Nutrition in the Carp with special reference to the morphology of the gastro-intestinal tract and to the effects of temperature.

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Master of Philosophy.

SUMMARY.

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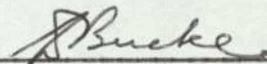
An enclosed recirculating aquarium system was constructed to hold carp which were subjected to variations in feeding regimes and water temperatures. The digestive tracts of these fish were examined for changes, by histological, histochemical and biochemical methods. These carp were considered to be derived from varied stocks, therefore, from previous experience on the histology of wild fish, and the variables which could occur, a sample of the carp were examined histologically to provide baseline data for the experimental samples, also the techniques for sampling and biochemical enzyme analysis were investigated.

There was no significant differences detected in the digestive tracts of carp fed two diets containing different protein levels, nor were there significant differences detected in fish which had been fed or starved at a temperature of 17°C. However, when the temperature was raised to 27°C, significant changes were recorded. These were increased cellular activity, more mucin production, a greater fluctuation in liver glycogen levels and a higher enzyme activity of the digestive tracts. A further study also showed the total serum proteins were increased at the higher temperature.

The overall conclusion was that when the temperature was raised sufficiently, the metabolic rate was increased, thus enabling the carp to absorb proteins more efficiently, and this was particularly evident when carp were starved at the higher temperature and tissue degeneration and depletion occurred.

Declaration.

I hereby declare that the whole of the work submitted in this thesis is the result of my own investigations except where reference is made to published literature and where assistance is acknowledged.



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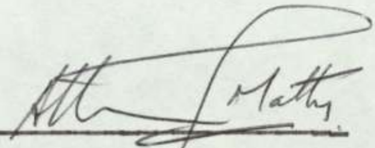

Director of Studies.

Certificate.

I hereby declare that the work embodied in this thesis has not already been submitted in substance for any degree, and is not being concurrently submitted in candidature for any degree.

Signed [signature]

Date 13th October 1976.

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1. INTRODUCTION

1. INTRODUCTION.

1.1. General introduction and objective.

Interest in aquaculture as a protein source has increased substantially in recent years. As a result, research into fish nutrition has intensified and fisheries' management now enjoys a similar status to other methods of animal husbandry in many parts of the world. The utilisation of freshwater fish as a food source is generally only justified economically when intensive methods of culture are used, and since these procedures are alien to the natural ecology of the relevant species, consideration must be given to the many interdependant variables which may influence fish growth for example, water quality, temperature and diet.

Any effects caused by changes in these conditions can be studied in a variety of ways, and Follis (1948) considered that for the histologist and histochemist much has, and can be gained, by examining alterations in the morphology of tissues after such changes have been applied.

Most of the data acquired by histological and histochemical techniques is qualitative, and although this may be interpreted by the experienced pathologist, it is of considerable value to apply some form of quantitation. This will probably correlate physiological and morphological changes and this is where the science of biochemistry is important.

With applied and basic research it is often difficult to visualise an end result. It was Woodall (1959) who suggested that such knowledge gained, forms the basis for solution of future problems, and he gives us the encouraging thought that while we see a result such as the discovery of polio vaccine, it was only

possible after collecting information from thousands of non-directed basic research projects.

The carp Cyprinus carpio was chosen for this research in preference to other species of fish because of its ease of handling in the facilities available. Experience in handling other fish, i.e. trout has shown that a closed circuit aquarium system is not ideal for their husbandry for a prolonged period. The carp is farmed throughout Asia and Europe, and is considered to be one of the most important food fish in the world (Mann, 1961), although there is little chance of this species becoming of economic importance in Great Britain, because of the temperature variations which do not aid the growth and fecundity of this fish. Probably the extensive farming of carp depends on a supply of heated water for example utilising the effluent of power stations. Nevertheless, the carp has existed as a wild fish in this country since the middle ages, and was first mentioned in "Treatise on Fishing with an Angle" written by Dame Juliana Berners in the 15th Century.

In view of the paucity of information on the correlation of temperature and feeding with intestinal morphology and biochemistry in the carp, this research project was concerned with the study of changes which occurred in the digestive tract of these fish after alterations in feeding regimes and water temperature had been applied. Certain techniques incorporating histology, histochemistry and biochemistry have been investigated and the results presented qualitatively and, where possible, quantitively.

1.2. Historical review of the digestive tract.

1.2.1. Morphology and histology.

Studies of the digestive systems of fish are not new, it is interesting to find Aristotle (384-322B.C.) evidently gave an accurate observation of the alimentary tract including the liver and gall bladder, in his book "Historia Animalum".

Dr. Charles Preston presented a communication to the Royal Society of London in 1697, on "A general idea of the structure of the internal parts of fish". Preston included a very brief description of the digestive tract of an unnamed species. Quote:

> "Their stomach is membranous; for fish swallow down small fish whole, and sometimes earth; where 'tis needful to have a power of contracting and straitning itself, forceably to break to pieces the hard matters contained therein. Their intestines make several great windings about; a sign the fermentation is but slow therein, which is made up by the length of the intestines."

He also described the liver as similar to that of other animals, and erroneously observed the fish to have up to forty four pancreases! Obviously he was describing pyloric caecae!

Georges Cuvier (1773-1838) famous for comparative anatomy, in one of his books; "Lecons d'anatomie comparee, (8 vols) volume IV, (1800 - 1805) described livers and pancreases of fish; volume V described digestive tracts of fish. This same author, with Valenciennes (1828 - 1840) wrote "Histoire Naturelle des Poissons" (22 vols), and in volume 1, they described the internal organs of the perch. It is interesting to note that Jonathan Couch

in his "Fishes of the British Islands" (1865) mentioned the action of rumination on food in the intestines of the Cyprinidae, and he made a special point that this occurred in carp (Cyprinus carpio), Tench (Tinca tinca), and bream (Abramis brama).

Dr. H. von Eggeling, who was Professor of Anatomy at the University of Jena, Germany, described and compared the mucosal surface of the intestines of some 179 bony fishes, including C. carpio (1908). A compatriot of his, also at the University of Jena, Dr. Edward Jacobshagen, (1913) gave his own description of the anatomical structure of fish intestines including that of C. carpio. He categorically stated that the carp has no stomach or pyloric appendages, but has a long winding intestine, with five loops; also he described the hepatopancreas.

However, to obtain a detailed knowledge of the anatomy of the digestive system related to the feeding habits of fish, Yasuo Suyehiro (1942) must be consulted. His descriptions of 150 species and over 500 research publications related to the alimentary tracts of fishes are a valuable source of reference.

The histological descriptions of the digestive systems of fish began at the same time as the science of histology and the establishment of the microscope, noteably the mid-point of the 19th and the early part of the 20th century. Many famous histologists studied the alimentary tract of fish, for instance E.H. Weber renowned for his discovery of Weberian Ossicles in man, also described the hepatopancreas of the carp (1827). Eberth and Muller (1829) described, for the first time, the pancreas cell (Islets of Langerhans) in Esox lucius. Von Kupffer, well known for his description of the Kupffer cell in the liver, outlined the development of the pancreas and spleen in fish (1892).

Around this period of time, workers specialising in histological research of the alimentary tract of fish, began to publish their results. Valatour (1861) described the gastric glands and the submucosa of the intestine of teleost fish. Gulland (1898) recorded a 'catarrhal' change in the mucosal epithelium of Salmon Salar salar when it returned from the sea to the river, and the change of the mucosal epithelium when the fish migrated to the sea. This is interesting, because Barton (1902) made a study of the digestive tracts of Salmon kelts describing fat in the mucosal epithelium of the intestine of starved fish. With this discovery, he suggested the columnar epithelial cells of the intestine both secrete and absorb fat.

Pictet (1909) made a detailed histological description of the intestines of five cyprinid fish. The carp was amongst these species. He reviewed the subject of mucous secreting cells and cited work by Oppel (1900). Pictet, noted goblet cells ('cellules caliciformes') to be more abundant in the crypts of the mucosal folds than at the tips.

Greene (1912) described the changes which occurred during spawning migration of Oncorhynchus tschawytscha. He made an interesting hypothesis on granular cells which he found in the stratum granulosum layer of the intestine, and considered the granules to be zymogen granules, whereas Gulland (1898) had termed these cells "eosinophilic leucocytes." Oskar Keil (1917) wrote a dissertation of the histology of the "Cyprinidenpankreas", and he gave technical details of fixatives and staining methods he used to demonstrate the various cellular elements.

Dawes (1930a) made a study of the alimentary tract of the plaice Pleuronectes platessa and stated that the intestinal musoa is folded to simulate crypts and villi, but are not true crypts or villi.

Blake (1930) studied the histology of the alimentary tract of the base <u>Centropristes striatus</u> and described peptic glands in the mucosa of the stomach.

The development of the carp in the larval stages was documented by Smallwood and Smallwood (1931) who described the development of the intestinal canal from the egg stage. It is interesting to note that the carp had then only been in the United States of America for a hundred years, but because of its adaptive habit to breed in polluted streams it had, even at that time, become a menace to sports fisheries. Smallwood and Smallwood recorded how mucous cells are formed in nuclei close to the basement membrane, and migrate towards the intestinal lumen. Smallwood and Derrickson (1933) described the development of the hepatopancreas, islets of Langerhans and the spleen carp. They observed that the liver originates on the day of hatching as two ventrolateral evaginations from the mid-gut; the 'principal islet' tissue developed from the biliary vesicle; and the spleen originates from the dorsal wall of the intestine. Following Blake's work on the plaice (1930) at University of Nebraska, Rogick (1931) described the histology of the alimentary tract of a minnow <u>Campestoma anomalum</u> and Curry (1939) the histology of the intestine of the carp. This author stated that in the seven carp she examined there were six loops of intestine instead of the five described by von Eggeling (1908). She described the mucosal epithelium well, including small nucleated dark staining cells between the columnar cells.

Klust (1939) made a comparison of the length of the alimentary tract in fish, including <u>C. carpio.</u> to their feeding habits. He stated that the length of tract could vary with the type of food. McVay and Kaan (1940) made a histological description of the digestive tract of the goldfish <u>Carassius auratus</u>, and found goblet

cells to be more numerous in the folds of the mucosal epithelium of the intestine, but not in the tips.

Since the review of Suyehiro (1942), a considerable amount of informative research has been given by Al-Hussaini (1945, 1946, 1947a,b, 1949a) when the author described the anatomical and histological structure of the alimentary tracts of both marine and freshwater fish. He made comparisons of the various species, related to their feeding habits. One of his publications (1949a) described the histological structure of the alimentary tract of C. carpio, where he compared this fish with Rutilus rutilus, and Gobio gobio and considered C. carpio to be a herbivorous fish. Al-Hussaini and Kholy (1953) critised a paper by Girgis (1952) who had rejected Al-Hussaini's (1949a) use of the term 'rectum' in describing the posterior intestine. It is interesting to note that Al-Hamed (1965) also rejected the term 'rectum' but Singh (1966, 1967) considered it should be applied.

Kristal (1946) described the dispersed exocrine pancreatic tissue, (the hepatopancreas), found in some fish and compared it with the diffuse exocrine pancreatic tissue found in other fish, C. carpio was not one of the species examined. Amlacher (1954), published a description on the anatomical structure and formation of the 'Karpfenleber' of C. carpio. In this work much useful histological detail is shown on the vascular and biliary structures.

Barrington (1957) reviewed the work on the alimentary tracts of fish including enzyme chemistry, absorption, diet and drug stimulation. Burnstock (1959) described the histology of the gut in Salmo trutta and made a particular study of the stratum compactum. Chaudrey and Khandewal (1961) confirmed that gastric glands were absent in cyprinid fish. Gohar and Latif (1961) compared the histological structure of Scarid and Labrid fish, and observed

granular cells in the submucosa in both fed and starved fish. Weisel (1962) assumed that the function of the granular cells of the epithelial mucosa could be either absorption, digestion, secretion or phagocytosis. Khanna (1963) gave a useful review of work on the endocrine pancreas in fish, and his own work included clear technical methodology on demonstrating islet cells. Pasha (1964 a,b) described glandular structures in Mystus gulio and suggested their function was zymogen secretion. Whereas, Bishop and Odense (1966) described intestinal glands in Gadus morhua but could not agree on their function.

It was inevitable that the ultrastructure of the intestine would be investigated and Yamamoto (1966) examined the fine detail of the columnar epithelial cell of C. auratus and Salmo gairdneri. This author described a difference in the columnar epithelial cell of the anterior intestine from that of the posterior intestine, also evidence of a muscularis mucosal layer which McVay and Kaan (1944) had considered absent.

Comparative anatomical and histological studies of the alimentary tract have been made on a catfish, Pimelodus macrulatus, by Godinho (1967) and Channa sp. by Tandon and Goswani (1968); the last authors related on increase in mucous cells to adaptive modifications to feeding habits. Also Vasilieva and Korovina (1968) made histological comparisons of the alimentary tract of some species of Cyprinidae, Osmoridae and Salmonidae. Bucke (1971) described the anatomy and histology of the alimentary tract of the pike Esox lucius and quantified the area of mucosubstance in the tract.

De Groot (1971) made an assessment of the interrelationships between morphology of the alimentary tract and feeding behaviour in "pleuronectiformes". This study however, did not include a histological study, but he did examine diurnal activity and its

effect on feeding.

Sinha and Moitra (1975) described the histological structure of the mucosal epithelium of the intestine in Cirrhinus mrigala, and discussed the effects diet had on cellular changes in the mucosa. Yamada and Yokote (1975) did not quantify the results of their studies on mucus cells in the intestine of Anguilla japonica because they considered stereological complexities might distort the results of statistical analyses.

1.2.2. Mononuclear cells.

The mononuclear cells which appear to 'wander' from the submucosa to the mucosa, and occasionally on the edge of the mucosal surface, have interested histologists for three quarters of a century. Greene (1912) described these cells as lymphocytes. Michels (1923) and Bolton (1933) suggested they were mast cells, whereas Jordan (1926) proposed they were eosinophils. The eosinophils of fish have been described by Drury (1914) and Catton (1951), and the literature on blood cells and their formation has been reviewed by Hawkins and Mawdesley-Thomas (1972). Vickers (1962) described lymphocytes in the intestine of C. auratus by their ultrastructural characteristics.

1.2.3. "Rodlet" cells.

This cell type has caused considerable controversy since histological descriptions of fish intestines began. Thélohan (1892) presented a remarkable publication on "Sur quelques coccidies nouvelles". A study of a cell type described previously by Laguèsse (1891) in his description of the pancreas and hepatopancreas of fish. Thélohan described these 'rodlet' cells as a species of coccidia. A description by Plehn (1906a,b) suggested this cell to be a unicellular gland "stabchendrusengellen" which discharged into the intestinal lumen. Laguèsse (1906)

promptly replied that this cell type was a sporozoan, Rhabdospora thélohani, and the argument still progresses because some workers have backed the unicellular gland theory, including Klust (1939), Catton (1951), Al-Hussaini (1959), Bullock (1963, 1967), Chaicharn and Bullock (1967) and an ultrastructural description recently by Leino (1974), and other workers have backed the sporozoan theory Hale (1965), Bannister (1966) and an ultrastructural description recently by Anderson et al (1976). It is even more interesting to note that Riley (1965) cited a paper by Winkler and Portela (1962) where the 'rodlet' cell was described as a mast cell in R. rutilus. Riley described the cell as a typical mast cell of the teleost fish.

1.2.4. Digestive enzymes.

Enzyme histochemically studies of the digestive tract did not appear to be investigated until Weinreb and Bilsted (1955) examined several enzyme systems including acid and alkaline phosphatase activities in the digestive tract of rainbow trout. They suggested there was a correlation between mucus and sites of alkaline phosphatase. Makino (1963) investigated activity of alkaline phosphatase in the digestive tract of a loach Misgurmus anguilliacaudatus and reported less activity in starved fish than fed fish. Srivastava (1966) described the similar distribution of glycogen to alkaline phosphatase in the digestive tract of several teleosts.

A comprehensive review of enzyme studies in fish was given by Barrington (1957), has already been mentioned previously. Acid and alkaline phosphatase activities were examined in livers of S. trutta and S. gairdneri by Mawdesley-Thomas and Barry (1970). This study was made to differentiate the two species in their resistance to aflatoxins. Western (1971) described activities of various enzymes in part of the digestive tract of two cottid fish. Alkaline phosphatase and leucine aminopeptidase enzymes were included in this investigation. Western did not find evidence of alkaline

phosphatase activity in the posterior intestine of Cottus gobio.

Goel and Sastry (1973) examined the distribution of alkaline phosphatase in the digestive systems of certain teleost fish. These authors also examined activity of this enzyme in the hepatopancreas. Lipase activity in the digestive systems of an omniverious and a carnivorous fish had been examined histochemically by Sastry (1974). He stated that there was less activity in the omnivorous fish than the carnivorous fish. Hinton et al (1972) demonstrated activities of alkaline phosphatase and glucose-6-phosphatase in the central veins and sinusoids of the liver of Micropterus salmoides.

Possibly the first recorded electron microscopical study of the enzymes (catalase and non-specific phosphatase) in the hepatocytes of C. carpio was made by Kramer et al (1974). These authors were making a study of peroxisomes in liver.

Biochemical studies on enzymes in fish have been reported by Al-Hussaini (1949b) who recorded tryptic and lipolytic effects from the intestinal mucosa, thus suggesting that secretions did not only occur in the pancreas. Sarbahi (1951) compared digestive enzymes in C. auratus and M. salmoides. His report indicated that carbohydrases were prominent in the former fish compared to the amounts of proteinases and lipase in the latter fish. This indicated the fact that omnivorous fish can utilise animal foods more readily; a fact, that was recorded by Vonk (1941) a decade earlier. Fish (1960) recorded the presence of proteolytic enzymes in Perca and Tilapia. Gohar and Latif (1961) observed pancreatic enzyme secretion in the intestinal mucosa of fish and later, Gohar and Latif (1963a) examined proteolytic enzymes including aminopolypeptidase in scarbid and labrid fishes, and in a later investigation (1963b) studied intestinal absorption of olive oil and oleic acid in

Clarius lazera.

