o-cresol

30.009
27.608
24.225
26.081
33.937

Mean
28.372

5% o-cresol

37.757
27.827
25.208
27.499
30.118

Mean
29.681

2% m/p-cresol

27.828
28.326
27.127
31.864
25.971

Mean
28.223

5% m/p-cresol

27.062
39.939
36.993
30.118
41.413

Mean
35.105

5% 2,4/2,5-XYLENOL

25.753
28.372
31.646
30.564
29.889

Mean
29.244

5% 2,4/2,5-XYLENOL

29.027
24.335
39.394
44.959
35.356

Mean
34.614

10% L.B.X.

37.866
37.102
34.047
34.156
31.209

Mean
34.876

10% H.B.X.

46.923
45.638
48.451
50.306
46.487

Mean
47.561

10% X. L.

25.862
28.045
29.573
31.209

Mean
28.672

10% X.X.L.

37.839
29.682
31.755
33.392
40.485

Mean
34.630

TABLE 8
CONTROL

As for Table 7

5% L14/13

37.905
33.532
37.593
36.760
40.092
39.780
38.843

Mean
37.786

10% L14/13

32.074
36.526
35.198
32.074
42.487
42.383
32.281
36.958
26.867

Mean
35.205

5% L14/19

36.239
35.198
26.867
33.844
31.553

Mean
32.740

10% L14/19

31.137
37.176
35.406
32.594
31.657
34.677

Mean
33.774

5% L14/47

34.157
27.618
32.386
33.428
31.345
34.990

Mean
32.320

10% L14/47

49.360
48.735
37.593
46.861
40.821
29.366
48.840

Mean
43.082

5% L14/56

28.846
28.637
28.950
30.304
36.135
30.199
33.001

Mean
30.867

10% L14/56

42.487
44.674
42.071
27.960
43.321
39.051

Mean
39.927

TABLE 8 (cont/d.)/9/10

5% L16/38

36.656
41.029
43.112
44.674
31.449
41.186
38.322

Mean
39.489

5% L16/53

37.490
31.137
46.757
38.947
36.864
44.570
43.008

Mean
39.824

10% L16/53

33.363
40.509
51.755
40.092
45.716
38.530
38.114

Mean
41.154

5% L16/70

29.731
37.176
39.259
34.261
48.319
51.651
45.716

Mean
40.873

10% L16/70

50.714
55.088
39.884
41.134
40.509
41.238
39.155

Mean
43.960

TABLE 9
CONTROL

29.085
25.305
25.826
25.721
31.449

Mean
27.477

5% A.O.A.'200'

33.948
30.324
39.358
38.350
35.406
34.175
30.408

Mean
34.567

10% A.O.A.'200'

44.883
42.071
36.447
38.947
31.657
33.219
48.215

Mean
39.348

5% A.O.A.'225'

30.213
33.219
31.137
46.028
31.970
32.699

Mean
34.211

10% A.O.A.'225'

32.907
36.031
40.509
39.260
31.241
33.844

Mean
35.632

5% A.O.A.'290'

40.717
43.112
40.509
35.823
32.907
33.532

Mean
37.766

10% A.O.A.'290'

37.281
45.091
35.406
46.757
49.256
44.778

Mean
43.094

5% A.O.A.'330+'

27.492
30.303
27.283
38.114
37.697
29.783
35.718

Mean
32.341

10% A.O.A.'330+'