Noda and Tachino (1965) recorded acid and alkaline phosphatase in fish and Utida and Isono (1967) examined alkaline phosphatase activity in the intestinal mucosa of A. japonica which were acclimatised to freshwater and sea water. Western and Jennings (1970) demonstrated HCl in the gastric tubules of cottid fish. Proteases have been recorded in a carnivorous teleost, Dicentrarchus labrax by Alliot et al (1974).

Since Field et al (1943) described the blood constituents of carp and trout by lengthy laboratory processes, the use of electrophoresis for determining proteins and enzymes has speeded up this methodology, and much work was reviewed by de Ligny (1969). Lysak and Wojcik (1960) studied serum proteins in C. carpio after they had been fed different amounts of protein. Fujiya (1961) suggested the use of electrophoresis for detecting toxic changes in fish. Meisner and Hickman (1962) examined serum proteins after S. gairdneri had been acclimatised to two different temperatures and reported that the albumin/globulin ratio was greater in trout acclimatised to 8°C than 16°C. Thurston (1967) examined the serum protein from S. gairdneri after the fish had been subjected to various stress conditions. It appeared that sexual maturity affected the results considerably.

Love (1970) in his publication "Chemical Biology of Fishes" comprehensively reviewed the chemical analysis of fish tissues, this work has been a useful source of information.

1.2.5. Absorption

Greene (1913) described absorption of fat in the alimentary tract of king salmon, and a careful study of fat absorption in Pleuronectes platessa was made by Dawes (1930b). However, one of

the first *in vivo* studies on absorption in fish was made by Wislocki (1917), who injected coloured dyes into the peritoneum of *C. auratus* and studied the appearance of the dyes in the surrounding tissues. Mackmull and Michels (1932) examined peritoneal absorption after carbon had been injected into the peritoneum of teleosts, and confirmed the results of Wislocki. Allee and Frank (1948, 1949) observed the way colloidal material and minute food particles were absorbed in the intestine of *C. auratus*. *In vitro* absorption tests were made by Smith (1964) who dissected lengths of intestine and placed them in a suitable incubating medium, and measured the sodium, potassium and water content. Glucose absorption in the gut of *S. gairdneri* was examined by Stokes and Fromm (1964), fat absorption in *Tilapia mossambica* (Peters) by Sivadas (1965).

The ultrastructure of absorptive cells of the intestinal mucosa have been examined by Yamamoto (1966) in *C. auratus* and *S. gairdneri*. Iwai (1968a,b) followed up this work by examining the columnar epithelial cells of fry in these fish species. Gauthier and Landis (1972) further investigated the ultrastructure of the epithelial cells in *C. auratus* intestine and considered that protein was absorbed in the most distal region of the intestine.

The ultrastructure and comparison of the brush borders on the enterocytes of three cyprinid fish was reported by Cartier and Buclan (1973). These authors have paid special attention to the nutritional function of the cells. Nouillac-Depeyre and Gas (1974) made an ultrastructural study of enterocytes in *C. carpio* with particular reference to fat absorption, which they recorded took place in the anterior intestine.

1.2.6. Starvation.

Smallwood (1916) recorded twenty months of starvation in

a bowfin Amia calva. This fish had existed in an observation tank, and received no food, and therefore survived on food energy derived from the body muscles. No mention of changes in the digestive system were made. Honma and Matsui (1973) described the histological changes that occurred in a specimen of A. japonica after it had been starved for 522 days. A description of the digestive tract was included, and marked atrophy occurred in the intestine, exocrine pancreas and liver. Between these two publications Borek (1958) studied lipids in C. carassius after experimental starvation and hibernation, and reported an increase in lipid content of the muscles of starved fish, but a general total reduction of lipid. Love (1958) examined the organ weights of Gadus sp. after 78 days starvation, and recorded a drastic reduction of liver weight. Sorvachev (1959) studied serum proteins using electrophoresis in C. carpio during hibernation, and recorded a general decrease in proteins. Leibmann (1960) recorded a total loss of serum albumin in C. carpio after starvation. Hanna (1962) examined organ weights and liver glycogen of Clarius lazera starved for periods up to 7 months. A general reduction in organ weights plus a loss of liver glycogen was recorded. Smirnova (1965) studied changes in the blood indices of Lota lota after 20 days starvation, and recorded a loss of haemoglobin and leucocytes. Steffens (1964) considered that physiological problems concerning hibernation of C. carpio led to diseases, particularly dropsy (Spring viremia of carp). Creac'h and Cournéde (1965) recorded that after starvation, atrophy occurred first in the intestine, then in liver, spleen, muscle and heart. Creac'h and Serfaty (1965) reported considerable amounts of peritoneal fluid in starved C. carpio (a possible source of the dropsy mentioned by Steffen (1964)). Inui and Oshima (1966) studied starvation of A. japonica and noted the weight loss more

rapid at a high than a low temperature. Hamra (1966) recorded liver glycogen loss in starved *gadus* sp. Changes caused by short term starvation were a sharp decrease in glycogen after 24 hours but a sharp increase at 48 hours, according to Sundaraj et al (1966).

Inui and Eausa (1967) studied histological changes in the liver of the glass eel, and the main changes were loss of glycogen and hepatocyte changes. Bilinski and Gardner (1967) examined free fatty acids in the muscles of *S. gairdneri* and Nagai and Ikeda (1971a) described changes in the hepatopancreas of *C. carpio* after starvation. Lipids and glycogen were critically examined.

The effects of short term starvation in *Fundulus heterochitus* were recorded in a histological study of the intestinal tract by Ciullo (1975). Changes in alkaline phosphatase, neutral lipids and 'rodlet' cells were recorded. Hess (1935) noted a reduction of islets of Langerhans in *S. gairdneri* by reducing the protein content of the diet and adding fat and carbohydrates. This type of study was repeated by Donaldson (1934) in Chinook salmon, who recorded similar changes. Wood et al (1957) considered that salmonid fish required a high protein diet to prevent disease. Judd and Cross (1966) recorded tissue changes in *Ictalurus punctata*; accumulations of fat in the liver were the main changes. Yokote (1970a,b,c,d,e) made a study of 'Sekoke' disease or spontaneous diabetes in *C. carpio.* Histological changes in pancreas, liver, adrenocortical tissues, thyroid and eye were recorded. Kawai and Ikeda (1971, 1972, 1973a, 1973b) studied enzymic changes of the digestive systems of *C. carpio* and *S. gairdneri* after changes in dietary composition. Nutritional diseases have been comprehensively reviewed by Sniezko (1972), and again this important work has been a valuable source of information.

1.2.7. Temperature

Cheng (1931) described the histological differences between the intestinal mucosa of *C. auratus* in spring and winter, and clearly showed that when the fish was not feeding, in the winter, the epithelial cells were at a resting stage, and when feeding in the warmer weather, the epithelial cells were more active. Hoar and Cottle (1952) examined the biochemical composition of *C. auratus* acclimatised to different temperatures. This study was followed by a further biochemical examination of *C. auratus* (Irvine et al, 1957) in which dietary phospholipid and cholesterol were measured in relation to temperature resistance.

Ananichev (1959) studied digestive enzymes and seasonal variabilities in various freshwater fish, and found an optimum of activity at periods of intensive feeding. A similar study was made by Chepik (1964). Dean and Goodnight (1964) also examined biochemical parameters of fish stressed by temperature and exercise, and one of these parameters was liver glycogen. Hormonal activity and temperature acclimatisation in *C. auratus* were studied by Klicka (1965).

Mepham and Smith (1966) described the transport of amino acids in the intestine of *C. auratus* acclimatised to different temperatures. This type of investigation was also made by Kitchen and Morris (1971).

Temperature induced alterations in the hepatocytes of *S. gairdneri* have been recorded by Berlin and Dean (1967). This was an ultrastructural examination, and particular attention was made to the golgi complex and rough endoplasmic and secretory activity of hepatocytes.

Smit (1967) investigated the influence of temperature on gastric

juice secretion in Ictalurus nebulosus, higher secretion was dependent again on higher temperature. The haematological values of C. carpio and thermo-acclimatisation variations were investigated by Houston and De Wilde (1968). It was pointed out that in this study the various groups on test varied in their nutritional state. A more straightforward approach to temperature variation was made by Gammon et al (1972) when they investigated the histological changes in the digestive tract of I. punctatus in freezing winter and hot summer conditions, and in a most comprehensive report, Haschemeyer (1973) discussed protein synthesis when toadfish Opsamus tau were exposed to low temperatures. Here the author had investigated the time enzymes take to adjust to temperature changes so that the normal functional metabolism processed can carry on. Also Villanueva and Marcus (1974) looked at the effect temperature had on fructose 1,6-diphosphatase in liver from C. carpio. This enzyme was considered temperature dependent.

2. MATERIALS

2. MATERIALS

2.1. The Aquarium System

An enclosed continuous circulating aquarium system ultilising multiple tanks was developed. The system circulated water from a stock tank, into six separate experimental tanks, and back into the stock tank. (Fig. 1) The stock tank had 50 gallons capacity (227.3 litres) and water was pumped from the tank with the aid of an electrical pump, (Charles Austin Pumps Ltd., model C25) into a plastic drain pipe, 10cm diameter sealed at each end, but with a hole 2.5cm diameter for access of the 2.5cm pipe from the pump. This pipe served as the overhead distributing pipe. Water was delivered from six points, through six triangular holes (2.5cm high) into separate 100 litre glass tanks. The water level was kept constant at 60 litres in these tanks with the aid of syphoning system. The syphoning system worked as follows :- 18" lengths of plastic pipes, 2.5cm diameter (electrical conduit piping), were bent into a 'U' shape. One end of each pipe was introduced into each 100 litre tank, the other end was sealed into the lid of a 5 litre plastic container termed a 'trap'. The end of each pipe was kept within 1cm of the bottom of the vessels. The suctioning end in the 100 litre tank was perforated with small holes (0.5cm diameter) to reduce the pressure and avoid a fish becoming wedged in the pipe.

The traps were placed on wooden platforms at the end of the 100 litre tanks, which were placed lengthways on a bench top. A plastic gutter (10cm diameter) was situated beneath the wooden platforms, blocked at one end, this enabled the water to run back into the stock tank. The gutter was raised 3cm at the blocked off end to obtain this run off.

The syphons worked by having 'L' shaped pieces of 2.5

diameter plastic pipe let into and sealed in the traps at the 60 litre water levels on the glass tanks. The water flowed when the levels were equal in the two vessels, and the surplus air was sucked off at the end of the 'L' shaped pieces. Therefore, water was pumped from the stock tank, into the overhead distributing pipe, out into six individual tanks, syphoned off at the same rate, into a gutter which led back into the stock tank. The flow rate of water from the pump had to be regulated, as the pump output was faster than the syphons output. A by-pass was let into the discharge pipe from the pump, and led back into the stock tank. This by-pass was easily regulated, and had proved useful when flushing out the head pipe. Aeration occurred, when the returned water entered the stock tank, where the by-pass water entered the stock tank, where individual water entered the glass tanks, and also from the syphon discharge. An independent aeration system was also installed, using an aquarium aeration (type Eheim) with air stones in each glass tank, but this was later found to be unnecessary and dismantled.

The water in the stock tank was continually filtered with the aid of two 'Eheim' type filters. The containers held glass-wool floss and activated charcoal chips. The water discharged from the gutter passed through a gauze filter before entering the stock tank. Each trap acted as a reservoir for almost all of the solid waste by the syphoning action. The charcoal filters were changed every 15 days, the crude gauge filter every day and the traps were emptied every 3 days; therefore approximately 30 litres of fresh tap water was exchanged in the system every three days and the total amount of water involved was 530 litres, and the restocking rate for just under 6%.

An anaylsis of the tap water was made by the Anglian Water Authority (See appendices 1), before the test started, and the main characteristics were again examined 4 weeks after the test

had started, and thereafter, at intervals of 4 weeks, but later discontinued as no deaths occurred in the year the system was in operation.

The apparatus was installed in a room with a thermostatically controlled electric fan heater. For the first experiments this heating system was set to give a water temperature of 17°C (±2°C). In later experiments the room temperature was raised to 27°C (±2°C).

This apparatus described above worked continuously for a year and was found to be releatively reliable. However, problems were encountered in an early experiment (Protocol 2) when feeding twice-daily caused uneaten food to accumulate in bends of the syphons, which subsequently led to flooding. This fault was remedied by cutting the feeding rate by half. Flooding also occurred after the temperature was raised to 27°C (±2°C), when accumulations of brown slime * were found on all bends of the syphons, and inside the overhead distributing pipe. The remedy was more frequent cleaning of the traps and overhead distributing pipe, combined with flushing of the system by closing off the by-pass on the pump discharge for short periods daily, giving a stronger flow of water.

* The brown slime was an accumulation of filamentous bacteria and suspended solids, identified by microscopical examination.

Fig. 1

Aquarium System.

1 - Stock tank. 2 - Pump pipe.

3 - Overhead distributing pipe.

4 - 100 litre tank. 5 - Syphon trap.

6 - Plastic drainage gutter.

Fig. 1

2.2. The Fish

A total of 200 common carp Cyprinus carpio (L.) 20 goldfish Carassius auratus (L.) and 20 crucian carp Carassius carassius (L.) were purchased from the Avon Coarse Fish Farm, Alresford, Hants., but possibly originated from Belgium. The common carp were from mixed races; either the elongated scaled strain, considered to be 'wild', or the 'mirror' or cultivated variety. All were between 10 and 18c.m. length, with an average weight of 62g. These fish were in good health on arrival, and were kept in two x 50 gallon tanks for acclimatisation to Huntingdon tap water. To the stock tanks, "Furanace" * (Nifurpririnol) was added at the rate of 0.1 p.p.m. as a chemotherapeutic method for the prevention of microbial infections. No mortalities wer incurred throughout the period of research. The stock fish were fed on "Tetramin" ** flaked goldfish food.

* "Furanace" - obtainable from Piscisan Ltd.,
Howard Chambers,
31, Church Street,
Enfield, Middx. EN2 6BA.

** "Tetramin" - obtainable from any Pet shop or
Tetre Werke,
454 Melle, Postfach 1580
W. Germany.

2.3. Diets

The following diets were formulated commercially, diet 'A' contained 31.5% protein and diet 'B' contained 19% protein. The preparations were pelleted to 0.5cm. size.

TABLE 1

	CARP DIET (HIGH PROTEIN) 'A' %	CARP DIET (LOW PROTEIN) 'B' %
White Fish Meal	10	2½
Meat Byproduct Meal	10	2½
Soya Bean Meal	20	5
Grass Meal	3¾	3¾
Fermentation Byproduct	10	10
Wheatfeed	43¾	73¾
Cod liver oil	1¼	1¼
Vitamin/Meal Supp.	1¼	1¼
Total %	100	100

Calculated Analysis

	%	%
Oil	5.0	4.5
Protein	31.5	19.0
Fibre	4.5	5.5
Ash	6.0	5.0
Nitrogen Free Extractives (Carbohydrate)	41.0	54.0
Moisture	12.0	12.0

3. METHODS.

3. METHODS

3.1. Protocol 1: The histology of the alimentary tract of control carp.

Here the initial studies were made to select suitable methods for demonstrating changes in the alimentary system of carp. Six carp were placed in a tank containing tap-water which had previously been aerated for three days. The water temperature was maintained at a temperature of 17°C (±2°) and the experiment was continued for 20 days. The water was continually aerated with a 'Eheim' aerator, and the tank was kept clean by manually syphoning debris once every 5 days. The diet was 'Tetramin' flaked goldfish food, and feeding was twice daily. At the end of this initial study, the fish were sacrificed and examined by the following procedures :-

The fish were anaesthetised in MS222 (Tricaine Methanesulphonate, 100mg/l of water) Jolly et al (1972), and killed by severing the spinal cord at the level of the 1st vertebra. An incision was made on the ventral aspect from the gill to the vent, and a macroscopical inspection was made. (Bucke, 1972).

Samples of intestine, taken at the oesophagel (anterior) medial and rectal (posterior) regions, hepatopancreas, including any adhering pancreas, spleen and kidney were taken for biochemical, histochemical and histological examinations (See Laboratory Methods). The reason for examining these samples was to establish the general morphological details in these tissues by a histological examination, and subsequently to establish the presence and activity of alkaline phosphatase and leucine aminopeptidase by histochemical and biochemical techniques. Alkaline phosphatase was chosen because of its importance in the process of absorption and transporting amino-acids across cell membranes in the intestine. Leucine

aminopeptidase was chosen because of its catalytic enzymic properties which hydrolyse peptide bonds, breaking a link in the polypeptide chain which is an important stage in the absorption of proteins.

3.2. Protocol 2.

A preliminary experiment to examine changes in the alimentary system of carp fed diets containing two different protein levels. The enclosed continuous water flow aquarium using six individual tanks, was used for this experiment, and the water was maintained at a temperature of 17°C (±2°). Six fish were placed in each tank, thus giving a triplicated experiment. Diet A (High Protein) was fed to fish tanks 1, 3 and 5 and Diet B (Low Protein) was fed to fish in tanks 2, 4 and 6. The fish were observed, and manually fed once daily at the approximate rate of 3% bodyweight. (Twice daily feeds were made intially, but complications arose when uneaten food blocked the syphoning systems.) The experiment was continued for 56 days only, because of a problem with mouldy diet. Samples of fish were sacrificed for laboratory investigations as follows :-

> the fish were anaesthetised in MS222 and killed by severing the spinal cord at the level of the 1st vertebra. An incision was made on the ventral aspect from the gill to the vent, and a macroscopical inspection was made. Samples of intestine, hepatopancreas, spleen and kidney were taken for biochemical, histochemical and histological examinations. (See Laboratory Methods and Protocol 1.)