39.363
40.717
42.487
45.820
54.151
50.611
46.757

Mean
45.700

TABLE 10
CONTROL

20.931
27.075
30.095
27.804
30.824
26.555
27.255
27.000
28.693

Mean
27.359

2% 2,3,5-trimethyl-phenol

27.284
27.700
32.698
28.325
33.011
26.867
30.720

Mean
29.515

5% 2,3,5-trimethyl-phenol

31.315
28.325
29.367
33.662
39.236
26.867
27.492

Mean
30.894

2% 2,4,6-trimethyl-phenol

27.492
25.866
26.665
26.659
25.513
31.345
25.617

Mean
27.022

5% 2,4,6-trimethyl-phenol

31.345
28.637
29.470
28.325
28.116
30.824
26.242

Mean
28.994

TABLE 10 (cont/d.)/11

2% 3-methyl-5-ethyl-phenol

32.178
26.450
25.617
37.338
27.075
28.117

Mean
29.462

5% 3-methyl-5-ethyl-phenol

29.470
31.032
27.179
28.429
26.450
27.388
39.884

Mean
29.976

2% 4-indanol

29.575
26.138
25.201
29.054
29.158

Mean
27.825

5% 4-indanol

26.971
26.659
35.094
40.925
34.989
25.617
32.594

Mean
31.835

10% 4-indanol

25.625
29.365
28.001
27.457
30.156

Mean
28.120

2% 5-indanol

27.566
27.729
26.405
36.729
30.121

Mean
29.710

5% 5-indanol

29.366
33.428
25.409
23.487
37.458

Mean
29.829

10% 5-indanol

25.626
32.526
31.256
29.001
30.021

Mean
29.686

2% 6-methyl-4-indanol

38.843
38.218
31.657
33.948
30.199
30.519

Mean
33.897

5% 6-methyl-4-indanol

30.616
32.906
32.074
33.323

Mean
32.229

5% 1-methyl-5-indanol

32.178
33.011
27.596
31.315

Mean
31.025

2% 7-methyl-5-indanol

32.490
29.679
28.325
28.429
30.512

Mean
29.887

5% 7-methyl-5-indanol

31.032
29.991
28.533
27.770
27.492
33.636

Mean
29.742

10% 7-methyl-5-indanol

35.623
36.293
40.152
38.256
39.554
37.252

Mean
37.855

TABLE 11
CONTROL

29.079
28.612
26.122
35.097
30.402

Mean
29.862

TABLE 11 (cont/d.)/12

10% A.O.A.

27.543
33.566
49.208
43.112
34.474
36.731

Mean
37.438

20% A.O.A.

68.484
66.458
68.041
46.277
52.969
40.544

Mean
57.128

10% A.O.A.'175'

37.380
40.518
44.965
35.849
35.434
36.861

Mean
38.501

10% A.O.A.'195'

31.154
40.518
26.199
35.201
35.227

Mean
33.659

10% A.O.A.'200'

30.505
34.942
33.099
32.607
37.665

Mean
33.763

10% A.O.A.'225'

40.778
41.841
40.778
46.355
37.224
39.533
42.256

Mean
41.252

10% A.O.A.'290'

53.722
52.139
60.259
53.774
54.733
35.201

Mean
51.638

10% A.O.A.'330+'

61.141
64.928
52.191
57.898
38.962

Mean
55.024

10% L14/13

28.612
46.149
63.361
49.260
43.501
39.429

Mean
45.052

10% L14/19

36.835
37.068
32.088
37.691
33.748
35.201

Mean
35.438

10% L14/47

34.267
33.904
26.381
34.993
32.477
33.955

Mean
32.662

10% L14/56

41.582
42.256
39.792
36.913
52.217

Mean
42.552

10% L16/53

52.217
42.229
59.714
46.123
52.010
40.881

Mean
48.862

10% L16/70

38.846
17.990
44.850
39.792
51.802
40.312
31.725

Mean
37.902

10% 3,4-dimethyl phenol

26.530
34.941
40.830
36.835
35.175

Mean
34.862

10% 2,4,6-trimethyl phenol

39.429
32.166
34.996
32.762
34.811
36.913
36.835

Mean
35.416

10% 2-methyl-resorcinol

40.518
38.184
44.254
52.217
34.941
34.007

Mean
40.686

TABLE 12
CONTROL

26.260
25.662
25.896
24.570
29.276
29.172
33.826

Mean
27.808

5% A.O.A.'<230'