3.3. Protocol 3

An experiment to examine changes in the alimentary system of carp fed diet 'A' and carp starved, at a temperature of 17°C (±2°). The enclosed continuous flow aquarium using six individual tanks, was used for this experiment, and the water maintained at a temperature of 17°C (±2°). Six fish were placed in each tank, thus giving a triplicated experiment.

a) High Protein diet was fed to fish in tanks 1, 3 and 5 and b) fish were not fed in tanks 2, 4 and 6.

The fish were manually fed, once daily at the approximate rate of 3% bodyweight. The experiment was continued for 80 days, when samples of fish were sacrificed for laboratory investigations as follows :-

> the fish were individually anaesthetised in MS222 and blood samples were taken by cardiac puncture for biochemical analysis (See Laboratory Method 3.7.1.). The fish were then killed by severing the spinal cord at the level of the 1st vertebra. An incision was made on the ventral aspect from the gill to the vent, and a macroscopical inspection was made. Samples of intestine, hepatopancreas, spleen and kidney were taken for biochemical, histochemical and histological examination. (See Laboratory Methods and Protocol 1).

Note:- Blood samples were taken to make estimations of serum alkaline phosphatase and serum leucine aminopeptidase to gain an overall pattern of these enzymes in the fish.

3.4. Protocol 4

An experiment to examine changes in the alimentary system of carp fed on a high protein diet and carp starved at a temperature of 27°C (±2°). The enclosed continuous flow apparatus using six individual tanks was used for this experiment, and the water maintained at a temperature of 27°C (±2°) Six fish were placed in each tank, thus giving a triplicated experiment.

a) High protein diet was fed to fish in tanks 1, 3 and 5 and b) fish were not fed in tanks 2, 4 and 6.

The fish were observed, and manually fed once daily at the approximate rate of 3% bodyweight. The experiment was continued for 72 days, when samples of fish were sacrificed for laboratory investigations as follows :-

> the fish were individually anesthetised in MS222 and blood samples were taken by cardiac puncture for biochemical analysis (See Laboratory Methods 3.7.1.) The fish were then killed by severing the spinal cord at the level of the 1st vertebra. An incision was made on the ventral aspect from the gill to the vent, and a microscopical inspection was made. Samples of intestine, hepatopancreas, spleen and kidney were taken for biochemical, histochemical and histological examination. (See Laboratory Methods and Protocol 1).

Note:- Blood samples were taken as in Protocol 3.

3.5. Protocol 5

A preliminary experiment to examine the levels of serum protein in carp fed differing protein diets and starved, at two different temperatures. The enclosed continuous flow aquarium using six individual tanks, was used for this experiment, and the experiment was run in two parts.

Part 1 The water temperature was maintained at 17°C (±2°). Four fish (4-6cm in length) were placed in each tank, thus giving a duplicated experiment.

a) High protein diet was fed to fish in tanks 1 and 4. b) Low protein diet was fed to fish in tanks 2 and 5 and c) fish were not fed in tanks 3 and 6.

Part 2 The same system to that described in Part 1 was followed except the temperature was maintained at 27°C (±2°). Both experiments continued for 20 days, when samples of fish were sacrificed for laboratory investigation as follows :-

> the fish were individually anaesthetised in MS222 and blood samples were taken by cardiac puncture for serum protein analysis. (See Laboratory Method 3.7.2.) The examination of serum protein by electrophoresis was made because of the usefulness of this method as an indicator with considerable sensitivity. The method can show changes that arise in the animal organisms, and are routinely used in analytical, diagnostical and prognostical studies in mammalian studies, and it appeared feasible to investigate the usefulness of such a diagonostic tool in the above experiment. Therefore, three methods of electrophoresis investigation were made. See Laboratory Methods 3.7.2.

3.6. Laboratory Procedure: Histology

3.6.1. General histology

The procedure for histological processing was similar to that described by Bucke (1972).

Fixed samples of anterior, medial and posterior intestine, with hepatopancreas and any abnormal organs, were trimmed to 2-3mm thickness and processed into paraffin wax (56°M.P.) on an automatic tissue processor. The intestine was embedded to give transverse sections, and from these and the hepatopancreas, serial sections were cut at 5µm on an M.S.E. Sledge Microtome. The sections were floated on warmed distilled water (45°) to which 0.02% egg albumen had been added. Then placed on glass microscope slides and dried out at 37°C overnight for staining.

Staining methods

A set of the following staining methods were applied routinely to tissues from each fish sample.

a) Haematoxylin and Eosin for general morphological detail.

b) Van Gieson's stain for connective tissues.

c) Periodic acid Schiff, with and without diastase digestion for glycogen, neutral mucopolysaccharides and lipo-pigments.

d) Alcian blue-periodic-acid Schiff for acid and neutral mucopolysaccharides (intestine only).

The following staining methods were applied to certain tissues for pigmented material indentification.

e) Schmorl's reaction for lipofuscin and melanin.

f) Fouchet's reaction for bile pigments.

g) Ziehl-Neelsen stain for exogenous pigments.

h) Perls' Prussian blue reaction for haemosiderin material.

i) Mallory's bleaching method for identifying melanin.

Techniques for the above staining reactions can be found described in "Histological Laboratory Methods" Disbrey and Rack (1970).

3.6.2. Quantitative histology

A quantitative analysis of the area of mucin and numbers of mucin producing cells was made using an image analyser, the Quantimet 720 (Metals Research Ltd., Royston, Herts). This system is simply explained as follows :- A specimen is imaged by a microscope which projects this image on a Vidicon TV camera, which in turn produces the image on a monitor screen. Electrical information describing the sample passes to a detector module which selects the features to be measured according to their contrast (grey level) and passes this information on these chosen features to the computer.

The specimens measured by this equipment were sections of intestine, (anterior and posterior levels cut at 5µm) stained with Alcian blue/PAS.

i) The cell areas were contrasted and counted.

ii) The goblet cells were contrasted and counted.

Comparisons were made between the mucin area and goblet cell counts from the anterior and posterior intestine from each experiment. For this examination, the x 10 objective was used and measurements made over a 300 x 300 picture point "live frame" (PP) on the monitor screen. From every section, 10 different fields of intestinal mucosa were counted, for both area of mucin and number of mucin producing cells.

Note. 1pp = 1.42 linear = 2.046 μm^2 area

3.6.3. Enzyme histochemistry

Techniques for the detection of certain enzymes in samples of intestine and hepatopancreas are described previously by Bucke (1972). Dissected samples of anterior, medial and posterior intestine were cut transversally, and samples of hepatopancreas were placed immediately into a bath of acetone cooled to -80°C with solid CO_2. The frozen tissues were stored at -20°C until required for sectioning.

Sections (at 8µm) of the fresh frozen samples were serially cut at -15°C in a cryostat and collected on warm miscroscope slides. These sections were ready for the demonstration of certain enzymes.

The methods selected were taken from Pearse (1961).

1) Alkaline phosphatase. (Burstone, 1958).
2) Leucine aminopeptidase. (Nachlas, Crawford and Seligman, 1957).
3) Acid phosphatase. (Gormori, 1950).

The 'stained' slides were examined microscopically for any pathological changes.

3.6.4. Microscopy and Photography

Stained sections were examined on a Zeiss "Ultraphot 11" microscope. Photomicrographs were taken both for colour and black and white on Kodak "High Speed Ektochrome" and Kodak "Panatomic-X" Films.

The black and white photographs were developed in D76 developer and prints were made using a Durst DA 900 enlarger in combination with a Radidoprint LD 37 printer.

The colour photomicrographs were developed and printed professionally.

3.7. Laboratory Procedure: Biochemistry

3.7.1. Enzyme studies

Samples of the three levels of intestine and hepatopancreas were dissected from the carp autospy and immediately frozen, by placing them into acetone cooled to -80°C with solid CO_2. These frozen tissues were stored at -20°C until required, and then completely thawed and weighed. Each sample was diluted with 1ml of normal saline and homogenised in a laboratory blender. Each homogenate was split into two equal amounts for estimation of the activities of alkaline phosphatase and leucine aminopeptidase.

Samples of serum were obtained from anaesthetised carp, by taking blood from the heart (using a 2ml disposable sterile syringe and a No. 21 gauge needle), and placing it into dry 1.0 ml plastic tubes at 4°C for six hours, allowing the blood to clot, thus releasing the serum. The serum was syphoned into clean tubes and centrifuged at 3000g. for 10 minutes. The supernatent serum was stored at -20°C for estimation of alkaline phosphatase and leucine aminopeptidase activities.

a) Alkaline phosphatase estimation :-

An automated technique, using a Technicon Autoanalyser was applied. This was a modification of the King-Armstrong technique (Powell, 1954; King and King, 1954). The tissue homogenates were diluted in normal physiological saline, 1:40, 1:80 and 1:60, and the serum samples were diluted in normal physiological saline, 1:10 until suitable absorbance curves were obtained. The results were measured colorimetrically at 505μm and compared with standards, these results were expressed in arbitary King-Armstrong (K-A) units per mg. of sample.

b) Leucine aminopeptidase estimation

The method used was manual (Goldberg and Ratenburg, 1958).

although reagents manufactured by Sigma Chemicals Ltd. were used for the reaction, and was, therefore, slightly modified. The homogenates and serum samples were diluted at the ratio of 1:75 in distilled water before applying the method. After the reaction was completed, the samples were measured colorimetrically at 580μm wavelength and a curve plotted in arbitary Goldberg and Ratenburg (G-R). units per mg. of sample.

3.7.2. Serum protein analysis

The carp were anaesthetised and blood samples were obtained by exposing the heart and withdrawing blood from the ventricular chamber with an ultra-thin capillary tube. By applying a little pressure, a few microlitres of blood could be withdrawn as the heart gave a beat. When a suitable blood sample was obtained, the tubes were heat-sealed and centrifuged at 3000g for 10 minutes. The serum was obtained by breaking the tube at the junction of the red cells and serum. The methods of analysis of serum proteins were as follows :-

a) Electrophoresis in a prepared thin-layer polyacrylamid gel and for this technique, the LKB1804-101 Ampholine PAG plate kit was used. (LKB, Productor AB, S-161 25, Bromma-1, Sweden).

b) Electrophoresis by a cellulose acetate Phoroslide and for this technique the Millipore Zone Electrophoresis system was used. (Millipore Bromedica, Acton, Massachusetts 01720, U.S.A.)

c) Electrophoresis using agarose film, and for this technique the Corning Eel Agarose film cassette system was used. (ACL - Corning, Corning Glass Works 490, San Antonia Road, Palo Alto, California 94306, U.S.A.)

4. HISTOLOGY OF THE GASTRO-INTESTINAL TRACT OF CONTROL CARP.

4. HISTOLOGY OF THE GASTRO-INTESTINAL TRACT OF CONTROL CARP.

4.1. General histology

Although the digestive tract has been described in the carp by Pictet (1909), Curry (1939) and Al-Hussaini (1949), it was necessary to describe the normal histology of samples from the carp used in this investigation for interpretation of the test samples.

4.1.1. The intestine (Figs. 2 - 10)

The intestinal mucosa was thrown up into folds or rugae which consisted of the epithelium and some connective tissue. The widest part of the intestine was the anterior with a narrowing towards the posterior sections. In the anterior intestine, the mucosal folds were elongated, with rounded ends, almost touching across the lumen. The mid-intestinal mucosa also had elongated folds and here the tips had small invaginations. The posterior intestinal mucosa was thrown up into small folds, which were elongated rather than filiform. (Figs. 2 - 4)

The main cell types of the mucosa were the columnar and the goblet or mucous secreting cells. These cell types could be seen to originate from a basement membrane. Other cell types seen in all levels of the intestinal mucosa were mononuclear cells and rodlet cells.

Underneath the mucosa was the lamina propria which consisted of a capillary network with cellular layers in between a granular layer of cells which lay behind the lamina propria, this was termed the stratum granulosum, and ran the length of the intestine. A layer of loose connective tissue was seen behind the stratum granulosum and this separated the muscular layers of the intestine.

Fig. 2

Anterior intestine. Elongated mucosal folds.

CE - Columnar epithelium. LP - Lamina propria.

SG - Stratum granulosum. CM - Circular muscle.

LM - Longitudinal muscle. S - Serosa.

H & E. X 160.

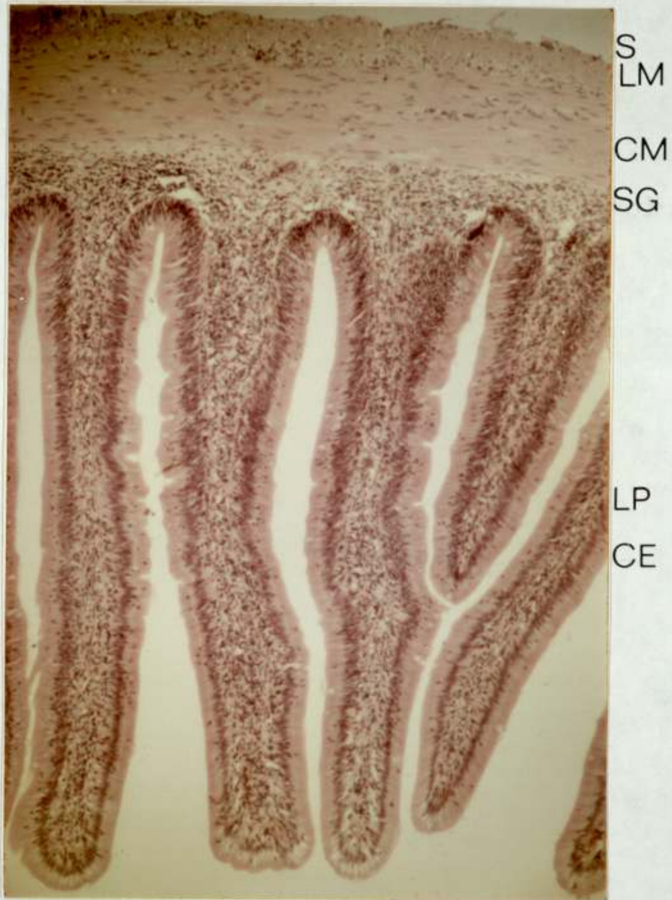


Fig. 2

Fig. 3 Medial intestine. H & E. X 80.

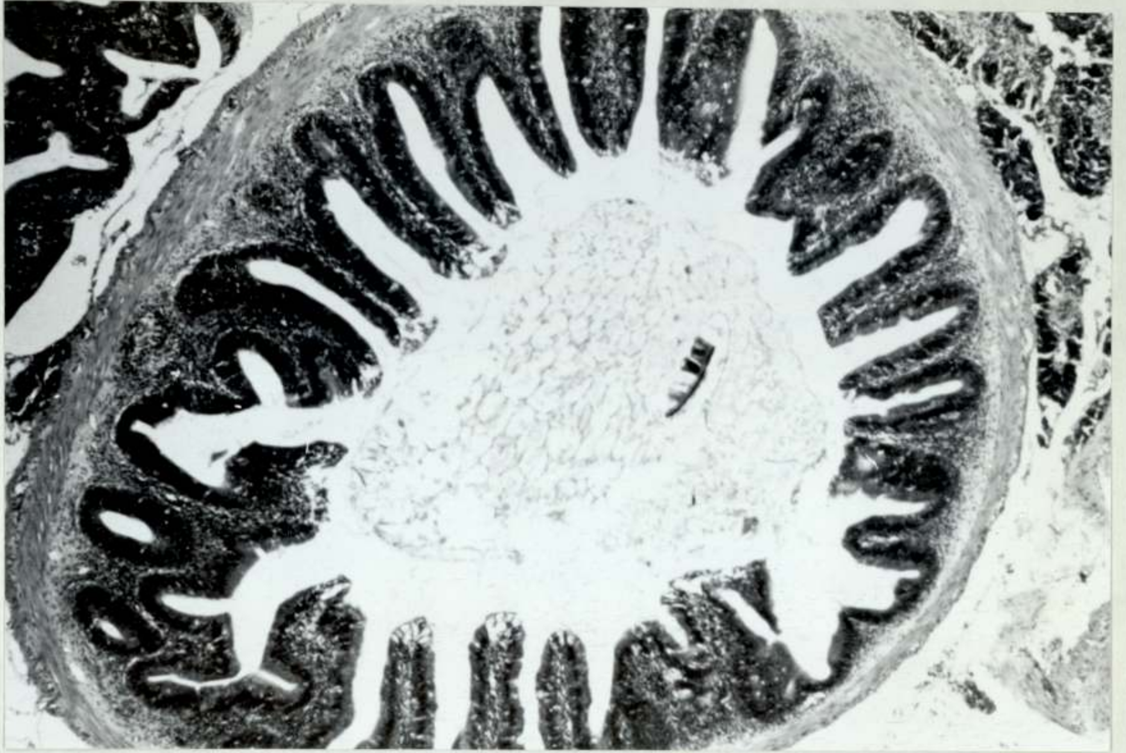


Fig. 3

Fig. 4 Posterior intestine. H & E. X 80.

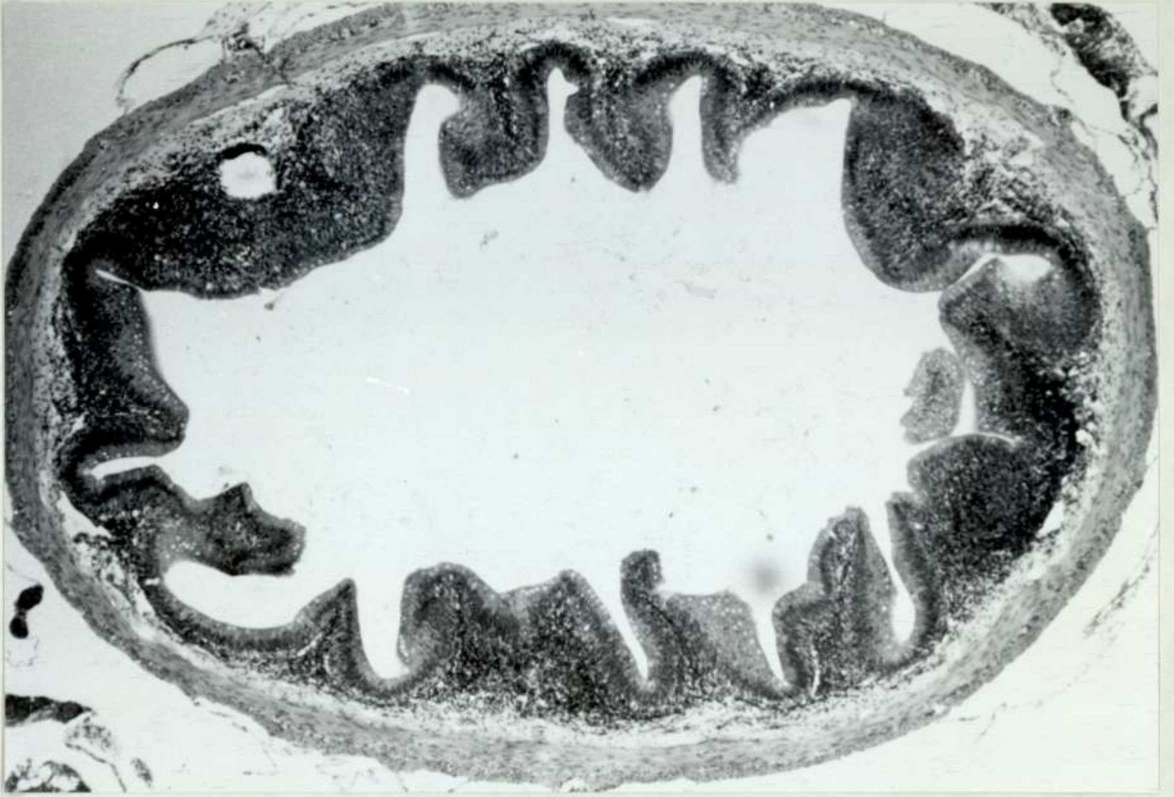


Fig. 4

The muscular layers consisted of an inner circular band, and separated by a thin strip of connective tissue was a thinner outer longitudinal band. This outer band appeared more cellular than the inner band of muscle.

The intestinal serosa consisted of a layer of connective tissue, sometimes detached by artefact from the outer muscular band. (Fig. 2) Adipose tissue, blood vessels, and mesenteric adhesions of the pancreas and hepatopancreas were attached to the serosal epithelium.

The columnar epithelial absorptive cells were the main cell type of the mucosal epithelium, and their function in fish was digestive activity, as described by Al-Hussaini (1949a). These cells had elongated nuclei situated in the lower third of the cells. The cytoplasm was pale eosinophilic, becoming darker towards the outer edge of the cell. (Fig 5) In some areas, notably the mid-intestine, microvilli could be seen on these cells. The cytoplasm contained globular material which was only sightly eosinophilic. The absorptive cells reacted for neutral mucopoly-saccharide with periodic-acid Schiff, and remained positive after diastase digestion. These cells were also positive for alkaline phosphatase activity, and this activity was more pronounced in the crypts than at the tips of the rugae. There was a positive reaction for leucine aminopeptidase activity on the outer mucosal surface. This was also more marked in the crypts of the rugae. The reaction was stronger in the anterior intestine than the lower part. (Figs.6-7) The goblet or mucous secreting cells were often unstained with haematoxylin and eosin, but occasionally slightly eosinophilic, these cells were interspersed randomly between the columnar cells, and they were more prominent in the crypts of the rugae, and more abundant in the posterior intestine than the anterior. Occasionally goblet cells could be seen to exude mucous into the lumen of the intestine.

Fig. 5 Anterior intestine. EC - Columnar epithelial cells. GC - Goblet cell. MC - Mononuclear cell. H & E. X 250.

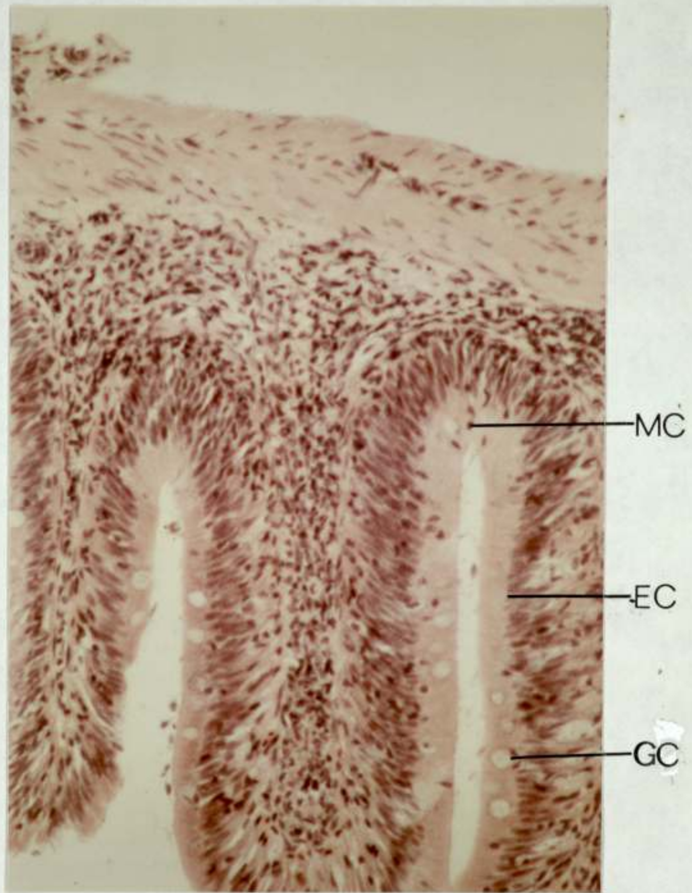


Fig. 5

Fig. 6 Anterior intestine. Alkaline phosphatase. Positive reaction along edge of mucosal epithelium and between muscle layers. X 160.

Fig. 7 Anterior intestine. Leucine aminopeptidase. Reaction limited to edge of mucosal epithelium only. X 160.

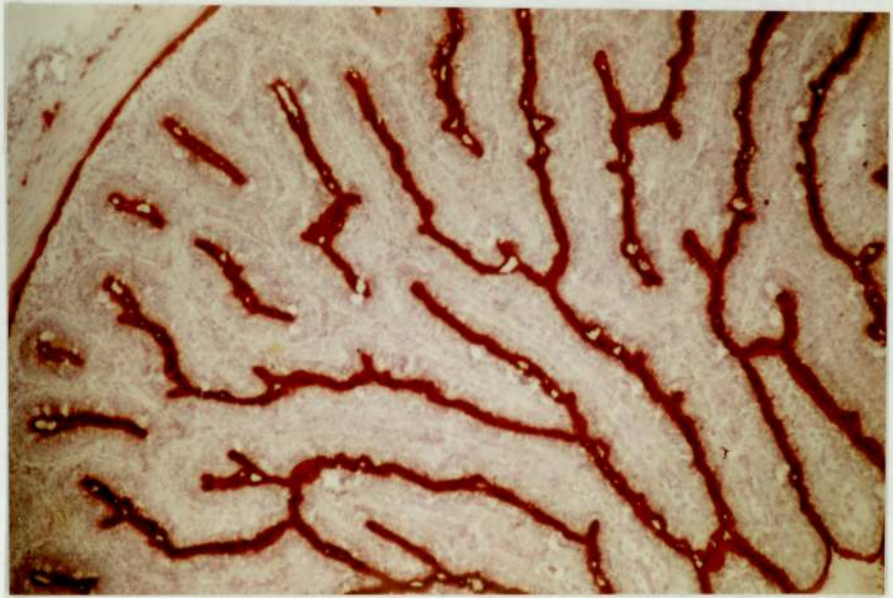


Fig. 6

Fig. 7

These cells stained positive for acid mucopolysaccharide, although cells at the base of the folds showed a mixed acid and neutral mucopolysaccharide reaction. (Fig. 8)

The mononuclear cells of the intestinal mucosa appeared to migrate from the stratum granulosum, through the lamina propria to reach the outer edge of the columnar cells and were shed into the intestinal lumen. These cells had very little cytoplasm and had the characteristics of lymphocytes. A few mononuclear cells with granular cytoplasm were also evident, some of these contained Schiff positive granules. (Fig. 9)

There were also macrophage cells present, and these were often congested with reddish brown pigment, which was Schiff positive, and gave most of the histochemical reactions for a lipo-pigment. (Fig.9) Occasional macrophage cells which contained cell debris were evident.

The "rodlet" cell was occasionally seen in the intestinal mucosa. (These cells are well described in the literature, see introductory review), and were typical, pear shaped, with a nucleus at the base. The "rodlet" cells were interspersed between the epithelial cells. The cytoplasm was slightly eosinophilic and sometimes distinct elongated rodlets with small nodules at the base were evident. These rodlets were positive for Schiff reaction and Ziehl-Neelson stain. (Fig. 10)

The lamina propria consisted of a thin acidophilic layer of connective tissue and stained positively for collagen with van Gieson and the Schiff reaction. This collagen layer extended the length of the mucosal folds. The capillary network followed the folds of the intestinal rugae. The cellular layer was composed of mononuclear cells, which were predominantly lymphocytes, plus granular cells. There were also strands of firbrous tissue, and occasional macrophage cells present in this area.

Fig. 8 Posterior intestine. Increased number of goblet cells in the crypts of mucosal folds. X 250.

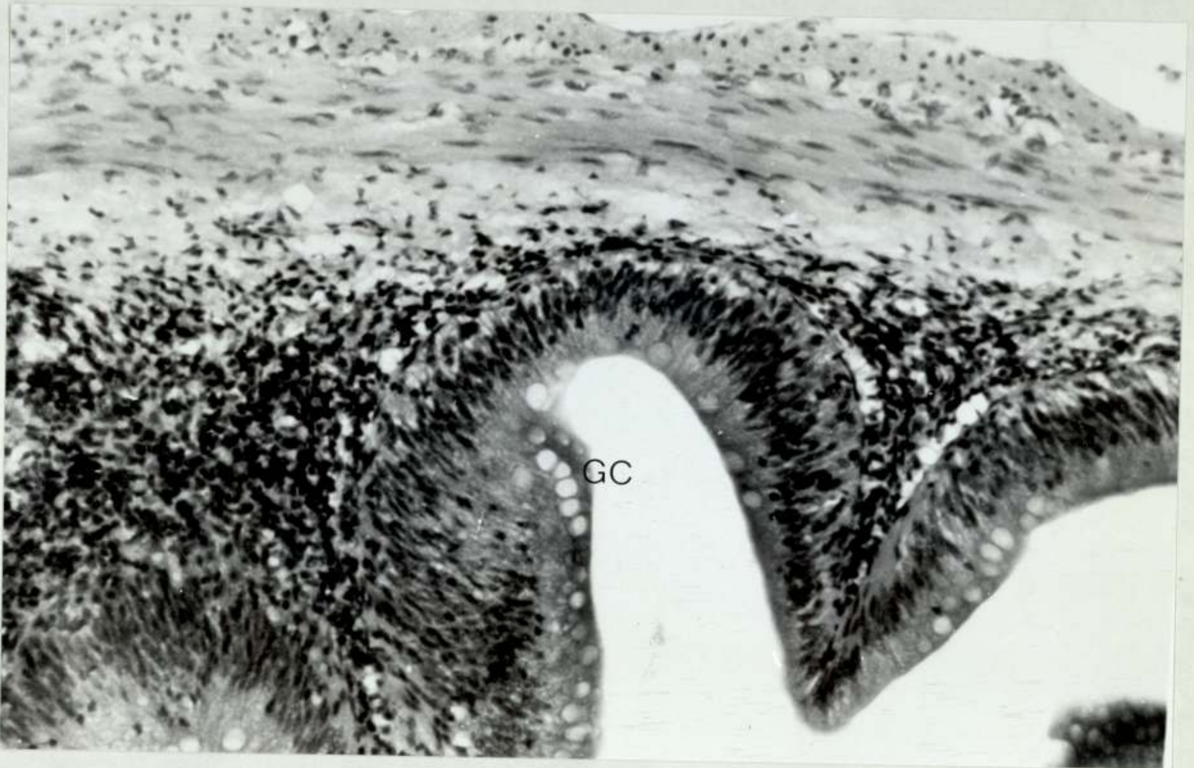


Fig.8

Fig. 9 Anterior intestinal mucosa. GrC - granular cells. M - Macrophage cell. Note goblet cells. Alcian blue/PAS. X 500

Fig 10. Anterior intestinal mucosa. R - 'Rodlet' cell. Note positive goblet cells and mononuclear cells. PAS. X 800.

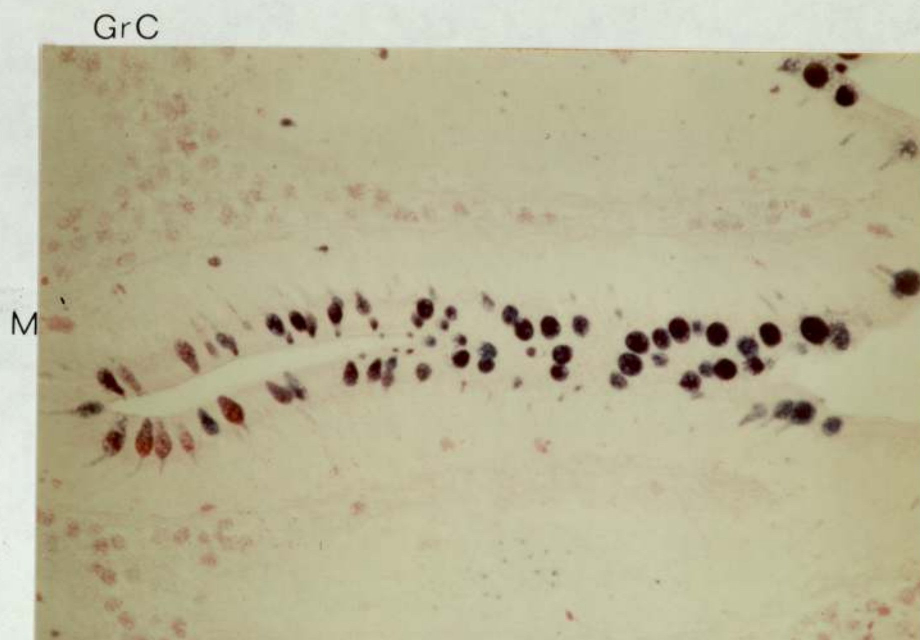


Fig.9

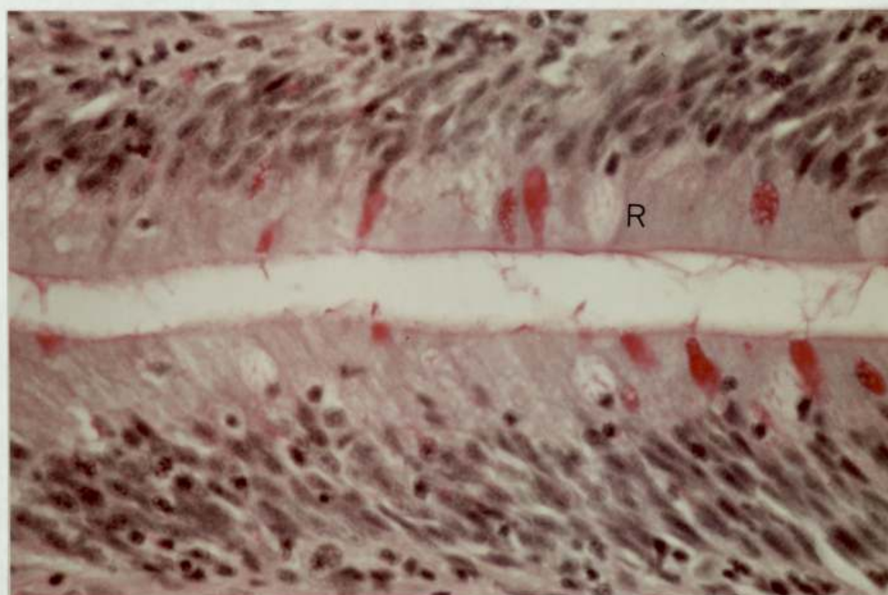


Fig.10

The stratum granulosum consisted of mononuclear cells, some of which contained acidophilic granules which reacted positively to the Schiff reaction. The other mononuclear cell types were not identified. Strands of loose connective tissue were also evident in this area, these stained positively for collagen with the van Gieson stain, it was also slightly Schiff positive. Blood vessels were evident in this region in abundance.

The muscular layers, both consisted of smooth unstriated muscle, the inner circular band showed occasional mononuclear cells with distinct Schiff positive granules. The outer longitudinal muscle band contained smooth muscle fibres, and in the posterior intestine particularly, an abundance of mononuclear cells containing Schiff positive granules were evident. A distinct band of alkaline phosphatase activity was evident on the inner edge of the circular muscle band, along the entire length of the intestine.

Pancreatic ducts could be seen to enter the longitudinal muscle and their pathway could be followed by a series of small Schiff positive granules, into the stratum granulosum. These granules consisted of what was probably proteinaceous material. Also vascular elements and nerve networks were seen in the section, along with ganglion cells.

The serosa consisted of a single layer of squamous epithelial cells, sometimes surrounding small blood vessels and connective tissue-adhesions of the pancreas and hepatopancreas. Mononuclear cells were associated with serosal tissue, and appeared to be loose in the peritoneum and mesenteric tissue.