28.756
33.930
31.626
27.222
28.626
26.156
25.922

Mean
28.891

TABLE 12 (cont/d)/13

10% A.O.A.'<230'	5% A.O.A. '250'	10% A.O.A.'270'	10% A.O.A.'300'
33.043	34.450	38.454	57.434
35.152	39.026	41.444	61.256
40.472	38.272	39.780	52.026
43.742	33.618	36.296	45.578
35.412	33.826	39.130	44.954
37.102	33.098	38.922	46.930
34.346	33.930	47.034	51.610
Mean	Mean	Mean	Mean
37.038	35.174	40.151	51.398

5% A.O.A. '230'	10% A.O.A.'250'	5% A.O.A.'280'	5% A.O.A.'310'
32.318	32.058	33.852	33.618
35.984	35.074	38.544	33.826
31.200	26.052	40.638	33.124
38.522	39.858	44.148	33.800
33.748	35.074	44.226	33.020
33.826	28.054	37.232	36.048
28.754	33.072	37.570	Mean
Mean	Mean	Mean	33.906
33.478	32.748	39.458	

10% A.O.A.'230'	5% A.O.A.'260'	5% A.O.A.'290'	10% A.O.A.'310'
31.980	33.852	44.278	47.814
35.148	36.426	39.234	52.572
39.134	39.000	46.592	46.748
35.022	51.740	37.908	52.026
28.782	33.462	38.558	46.514
31.278	36.140	46.254	Mean
33.392	33.592	40.268	49.134
Mean	Mean	Mean	
33.533	37.744	41.870	

5% A.O.A. '240'	10% A.O.A.'260'	10% A.O.A.'270'	5% A.O.A.'320'
28.751	31.720	49.296	50.674
28.834	36.244	49.920	40.040
31.278	37.108	57.304	52.130
33.670	32.500	57.538	63.596
28.626	27.898	39.234	39.884
28.674	41.054	46.150	36.322
33.696	44.536	54.834	Mean
Mean	Mean	Mean	47.107
30.504	35.865	50.610	

10% A.O.A. '240'	5% A.O.A.'270'	5% A.O.A.'300'	TABLE 13 CONTROL
46.592	31.278	36.478	25.948
39.078	33.644	44.343	23.322
51.974	33.982	48.984	26.104
37.362	36.634	49.478	27.798
40.716	33.618	51.689	26.234
49.452	33.862	61.412	31.200
48.334	32.578	57.460	25.662
Mean	Mean	Mean	Mean
44.786	33.656	49.977	26.609

TABLE 13 (cont/d.)/14/15a

10% Coalite H.B.T.A.

52.182
52.078
63.206
59.176
63.752
60.112
52.494

Mean
57.571

TABLE 14
CONTROL (DAY 1)

24.856
26.546
25.584
31.226

Mean
27.053

Day 2

25.698
26.124
30.125
26.544

Mean
27.122

Day 3

23.296
31.538
29.094
26.104

Mean
27.508

Day 4

31.486
28.600
26.026
28.678

Mean
28.697

CONTROL (DAY 11)

23.270
26.546
25.922
26.026

Mean
25.441

TABLE 15a
CONTROL
As for Table 2

PSOROX

38.918
43.231
44.270
56.584
66.145
40.944
46.660
47.232
46.037
49.778

Mean
47.979

TABLE 15b

INDIVIDUAL EPIDERMAL THICKNESSES, BODY WEIGHTS and TAIL STAINING

CONTROL

As for Table 5

Mouse	Weight	E.T.	Staining
LASSAR'S			
1	32	29.097	-
2	33	24.086	-
3	33	22.832	-
4	37	28.603	-
5	29	30.359	-
6	23	22.832	-
7	23	28.854	-
8	28	23.836	-
9	33	29.606	-
		Mean 26.678	
10% DITHRANOL/LASSAR'S			
1	27	58.711	+++
2	34	49.678	+++
3	25	55.951	+++
4	26	59.714	++
5	30	66.739	+++
6	29	67.241	+++
7	31	97.851	+
8	27	48.675	+++
		Mean 63.070	
10% DITHRANOL/Y.S.P.			
1	37	59.714	++
2	31	63.980	++
3	35	58.209	++
4	33	56.954	++
5	34	83.048	+++
6	38	55.951	+
7	30	66.489	++
8	32	94.840	+
9	31	66.489	++
		Mean 67.297	