4.1.2. <u>The pancreas</u>

The endocrine pancreatic tissue were situated in the free pancreatic ancinar tissue in the peritoneum. This organ was

disseminated and connected to the pancreatic tissue within the liver. Pancreatic ducts entered the intestine in the medial portion. The pancreas consisted of double cords of acina cells; which were typical of higher vertebrates, the inner edge of the acina cells were acidophilic, containing large eosinophilic granules, the outer edge was basophilic, and contained darkly stained nuclei. The islets of Langerhans were situated in the 'loose' pancreatic tissue and were distinct, often rounded, with pale eosinophilic cells. Amongst the exocrine acina cords there were blood vessels, pancreatic ducts, loose connective tissue and associated mononuclear cells, some of these had distinct Schiff positive granules. Occasional "rodlet" cells were evident in the lumen of the vascular ducts, and macrophage cells containing light brown pigment were seen in some areas of pancreatic tissue.

4.1.3. The hetopancreas (Fig. 11)

Some teleosts have a liver which contains exocrine acina cells and is termed the hepatopancreas. Many of the cyprinids have this type of organ and the carp is no exception.

The general picture of a section of hepatopancreas revealed a lobular pattern of hepatocytes, each lobule consisted of cords with double rows of cells with sinusoids and biliary canaliculi between. The pancreatic acina cells were also in double rows, and appeared randomly throughtout the hepatic parenchyma, although they appeared to be associated with the vascular system.

The hepatocytes had a centrally placed nucleus, and vacuolated, slightly eosinophilic cytoplasm. A positive reaction for glycogen was demonstrated with the Schiff reaction (which was found negative after diastase digestion). The cell walls were very distinct and stained very lightly for connective tissue. The sinusoids contained,

Fig. 11 Hepatopancreas. H - hepatocytes. P - exocrine pancreas. Note small number of mononuclear cells associated with the pancreas. H & E. X 180.

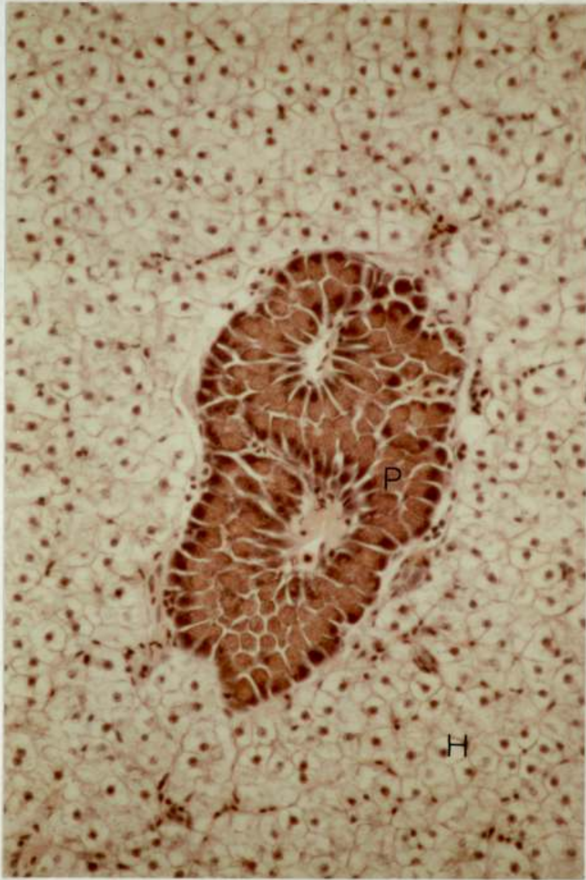


Fig.11

nucleated erythrocytes, and were lined with basophilic cells some of which had the characteristics of Kupffer cells. These Kupffer cells often contained yellow-brown pigment which were often positive for Perls' reaction, therefore suggestive of haemosiderin. Mononuclear cells were evident in the tissue adjacent to large vessels. Some of these cells had eosinophilic cytoplasm, which was also Schiff positive, others contained brown amorphous material, which was also positive for Schiff, and Schmorls' reaction but negative for the Fouchet and Perls' reactions. This material was most likely a lipo-pigment, and the cells were identified as macrophage cells. There were also small spherical foci of macrophage cells, containing brown pigment. In the large blood vessels, various types of mononuclear cells were evident, besides red blood cells.

The canaliculi gave a positive reaction for alkaline phosphatase activity, but no reaction for leucine aminopeptidase was evident in the liver.

The double cords of pancreatic acina cells were present along the main blood vessels. These surrounded by connective tissue fibres, and associated mononuclear cells. Some of these cells contained eosinophilic material, and others brown pigmented material. The acina cells were similar to the acina cells of the pancreatic tissue outside the liver, and had an exocrine function. Occasional foci of necrotic hepatocytes were evident, and sometimes granulomatous tissue, surrounded by fibrous tissue, these were probably sites of old parasitic infections.

4.2. Discussion

The carp used in the experiments were not specific pathogen free animals as would be used in mammalian experiments, but were derived from wild-stock. It was inevitable, therefore, that some

minor pathological changes would be found. For this reason, the histology of a sample of carp before testing was described and discussed.

The general picture of the histological structure of the intestine was typical of carp according to observations by Pictet (1909), Curry (1939) and Al-Hussaini (1949a), and the histology of the heptopancreas resembled the descriptions given by Keil (1917) and Amlacher (1954).

4.2.1. The intestine

The main cells types were columnar epithelial or absorptive cells and goblet or mucous secreting cells. The distribution of these cells in this batch of carp was similar to the distribution described by Al-Hussaini (1949a). Mononuclear cells which were either lymphocytes, granular cells, macrophage cells or 'rodlet' cells, were also described by Al-Hussaini (1949a).

The columnar epithelial cells have an absorptive function (Al-Hussaini, 1949b) and these cells occurring on the anterior intestine, absorb lipids, whereas epithelial cells of the medial to posterior intestine have a pinocytic function and absorb protein (Gauthier and Landis, 1971). Several workers (Chaicharn and Bullock, 1967; Weinreb and Bilsted, 1951; Bullock 1963; Srivastava, 1966) have demonstrated alkaline phosphatase activity in these cells and all agreed that the activity was present on the outer surface of the cells as was described in these carp. However, Chaircharn and Bullock (1967) who studied a Catastomid fish recorded more alkaline phosphatase activity at the tips of the intestinal folds and less in the crypts of the folds, a reverse of this was evident in the carp. Also Bullock (1967) recorded less alkaline phosphatase activity in the posterior intestine of *Gambusia affinis*. The distribution of

alkaline phosphatase in these control carp was roughly similar throughout all levels of intestine.

The epithelial cells were positive for neutral mucopolysaccharides, and according to Moog and Wenger (1952) alkaline phosphatase and neutral mucopolysaccharides (NPMS) occurred in similar sites in vertebrates. These workers considered the function of alkaline phosphatase was for transporting organic molecules. Western (1971) demonstrated alkaline phosphatase in the microvilli of the epithelial cells, and considered the enzyme to work both outside and inside the cell. In the fowl, alkaline phosphatase activity was seen as an even distribution along the brush borders of the crypts and tips of the mucosal epithelial cells. (Michael and Hodges, 1974). Therefore, distribution of this enzyme appeared to be similar in both carp and fowl.

Leucine aminopeptidase activity has not been demonstrated histochemically by many workers, although Western (1971) recorded this enzyme in the intestine of four fish species, Cottus gobio, S. trutta, Enophrys bubalus and Noemacheilus barbatulus. In all these control carp, the enzyme was present along the outer surface of the columnar epithelial cells, and Western's findings agreed with these results, and he also recorded a stronger reaction in the anterior intestine than in the posterior intestine of the fishes he examined. Leucine aminopeptidase is a proteolytic enzyme and plays a part in absorption, by acting on peptides and hyrdrolysing free amino-groups.

The mucous secreting goblet cells of the carp have been described by Pictet (1909), Curry (1939) and Al-Hussaini (1949a) and their findings agreed with the mucous cell distribution in this control sample of carp. There were predominantly more cells

in the crypts than the tips of the folds of the mucosa. Al-Hussaini suggested the function of the increased amount of mucous cells in the posterior intestine was to aid defaecation. Chaicharn and Bullock, (1967) considered the goblet cell was a unicellular gland which secreted mucus and formed mucus plus water. Weinreb and Bilsted (1955) proved that alkaline phosphatase activity was not present in the goblet cells of S. gairdneri, but the cells were positive for tests for acid mucopolysaccharide (AMPS). Bucke (1971) demonstrated that the mucus content of the goblet cells of Esox lucius intestine was predominantly acid mucopolysaccharide (AMPS) but there were mixtures of both acid and neutral mucopolysaccharides (NPMS) and a few goblet cells contained NPMS only. In this examination NPMS was only seen in a few mucous cells which lay close to the basement membrane of the epithelial mucosa. These mucous cells were much smaller than the goblet cells which secreted into the gut lumen, and were interpreted as immature goblet cells. This agreed with Vickers (1962) hypothesis that goblet cells were only capable of one cycle of synthesis, and signs of mucigenesis were restricted to the inner parts of the rugae.

The mononuclear cells consisted of lymphocytes, granular cells, macrophages and 'rodlet' cells. The lymphocytes which were evident in the intestinal epithelial mucosa of carp interspaced between the columnar epithelial cells, appeared to have migrated from the stratum granulosum to the lamina propria and through the basement membrane. These cells have been recognised by Hale (1964) and Al-Hussaini (1949a) although Curry (1939) did not mention them in her examaination of carp intestine. There is very little known about the function of lymphocytes in fish. Klontz (1972) suggested that they have phagocytic properties whereas Ellis (1976) considered that fish lymphocytes originated from two lymphopoietic organs,

which are the thymus and kidney and have similar properties to mammalian lymphocytes i.e. immunocompetant cells and therefore, could not have phagocytic properties. In these fish lymphocytes were evident in the intestinal lumen, and it was considered that they have responded to some stress response such as a parasitic infestation.

Al-Hussaini (1949a) was one of the first workers to describe these granular cells in the carp intestine, and he considered they migrated through the mucosal layers to reach the epithelium. Vickers (1962) described the granules to react weakly for the PAS demonstration and be acidophilic, and from this he deduced these granules contained zymogen and perhaps replaced the paneth cells found in higher vertebrates. These cells were certainly present in the intestine of the pike (Bucke 1971) although the granules were not so readily demonstrated. Weinreb and Bilsted (1955) called them "wandering cells" and they did not have the histochemical reactions for mast cells which had been previously described by Bolton (1936). Bullock (1967) found the granular cells more numerous in the posterior intestine of G. affinis and described the granules to be basophilic in the stratum granulosum, where these cells appeared to accumulate, and acidophilic in the epithelial mucosa. The granular cells in the carp intestine were acidophilic in all layers of the intestinal mucosa. Burnstock (1959a) suggested that granular cells of fish intestine were associated with collagen. However, the formation of collagen from these cells did not seem feasible, because it was possible to find them in all levels of intestinal mucosa, even in areas where collagen was not present, it could have been that Burnstock was working with S. trutta and not C. carpio, and this was the reason for the different results.

Lord Percy (1970) described 'wandering cells' in the epidermis of C. carassius, but these cells were derived from haemoblasts and were phagocytic and not secretory and did not have the same staining reaction as the granular cells of the carp intestine. The 'wandering cells' described in the mammalian intestines by Maximow and Bloom (1954) did not have the same characteristics as those of the carp. It is considered that these cells secrete zymogen, and this would agree with a report by Murayama and Takeuchi (1973) where it was said that zymogen was increased in feeding fish, a point which will be discussed later. From the paucity of published data, the macrophage cells have been neglected, in relation to investigations on intestines in fish. In the carp, and other cyprinids particularly R. rutilus, Leuciscus leuciscus and Abramis brama (unpublished observations) these cells were present in the epithelial mucosa and submucosa of the intestines (and in other tissues) and were often engorged with yellow to light brown pigmented material, and in some cases contained what appeared to be cell debris. The pigmented material, which was acidophilic, PAS positive, Schmorl positive, and removed after prolonged bleaching in hydrogen peroxide, yet Perls' negative, was considered to be a 'lipo-pigment'. The term 'lipo-pigment' is used because in fish there seems to be some controversy on the exact definition of the pigment, Wood and Yasutake (1955) considered it to be 'ceroid' comparable to 'ceroid' found in mammals. Roberts (1975) considered the pigment to be a visceral melanin and be part of the liponeuromelanins, as described by Edelstein (1971), and found in melano-macrophage cells. This could well be true, as Roberts (1975) described these cells as migratory having a function of oxidising lipid residues to lipofuscin, a type of wear and tear pigment. It was considered that macrophage cells which contained the dead cellular material, were acting as phogocytes, clearing up waste material. It was probable, that these

cells migrated to the liver, where accumulations of them occurred. Jacowska (1956) called the macrophage a circulating phagocyte of fish, and Ellis (1976) designated the criterium of a macrophage cell as avid phagocytosis, and proved this by observing carbon uptake by these cells in P. platessa. Phagocyte cells will be discussed further when the experimental data is examined.

Ever since Thélohan (1892) described the 'rodlet' cell as an unicellular parasite, there has been some controversy over it's identity. The cell was usually 'pear' shaped and contained a basal nucleus. In some forms, vertical rods extended from the base, and on each rod there was a small nodule which was weakly PAS positive. (Bullock (1963) also demonstrated these cells with Fast green). In carp, these cells were seen in the mucosal epithelium of the intestine, lining blood vessel walls in the pancreas and kidney, and the bulbus arteriosus. This cell is evident in most of the British freshwater cyprinids, and most abundant in R. rutilus (Bucke, unpublished observations). Ultrastructural examination by Leino (1974) has indicated this cell to have a secretory function, developed from an undifferentiated cell, finally releasing its contents, which was possibly for either water and electrolyte balance, pH controller lubrication. Anderson et al (1975) also made an ultrastructural examination of this cell in turbot, and considered it to be a protozoal parasite. Al-Hussaini (1959) described mitotic figures in this cell and considered that it was a type of goblet cell which had derived from either a wandering blood or connective tissue cell. It would seem that Al-Hussaini was agreeing with Catton (1951), although this author suggested the 'rodlet' cell to discharge coarse granulocytes at the surface of the lumen. Vickers (1962) working on C. auratus, described an increase in 'rodlet' cells after stimulation with metals in the intestine. It would appear from the available literature

that there is no definite role of the 'rodlet' cell in the teleost fish, and its presence may be likened to a foreign body which does no actual harm. This explanation was given by Flood et al (1975) when this unusual cell was examined by ultrastructural examination. These authors used Phehn's (1906a) term "Stabchendrusenzellen" and described the cell to be associated with epithelial tissue of the bulbus arteriosus of C. Auratus.

The term lamina propria was given to the layer of connective tissue fibres and capillaries which lie beneath the mucosal epithelium, this area also contained lymphocytes and granular cells. It appeared this area of intestine in the carp was comparable to that of other cyprinid fish. Chaicharn and Bullock (1967) confirmed that connective tissue was present, and they also described wondering leucocytes, granular cells and other mononuclear cells, plus a few macrophages in Catostomas commersoni. These authors also demonstrated a positive reaction foralkaline phosphatase in that layer of intestinal mucosa, this was interesting, because there was no reaction recorded in this region in preparations of carp intestine, from the current experiments, and no other workers had recorded the presence of this enzyme in this region.

The stratum granulosum was the section of intestine adjacent to the lamina propria, and was very cellular, the main cell types were granular cells interspaced with loose strands of connective tissue. McVay and Kaan (1940) described areolar and mast cells in this area, yet mast cells were not described by Al-Hussaini (1949a), only granular cells. This author considered granular cells migrated to other areas of the intestinal mucosa, also Vickers (1962) described migration of granular cells, but did not consider these cells to have the staining properties of mast cells. Mast cells do occasionally occur in the intestine of carp and have been described

in a previous study (Bucke, 1972).

There was no stratum compactum distinguishable in samples from this current experiment, although, this tissue had been demonstrated by Chaircharn and Bullock (1967) in certain cyprinids. Because of this, it was considered that the stratum compactum, which was dense collagen tissue, does not form until carp reached a certain size, and as these fish were only 1-2 years old, the structure had not formed. Burnstock (1959a) recorded the presence of a stratum compactum in salmonids, but not in pike, yet in a previous study (Bucke, 1971) this structure was easily distinguishable, which again appeared to follow the assumption that it was not present in younger fish species.

The histology of the muscle layers (muscularis mucosa) appeared to be similar to previous descriptions, basically consisting of an inner band of circular skeletal muscle, surrounded by an outer longitudinal band of skeletal muscle. Bullock (1967) described the presence of a band of alkaline phosphatase activity along the inner edge of the circular muscle band in G. affinis, a similar band of alkaline phosphatase was demonstrated in the carp in this experiment and has also been described in other fish by Chaircharn and Bullock (1967) but not in avians or mammals according to Michal and Hodges (1974). The presence of granular cells in the muscularis mucosa particularly in definite pathways, indicated that they were in the process of migration, probably to and from the mucosal epitheliem, through the pancreas. The granules of these cells, even after diastase digestion, were demonstrated with the PAS reaction, which excluded the possibility that they were glycogen globules. Al-Hussaini (1949a) considered the granular cells to contain zymogen, which would seem a feasible assumption.

The ganglion cells seen in the muscularis mucosa were part of

the plexus of Auerbach, described in the brown trout by Burnstock (1959b).

The description of the serosa section of the intestine corresponded to descriptions by other workers, noteably Hale (1965). However, previous workers have not mentioned the presence of mononuclear cells associated with the serosal epithelium. It was feasible that the mononuclear cells were present in response to an inflammatory reaction caused by some infectious agent, and were plasma cells, lymphocytes or macrophages which occur at sides of trauma and infection (Mawdesley-Thomas and Bucke, 1973).

4.2.2. <u>The pancreas and hepatopancreas.</u>

The term hepatopancreas was first given by Weber (1827) and has been also used by Keil (1917) Amlacher (1954) and Onishi et al (1973). The liver part of this organ in teleosts was very well described by Amlacher, as four separate lobes with occasionally many smaller lobes, all originating from a "liver centre". The folds of the intestine intertwined amongst these lobes, and other organs including the pancreas and spleen. The branches of the portal vein orginated from a plexas of vessels between the circular and longitudinal muscle layers of the intestine. These branches ran into the liver and were surrounded by pancreatic acina cells. The carp liver parenchyma consisted of two cords of cells arranged radially around hepatic veins, forming a lobular structure which were similar to mammalian liver. Smallwood and Derickson (1933) described the carp liver to consist of a group of hepatic lobules each containing a mass of cells interspaced with sinusoids lined with endothelium, the liver cells surrounding the sinusoids were polyhedral in shape and contained spherical nuclei with a reticular cytoplasm. Pancreatic alveoli were present in the liver and seen as columnar or polyhedral cells. Elias and Bengelsdorf (1952)

described the liver of the carp as being the extreme of the tubulosinusoidal type, with narrow sinusoids, making the liver look like a continuous mass of cells instead of being perforated by cylindrical lacunae, containing the sinusoids.

The pancreas in C. carpio was termed disseminated by Andrew (1959), whereas, the pancreas of E. lucius was termed compact by Bucke (1971) in a previous study, and the pancreas of Gadus was termed diffuse by Bishop and Odense (1966). The disseminated pancreas represented a fairly large mass of glandular tissue, widely scattered throughout the mesentery and was intersperced between the diffuse liver and spleen. Where the pancreas was found within the liver, it was termed hepatopancreas, and in a detailed description, Keil (1917) considered the acina cells were sometimes found as individual tubules or in compact masses. In the liver, the pancreatic cells did not have actual contact with hepatic tissue but were separated by two endothelial membranes. The tubular acina cells were branched but there was no anastomosis. These cells were polyhedral in shape and had coarse zymogen granules, in various stages of secretion, in the apical pole, and a nucleus at the basal end. Keil also described the many capillaries associated with the pancreas, and he even mentioned large vesicular cells which stained in various depths of colour.

Basically the histology of the pancreas in the current study was similar to these previous descriptions, but some additionaly findings have been discussed. These included the presence of a minimal number of "rodlet " cells present in the lumen of the vascular system of the pancreatic tissue, also considerable numbers of macrophage and mononuclear cells were seen in the interstitial spaces of the acinar walls. The "rodlet" cells appeared in the major blood vessels of cyprinid and other teleost fish, therefore

it was not surprising to find them in the portal vessels which run through the pancreatic tissue.

The mononuclear cells were mixed, some were similar to the 'wandering' granular cells of the intestine, others were leucocytes. Their presence was considered to be normal and Chaicharn and Bullock (1967) briefly described similar cells in the mesentery of catastomid fish. The macrophage cells, were seen singly or in aggregates, and these appeared to resemble the melano-macrophage cells described by Roberts (1975). Although he did not specifically mention these cells in the pancreas, he did, however, say that they were found in the haematopoietic and some other soft tissues of teleost fish. It was possible that these macrophage cells were present in response to a parasitic infestation, again Roberts (1975) noted an increase in these cells when there was such an infection.

Ashley (1975) commented that the liver of fish was less well organised than it was in higher vertebrates, and this certainly was the case in Cyprinid liver in the described experiment. He also stated that the hepatocytes revealed pale staining, and were vacuolated in and H & E stain, the vacuoles indicating the presence of glycogen. This was evident in the carp liver, stained with H & E, the cytoplasm was pale and glycogen was demonstrated in the hepatocytes with the PAS. However, in the carp liver there appeared to be an abundance of Kupffer cells and other macrophage cells, containing brown pigmented material, (lipo-pigment). The significance of numbers of these cells is uncertain. When they occurred in round clusters, they resembled the melano-macrophage cells described by Roberts (1975). Cahn (1975) described both Kupffer cells and macrophage cells to be present in the liver of the Atlantic Menhaden. In a previous study on the liver of pike (Bucke, 1971) Kupffer cells were seen, but no aggregations of macrophage cells. Hale (1964)

found Kupffer cells only rarely in Gasterosteus sp., yet Weinreb and Bilsted (1955) saw them frequently in S. gairdneri. Hinton and Pool (1976) made an ultrastructural examination of the cells of catfish liver and pancreas. In their study they described the fine structure of the Kupffer cells and confirmed that they were phagocytic. These authors also described hepatic macrophages, with eccentric nuclei displaced by phagocytised material. Simon et al (1967) described the normal liver of rainbow trout and stated that melanin was interspersed among or within lymphocytes. This seemed rather doubtful as lymphocytes do not have the ability for phagocytosis according to Ellis (1975).

From this varied research, it would appear that the presence of Kupffer cells and macrophage cells vary from one fish species to another, but appeared to have the same phagocytic function as Kupffer cells in mammalian liver described by Maximow and Bloom (1954).

The presence of alkaline phosphatase in the bile canaliculi of the carp liver, corresponded to a description of the distribution of this enzyme by Hinton et al (1972) who studied this enzyme in the bass M. Salmoides. As this enzyme was said to play an active part in dephosphorylation and absorption of glucose (Srivastava, 1966) and the active transport of metabolites (Western, 1971), it was no surprise to find the enzyme in these sites. No author appeared to have recorded histochemical reactions for leucine aminopeptidase in teleost livers.

The double cords of pancreatic acina cells surrounded by connective tissue and associated mononuclear cells in the hepatopancreas appeared to by typical for a teleost. It has been stated by Amlacher (1954) that the exocrine pancreatic cells followed the tract of the portal system throughout the liver, therefore it was feasible that

associated mononuclear cells were possibly lymphocytes and macrophage cells, containing lipo-pigment, and proteinanceous material, plus other leucocytes. The occasional faci of necrotic hepatocytes and granulomatous tissue were almost certainly evidence of tissue response to parasitic infestation.

5. RESULTS.

5. RESULTS.

1.5.1. Clinical and macroscopical results

The carp were healthy at the commencement of each experiment, and neither mortalities or distress occurred throughout the trials. The starved fish held at 27°C appeared emaciated, but otherwise were lively when disturbed. The carp used for the final experiment (Protocol 5) were considerably smaller than those in the previous experiments.

There were no significant clinical findings, although the starved fish at the higher temperature appeared to have thinner, and redder intestines and the hepatopancreases were paler in colour.

TABLE 2

The following fish were weighed before post-mortem examination.

Protocol 3 17°C		Protocol 4 27°C	
Group A	High Protein Diet	Group A	High Protein Diet
	Wt. in grams		Wt. in grams
Sample 1	74	Sample 1	121
2	57	2	89
3	99	3	73
4	57	4	69
5	102	5	75
6	120	6	59
7	60	7	80
8	62	8	60
Group B	Starved 17°C	Group B	Starved 27°C
Sample 1	88	1	54
2	48	2	66
3	60	3	93
4	46	4	67
5	60	5	94
6	65	6	52
7	38	7	54
8	54	8	64

5.2. Histopathology

5.2.1. Protocols 2-4. Histological details

Protocol 2:

Carp fed High and Low Protein diets at 17°C. Six specimens from each group were examined.

Group A. High Protein Diet.

The histological detail of the intestines and hepatopancreas was similar to that described for the control carp. Only slight changes were seen and these were associated with autolysis of the intestinal mucosa in one specimen. Glycogen was present in moderate amounts in the hepatocytes of the liver, sometimes its distribution was present in variable amounts in individual fish.

Group B. Low Protein Diet.

The histological details of the intestines and hepatopancreas were similar to that described for the control carp. Small changes seen in one specimen were occasional focal areas of degenerating hepatocytes, with associated mononuclear cells, some contained reddish/brown pigmented material, also an increase in the mononuclear cells of the muscular layers of the submucosa of the intestine was noted. Glycogen was present in minimal to moderate amounts in the hepatocytes of the liver, and its distribution was often variable in individual fish.

Protocol 3:

Carp fed High Protein diet and starved at 17°C. Eight specimens from each group were examined.

Group A. High Protein Diet

Hepatopancreas

The hepatic architecture was well defined in this group.

All specimens showed an increase in mononuclear cells, many of these contained reddish/brown pigmented material which had the characteristics of a lipo-pigment. The mononuclear cells were occasionally seen in focal areas or were associated with vascular elements and exocrine pancreatic tissue. In three specimens, granulomatous lesions were seen, these areas were surrounded by fibrous tissue, also unidentified protozoal parasites were occasionally encountered in the larger vessels. Glycogen was demonstrated in moderate amounts in the hepatocytes, the distribution was often variable between each individual fish.

Intestine

The histological detail of the intestines from this group of specimens was very similar to that described for the control carp. Small changes seen, were a few columnar cells containing eosinophils, PAS positive granules, and a minimal increase in numbers of mononuclear cells in the mucosa of all specimens. Some of these cell types contained reddish/brown pigmented material which had the characteristics of a lipo-pigment. Occasional rodlet cells were evident in the mucosal epithelial layers.

Group B. Starved Carp

Hepatopancreas (Fig. 12)

The hepatic architecture was well defined in this group. Mononuclear cells were seen in moderate numbers, some of which contained reddish/brown pigmented material, which had the characteristics of a lipo-pigment. Other mononuclear cells were seen to be associated with vascular elements and exocrine pancreatic tissue. Occasional focal areas of degenerating hepatocytes and granulomatous lesions were seen, also a few unidentified protozoal parasites were evident in the larger vessels. Apart from one specimen, where glycogen was minimal, moderate amounts of

glycogen were demonstrated, although the distribution varied in individual fish.

Intestine (Fig. 15)

The general histological detail showed no marked changes, however, minimal changes were recorded which were an increase in numbers of mononuclear cells in the mucosa and submucosa, some of these cell types were lymphocytes, and others were macrophage cells, containing PAS positive granules or reddish/brown pigmented material. The mucous secreting cells showed an increase, particularly in the posterior intestinal mucosa. Occasional 'rodlet' cells were seen in the mucosal epithelium.

Pancreas

There was an increase in fibrous connective tissue associated with granulomas and localised degeneration evident in one specimen.

Protocol 4

Carp fed High Protein Diet and starved at 27°C. The heptatopancreas and intestines of eight specimens from each group were examined.

Group A. High Protein Diet

Hepatopancreas (Figs. 13 and 16)

The hepatic architecture was well defined in this group of fish. The hepatocytes were eosinophilic and in one specimen, clear spherical vacuoles were present, this specimen showed a positive reaction for fat. In another specimen, the cytoplasm was 'foamy' and the cell membranes indistinct. Mononuclear cells were evident in moderate numbers, some of these cells contained reddish/brown pigmented material which had the characteristics

of a lipo-pigment, these cells were seen both in small focal areas, or associated with vascular elements and exocrine pancreatic tissue. Occasional granulomatous lesions were seen in the parenchyma and small numbers of unidentified protozoal parasites were evident in a few of the larger vessels. Glycogen was intensely stained in all, except one specimen, and that was the one with the 'foamy' hepatocyte cytoplasm.

Intestine (Fig. 18)

Histological changes in the group were a marked increase in the number of mononuclear cells in the mucosa and submucosa, and the distinct layers of PAS positive granular material demonstrated on the outer edge of the columnar epithelial cells of the mucosa, particularly in the posterior intestine.

The mononuclear cells were mostly lymphocytes, but many macrophage cells were seen in all levels of intestine. These cells contained large quantities of PAS positive material, which had the characteristics of a lipo-pigment, occasionally, macrophages containing degenerating cellular material were evident in the mucosal epithelium of the posterior intestine.

Group B. Starved Carp

Hepatopancreas (Figs. 14 and 17)

The hepatic architecture was well defined in this group, however, the hepatocytes appeared compact, and eosinophilic with indistinguishable cell membranes. There was a marked increase in the number of mononuclear cells, either containing reddish/brown pigmented material, some associated with vascular elements and exocrine pancreatic material. These mononuclear cells were not only scattered throughout the parenchyma but were seen in focal areas, and occasionally associated with areas of degenerating

hepatocytes. Granulomatous lesions were evident in five of the eight specimens and small numbers of unidentified protozoal parasites were present in the larger vessels. Glycogen was minimally demonstrated in most specimens.

Intestine (Figs. 19 and 20)

Several histological changes were seen in this group and included a marked increase in the numbers of mononuclear cells, and macrophages; an increase in the mucous secreting cells of the mucosal surface with desquamation of the epithelial lining in four of the eight specimens examined.

The mononuclear cells were seen at all levels of the intestine, and where they were of the macrophage type, reddish/brown, PAS positive pigmented material was evident. The mucous secreting cells were seen to be increased mainly in the posterior intestine, and it was here that desquamation of the epithelium was evident. Other changes were occasional areas of focal degeneration of the mucosa, associated with an increase in erythrocytes and inflammatory cells, in these areas columnar epithelial cells appeared vacuolated and also contained basophilic granules. One specimen had a mucin plug filling the lumen of the posterior intestine.

Pancreas

In one specimen, several granulomatous regions were present, and there was an increase in surrounding connective tissue and mononuclear cells of this particular specimen.

TABLE 3

Amounts of mononuclear cells seen in the hepatopancreas and intestines of carp fed and starved at two temperatures

	Fed 17°C	Fed 27°C	Starved 17°C	Starved 27°C
Hepatopancreas	*	*	*	***
Intestine	*	***	**	***

* = Minimal number

** = Moderate numbers

*** = Marked numbers

Fig. 12. Hepatopancreas. Starved (17°C). Hepatic architecture well defined. M - Mononuclear cells containing pigment associated with pancreatic tissue. H & E. X 180.

Fig. 13. Hepatopancreas. Fed. (27°C). Hepatic architecture well defined. M - Mononuclear cells containing pigment. H & E. X 180.

Fig. 14. Hepatopancreas. Starved. (27°). Hepatic architecture well defined. M - Mononuclear cells containing pigment. Hepatocytes compacted with poor definition. H & E. X 180.

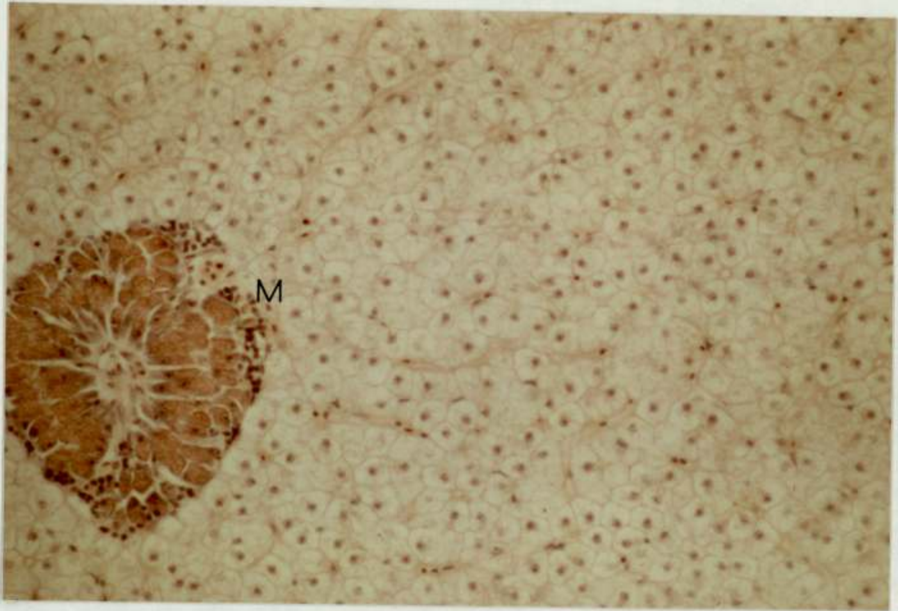


Fig. 12

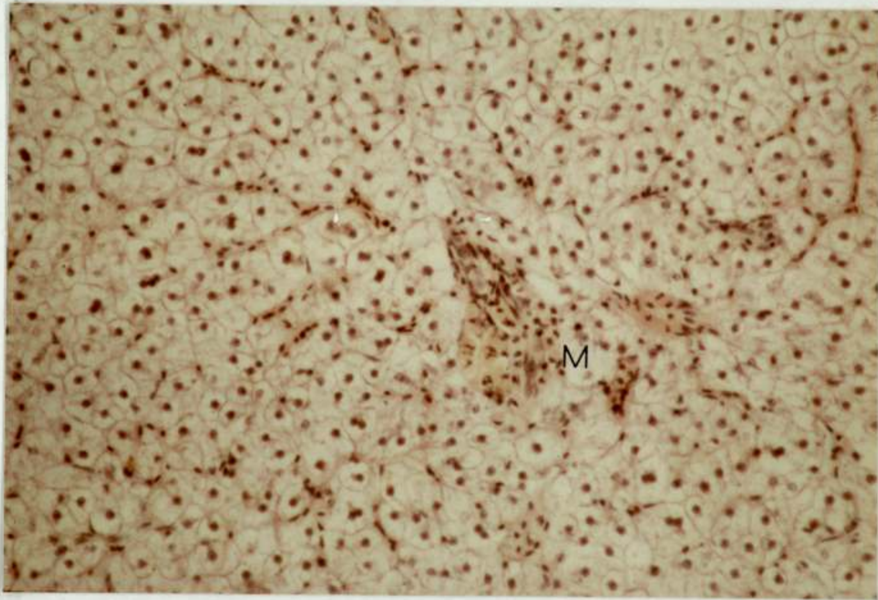


Fig. 13

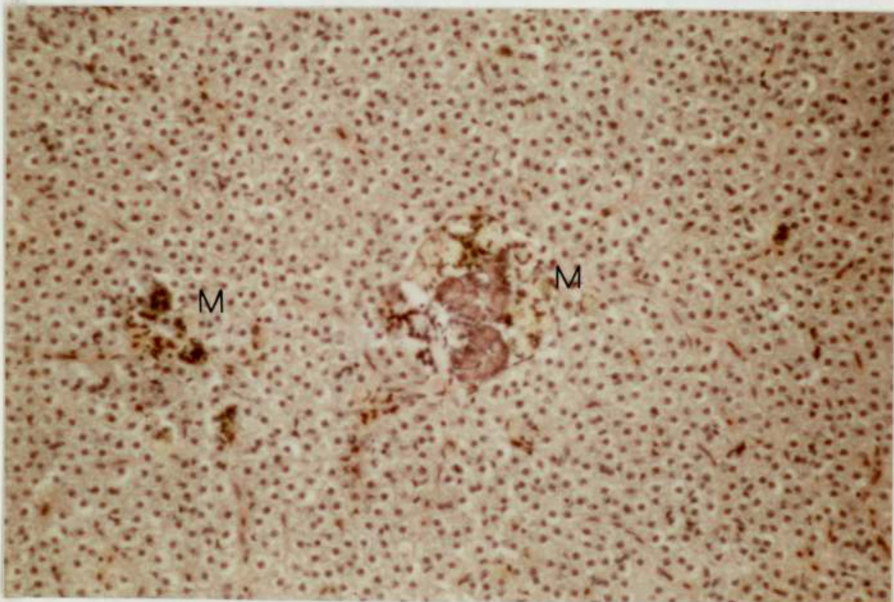


Fig. 14

Fig. 15. Medial intestinal mucosa. Starved (17°C).