TABLE 18/19

CONTROL and VEHICLE

As for Table 2

COTTON WOOL ONLY

31.176
30.656
33.566
32.942
36.008
35.333
35.125
41.204

Mean
34.501

10% A.O.A. '225P'

50.023
41.118
46.649
48.848

Mean
46.659

10% A.O.A. '290P'

46.649
41.457
52.337
43.205

Mean
45.912

10% A.O.A. '225F'

34.856
35.352
31.757
30.234
26.009
27.072

Mean
30.880

10% A.O.A. '290F'

26.690
35.352
29.273
35.378
29.393
39.359
31.282

Mean
32.389

TABLE 19

CONTROL

25.829
28.699
28.751
29.299

Mean
28.144

10% A.O.A. '225Y'

35.378
55.180
38.882
43.733
46.672
45.527
40.496

Mean
43.709

10% A.O.A. '290Y'

40.909
33.434
46.649
49.545
32.274
35.352

Mean
39.693

PLASTIBASE

29.073
26.042
24.993
31.021
30.077
25.025

Mean
27.705

TABLE 19 (cont/d.)/20

F.A.P.G.	2,3,5-trimethyl phenol	5-indanol
31.077	30.111	29.997
32.099	33.722	28.635
23.303	31.323	24.009
25.090	29.690	25.797
27.995	27.993	30.235
30.111	35.053	31.009
31.023	38.002	32.990
		30.665
Mean	Mean	Mean
28.671	32.270	29.167

TABLE 20

CONTROL

	2,4,6-trimethyl phenol	Vehicle 4
31.252	27.886	31.352
30.667	29.013	37.004
26.428	27.665	29.090
35.073	35.024	30.111
31.021	31.937	33.572
30.722	32.345	28.778
	31.073	29.999
Mean	Mean	Mean
30.860	30.706	31.415

3,4-dimethyl phenol

	3-methyl-5-ethyl-phenol
27.093	28.992
29.994	27.777
34.445	26.304
33.262	35.073
30.095	38.999
38.021	33.074
30.077	30.007
Mean	Mean
31.855	31.460

APPENDIX II

MICROTOME KNIFE SHARPENING

Microtome knives for the Base Sledge and Cambridge Rocking (Cryostat) Microtomes were sharpened on block-board impregnated with "HYPREZ" Diamond Compounds* of different particle sizes. Three boards impregnated with respectively 14μ, 6μ, and 1μ diamond compounds were used in this order to hone the knives. Prior to honing, the boards were sprayed with "HYPREZ FLUID" (Type OS)*, released and exposed the diamond particles from their resins. This method consistently produced an extremely sharp knife, which was essential for the cutting of the mouse tail skin used in these experiments.

The above method was first introduced by Mr. Allan Ayres of the Department of Histology, and Morbid Anatomy, General Hospital, Birmingham.

* Supplied by Engis Ltd.

APPENDIX III

SOURCES OF HISTOLOGICAL AND HISTOCHEMICAL REAGENTS

Reagent	Source
Carmalum (Mayer's)	Raymond A. Lamb
Congo Red	Raymond A. Lamb
3,4-Dimethylphenol	Koch - Light
Eosin (Yellowish)	Hopkins and Williams
Fast Red T.R. Salt	Raymond A. Lamb
Fluorlite	Raymond A. Lamb
Haemalum (Mayer's)	Raymond A. Lamb
Haematoxylin (Ehrlich's)	B.D.H.
Methyl Green	Raymond A. Lamb
2-Methylresorcinol	Koch-Light
α-Naphtyl acetate	Koch-Light
Sodium β-glycerophosphate	B.D.H.
Thioflavin T	Raymond A. Lamb
Tissue-Tek	Raymond A. Lamb
Tru-Touch Disposable Vinyl Gloves	Bard Parker

Photographs were taken on Kodacolour-X colour negative film.

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