Increased numbers of mononuclear cells.

H & E. X 250.

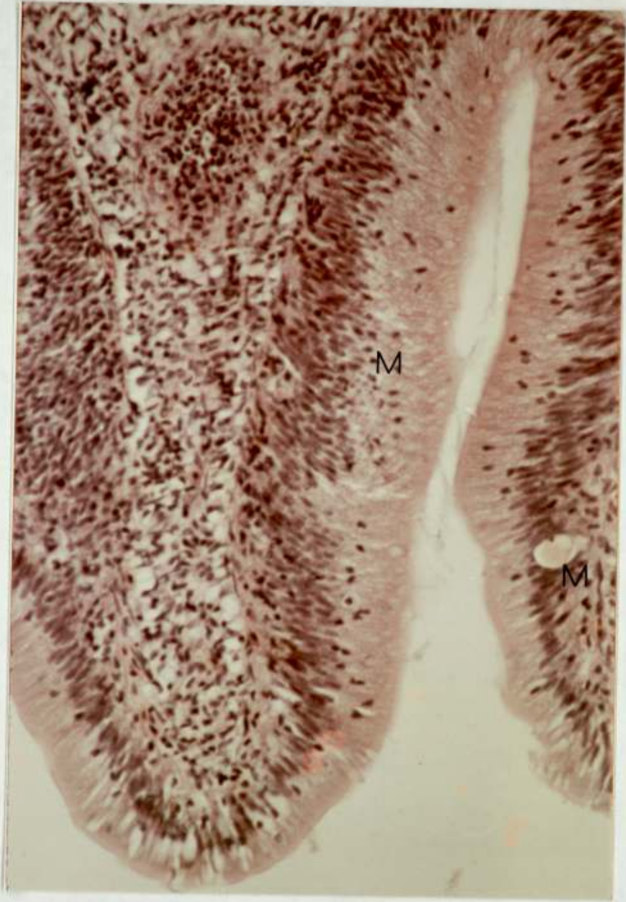


Fig. 15

Fig. 16. Hepatopancreas. Fed (27°C).

Glycogen well demonstrated.

PAS. X 180.

Fig. 17. Hepatopancreas. Starved. (27°C).

Almost complete absence of glycogen.

Pigmented material demonstrated.

PAS. X 180.

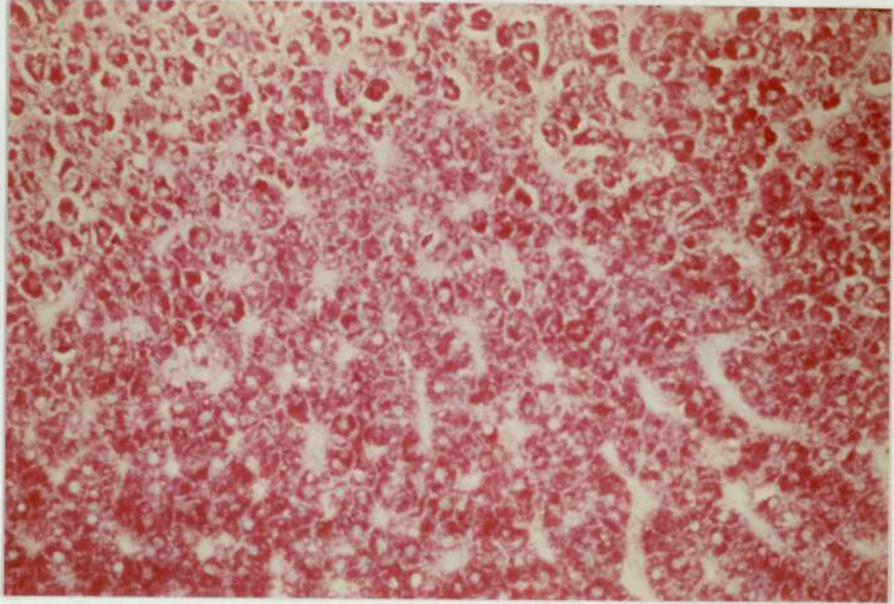


Fig.16

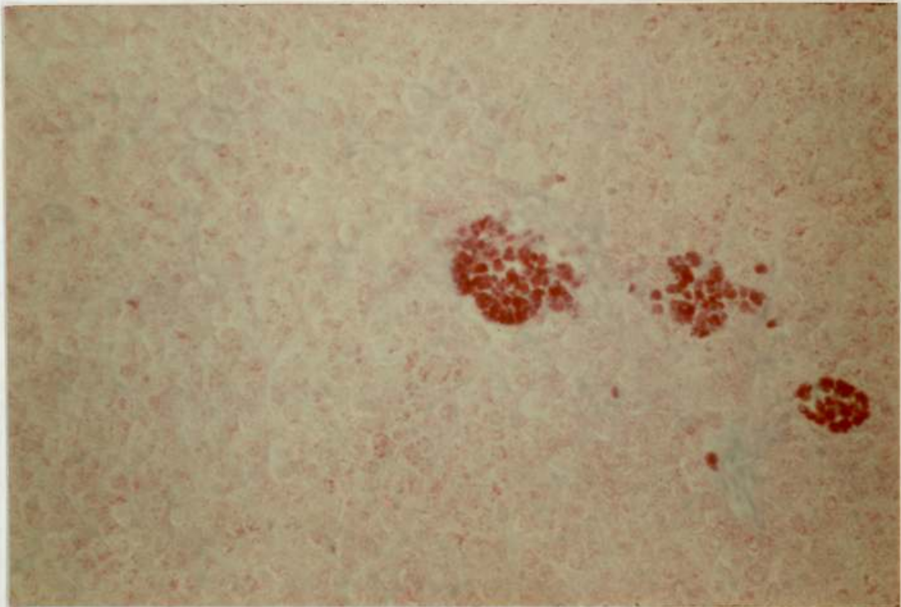


Fig.17

Fig. 18 Anterior intestinal mucosa. Fed. (27°C).
M - Macrophage cells containing PAS positive material. PAS positive material also demonstrated at outer edge of absorptive cells. Goblet cells stained blue.
Alcian blue/PAS X 250.

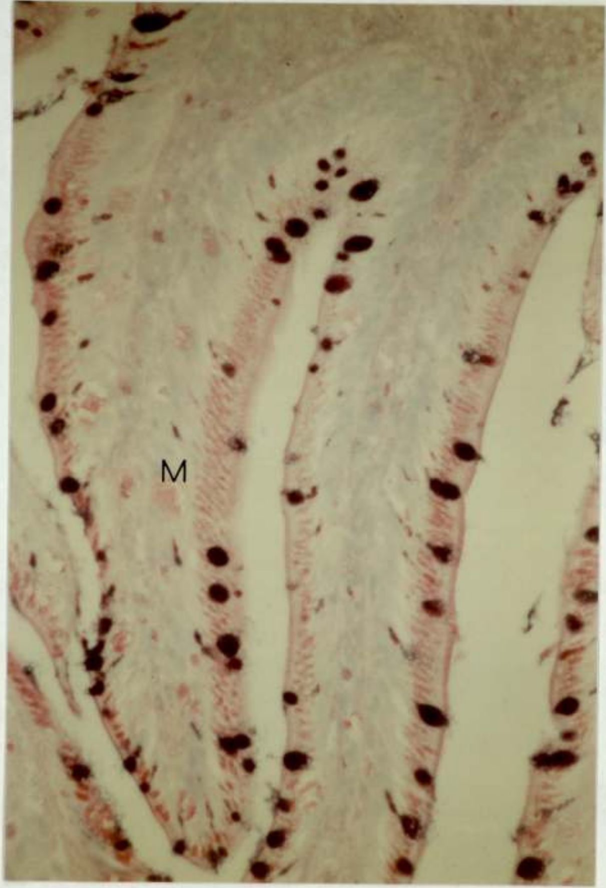


Fig. 18

Fig. 19 Posterior intestinal mucosa. Starved (27°C).
V - Vacuolated absorptive cells. Marked increase in cellular activity including engorged macrophage cells.
H & E. X 250.

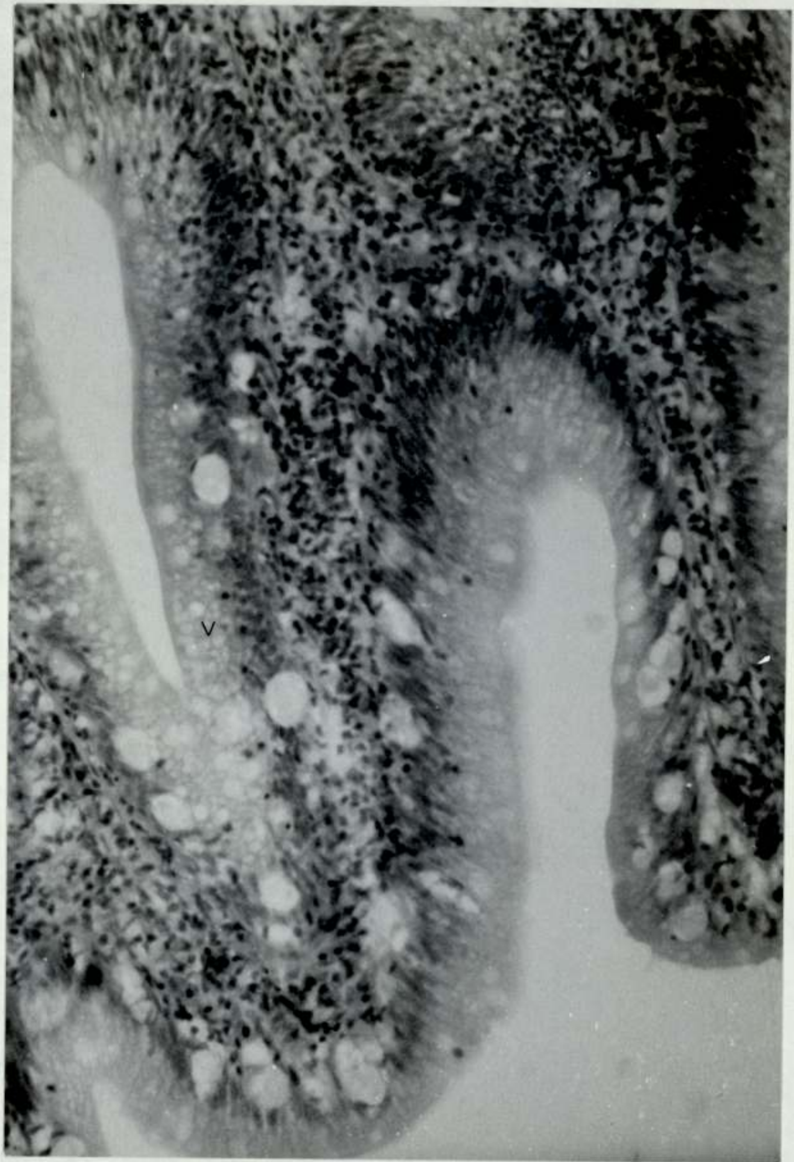


Fig.19

Fig. 20 Medial intestine (T.S.) Starved ($27^{\circ}C$).
Abundance of goblet cells and sloughed mucosa. Alcian blue/PAS. X 80.

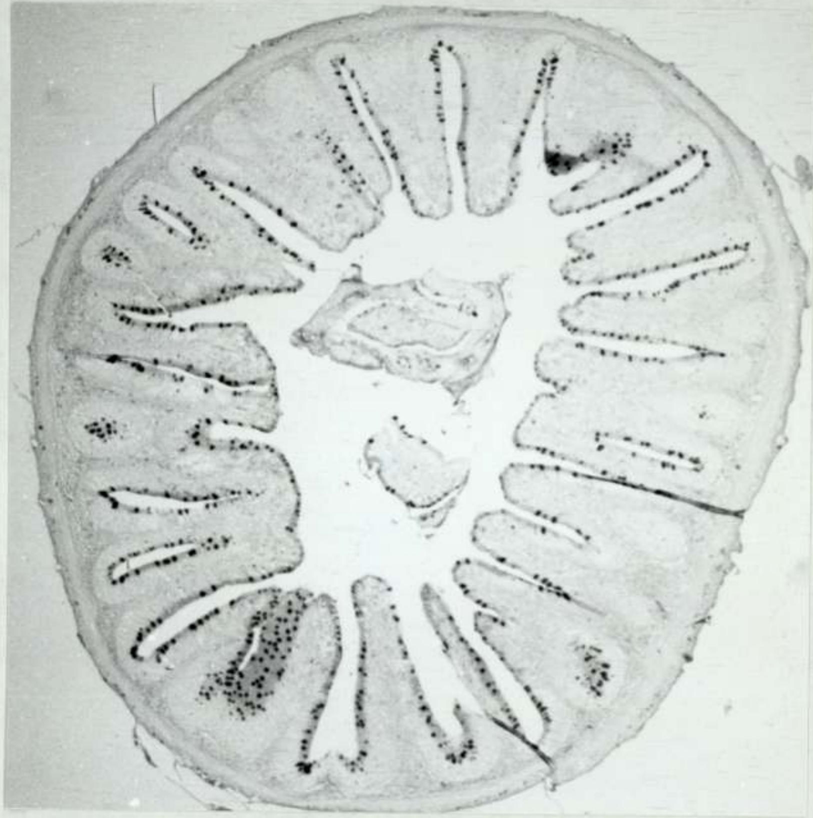


Fig. 20

5.2.2. Quantitative histology

The quantitation of mucopolysaccharides
in intestines from Protocols 3 & 4.

The histological results indicated that there was a change in the number of mucous secreting cells from levels of intestines on the various experiments, therefore a quantitation analysis was applied, and at the same time an estimation of the total area of mucin was made. Table 4 shows the number of mucous or goblet cells counted in 10 different fields of two levels of intestine from the experiments in Protocols 3 and 4 using an image analyser (Quantimet 720).

TABLE 4

An estimation of the number of goblet cells in transverse sections of the anterior and posterior intestines from 32 carp fed and starved, held at two different temperatures. (10 different fields, using the X10 objective. Stained by Alcian blue-PAS.)

	Anterior intestine				Posterior intestine			
	Fed		Starved		Fed		Starved	
Temp.	17°C	27°C	17°C	27°C	17°C	27°C	17°C	27°C
1	182	120	202	261	580	310	230	433
2	182	131	128	143	193	262	451	741
3	92	78	110	209	193	240	311	462
4	181	218	242	499	383	381	160	488
5	230	71	290	232	440	332	222	532
6	222	171	101	82	522	829	511	370
7	98	156	332	291	243	714	310	343
8	108	207	301	498	299	639	373	462
Mean	162	144	213	314	357	463	321	478

The following table shows an estimation of the amount of mucopolysaccharide in 10 different fields of two levels of intestine from the experiments in Protocols 3 and 4 using an image analyser (Quantimet 720)

TABLE 5

An estimation of the area of mucopolysaccharide in transverse sections of the anterior and posterior intestines from 32 carp fed and starved, held at two different temperature ranges (10 different fields; X10 objective; staining Alcian blue-PAS).

	Anterior intestine				Posterior intestine			
	Fed		Starved		Fed		Starved	
Temp.	17°C	27°C	17°C	27°C	17°C	27°C	17°C	27°C
1	19578	4082	10529	15482	31130	18321	10013	97133
2	5381	8539	6720	4912	8093	22813	28069	42193
3	3602	2463	5561	3789	7480	10822	12162	23981
4	4881	20079	19722	38618	33273	32342	3308	40913
5	11581	2461	15162	8520	16348	39898	9463	40518
6	10922	5873	4142	2748	42511	76283	39202	17669
7	2818	7015	21662	16620	19491	13251	18053	35941
8	5681	5329	7328	16638	8590	18415	23163	26679
Mean	8055	7646	11353	13666	20864	29018	17929	40253

The mean count of goblet cells from each experiment and the mean area count of mucopolysaccharides are expressed as histograms (Table 6 and 7). It can be seen that the goblet cell count and area of mucopolysaccharide follow the same trends. These will be given the general term of mucosubstances for these results. The overall pattern seen was that there were more mucosubstances present in the posterior than in the anterior levels of intestine whether fed or starved at both temperatures.

The results were given as comparisons between a) feeding and starving a 17°C according to Protocol 3; b) feeding and starving at 27°C according to Protocol 4; and c) feeding at 17°C and 27°C; d) starving at 17°C and 27°C.

a) There was minimal increase in mucosubstances in the anterior intestine of the starved fish, and a slight decrease in the mucosubstances of the posterior intestine of starved fish, compared with fed fish at 17°C.

b) In this comparison there was a slight increase in mucosubstances in the intestines of starved fish at both levels of intestine.

c) Levels of mucosubstances in the anterior intestine of the fed fish at the two temperature ranges showed very little difference. However, the levels were higher in the posterior intestines of fed fish held at 27°C.

d) Levels of mucosubstances in the anterior intestine of the fish starved at the two temperature ranges showed very little difference. However the levels were higher in the posterior intestine of starved fish.

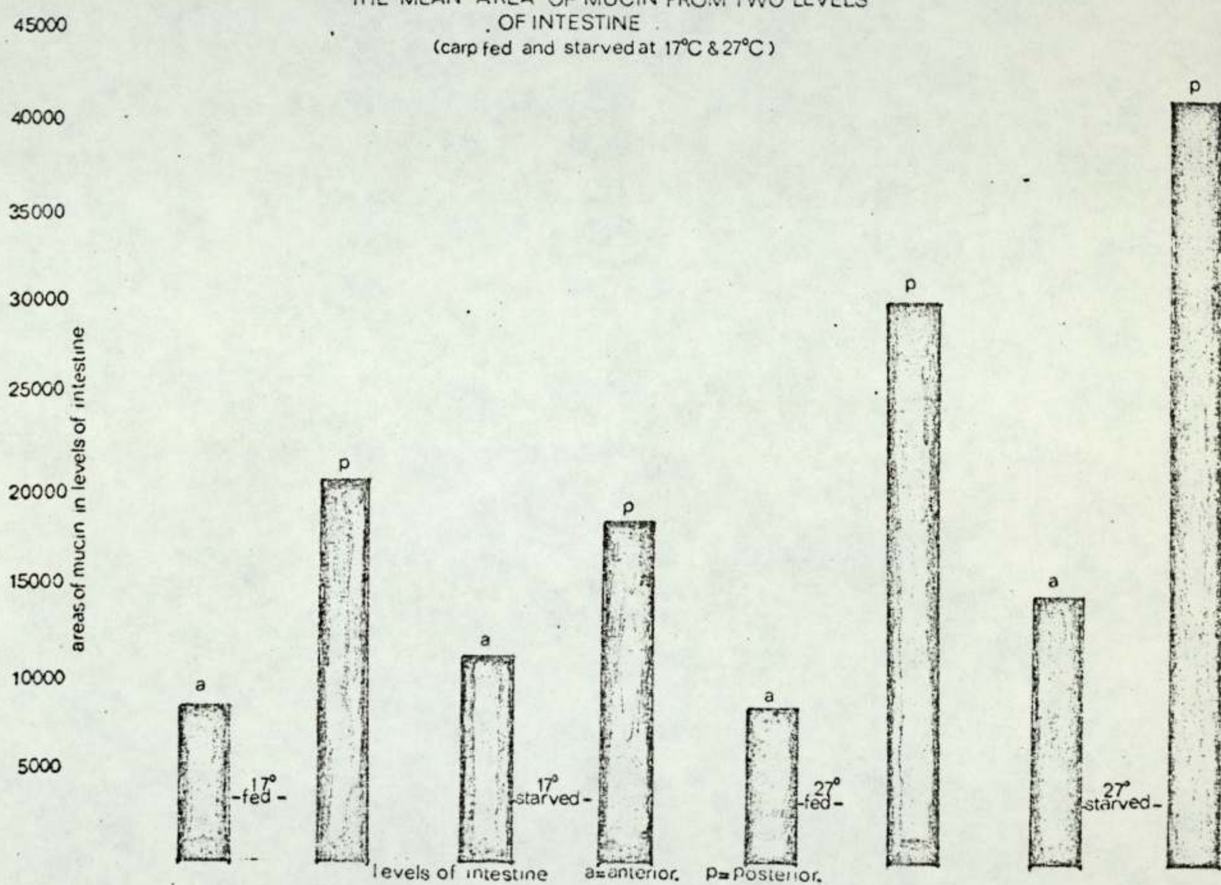
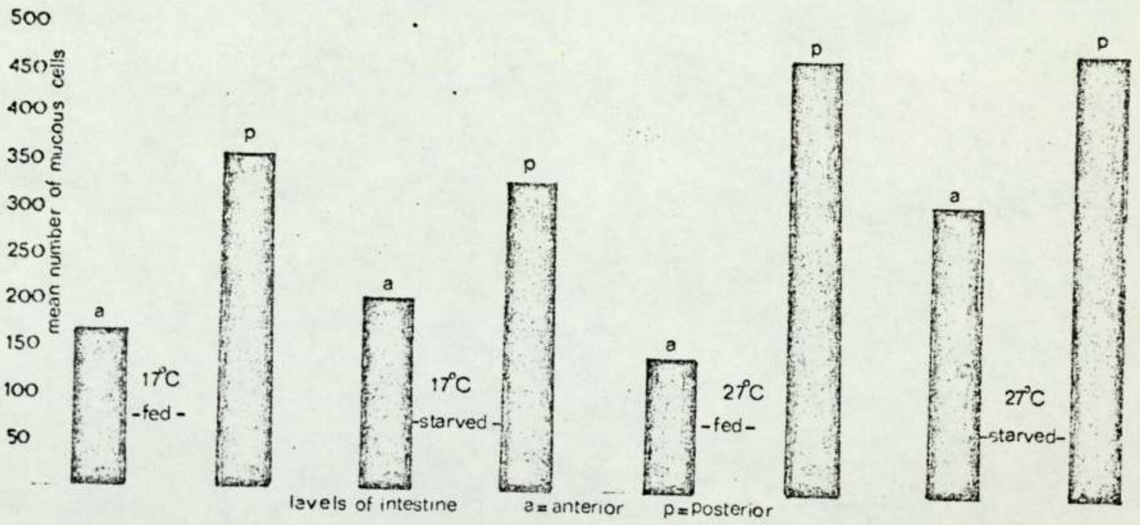


TABLE 6
THE MEAN AREA OF MUCIN FROM TWO LEVELS OF INTESTINE
(carp fed and starved at 17°C & 27°C)
45000
40000
35000
30000
25000
20000
15000
10000
5000
areas of mucin in levels of intestine
a
p
17° -fed-
17° starved-
27° -fed-
27° starved-
levels of intestine
a=anterior.
p=posterior.

TABLE 7
THE MEAN NUMBER OF MUCOUS CELLS
FROM TWO LEVELS OF INTESTINES

(carp fed and starved at 17°C & 27°C)



5.2.3. Enzyme Histochemistry

The results of the two enzymes investigated, alkaline phosphatase and leucine aminopeptidase were given. Samples from 8 fish in each group were examined.

1. a) Alkaline Phosphatase activity in the experiment from Protocol 3 - 17°C.

There was no significant difference detected in alkaline phosphatase activity from the tissues in fish that had been fed or starved. The activity in the hepatopancreas was strong in the canaliculi and areas surrounding granulomatous tissue. In the intestine the activity was restricted to the whole of the outer edge of the mucosal epithelial cells, and tended to be stronger in this area, in the posterior intestine, than the median and anterior intestine. There was a strong reaction for this enzyme in the connective tissue layer of the submucosa. Weaker reactions were seen in between the muscle layers. (Figs. 21 and 22).

b) Aminopeptidase activity in the experiment from Protocol 3 - 17°C

There was no significant difference detected in aminopeptidase activity from the tissues in fish that had been fed or starved, and the hepatopancreas did not show evidence of any activity. The activity in the intestine was restricted to the outer edge of the mucosal epithelial cells of all levels of intestine. The reaction was evident in the crypts of the mucosal folds and became weak to negative at the tips of the mucosal folds.

2. a) Alkaline phosphatase activity in the experiment from Protocol 4 - 27°C

Activity in the hepatopancreas was restricted to canaliculi and connective tissue surrounding granulamatous areas. Activity

Fig. 21 (17°C). Hepatopancreas attached to intestine. Alkaline phosphatase demonstrated in canaliculi of hepatopancreas and between muscle layers of intestine. X 180.

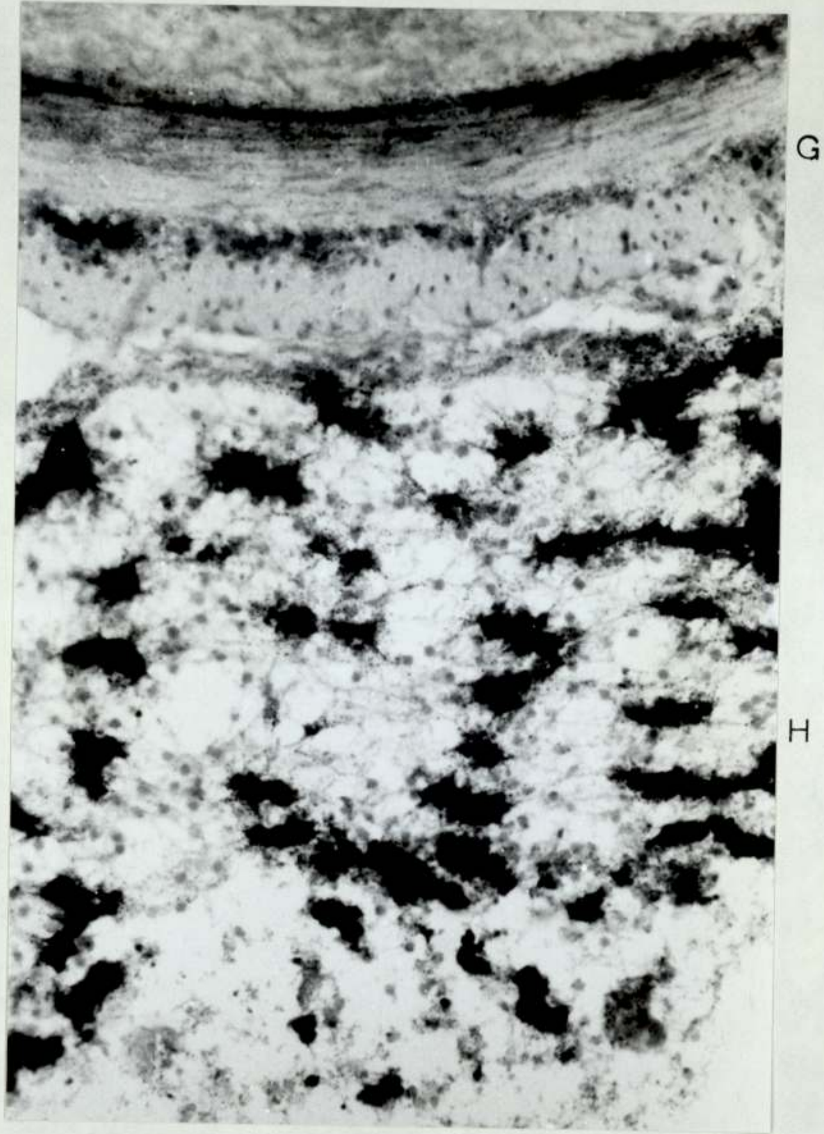


Fig. 21

Fig. 22 (17°C) Anterior intestine. Alkaline phosphatase demonstrated on outer edge of mucosal epithelium. X 180.

Fig. 22

in the intestine was evident along the outer edge of the mucosal cells, and the connective tissue layer of the submucosa adjacent to the muscle layers. The reaction was equally strong at all levels of intestine. It is also evident in this experiment, that the activity of alkaline phosphatase in the whole digestive system was stronger in fish that had been fed than starved. (Figs. 23 and 24)

b) <u>Leucine aminopeptidase activity in the experiment from Protocol 4 - 27°C</u>

No activity was recorded in the hepatopancreas. Whereas activity in the intestine was evident on the outer edge of the mucosal epithelial cells, and stronger in the crypts of the mucosal folds, becoming negative at the tips. It was evident in this experiment, that activity of leucine aminopeptidase was stronger in the carp that have been fed than starved, and in the posterior sections of the intestine, the reaction was less than in the anterior section of intestine. (Fig. 25)

TABLE 8

Amounts of enzyme activity detected by histochemical methods in tissues from carp, which had been fed and starved at different temperatures

	Protocol 3 - 17°C		Protocol 4 - 27°C	
Alkaline Phosphatase	Fed	Starved	Fed	Starved
Hepatopancreas	**	**	***	**
Intestine	**	**	***	**
Leucine aminopeptidase				
Hepatopancreas	-	-	-	-
Intestine	**	**	***	**

- = No activity observed

* = Minimal amounts of enzyme activity.

** = Moderate amounts of enzyme activity.

*** = Marked amounts of enzyme activity.

Fig. 23 Hepatopancreas. Fed (27°C). Strong reaction of alkaline phosphatase in canaliculi. X 120.

Fig. 24 Hepatopancreas. Starved (27°C). Weak reaction of alkaline phosphatase. X 120.

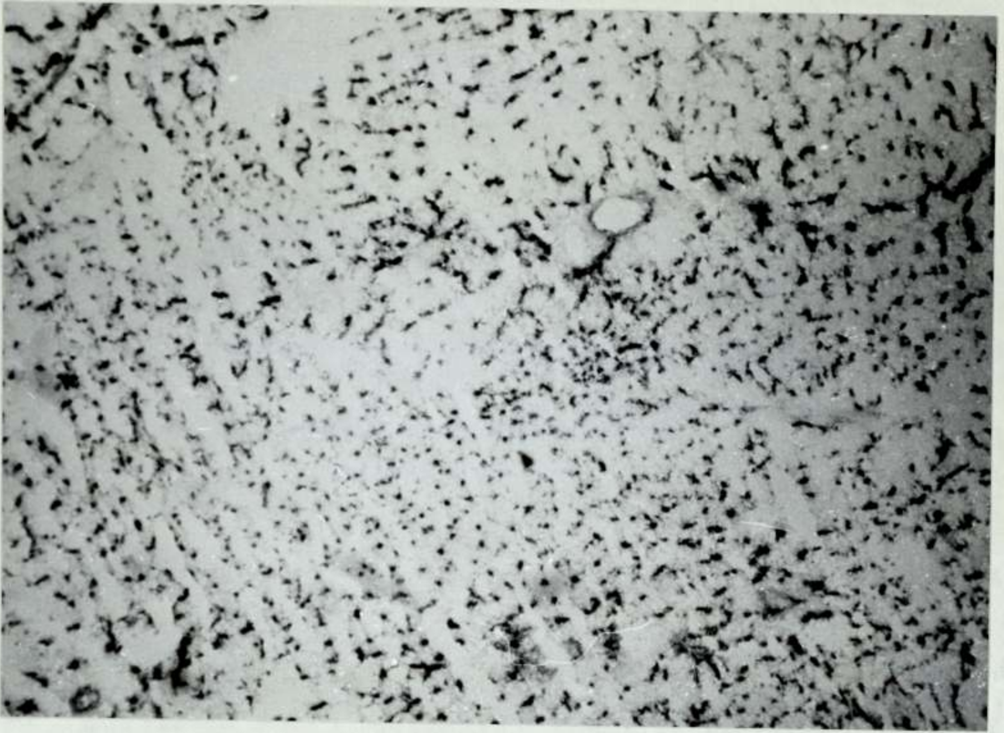


Fig. 23

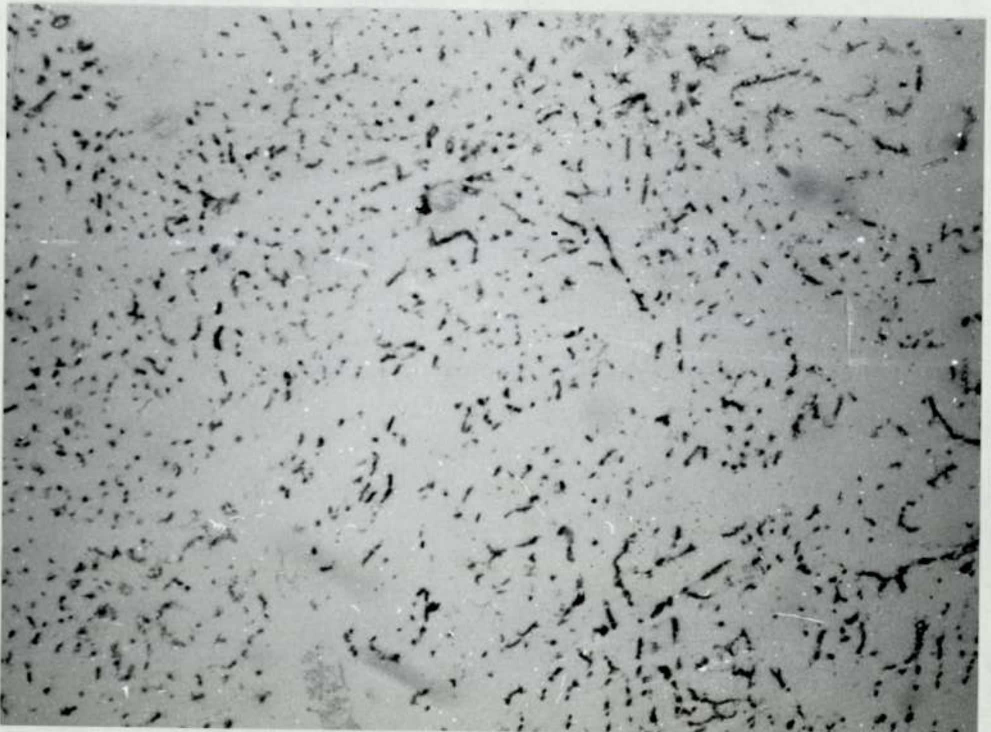


Fig. 24

Fig. 25 A - Anterior intestine. P - Posterior intestine. Fed (27°C). Stronger reaction of leucine aminopeptidase in the anterior intestine. X 80.

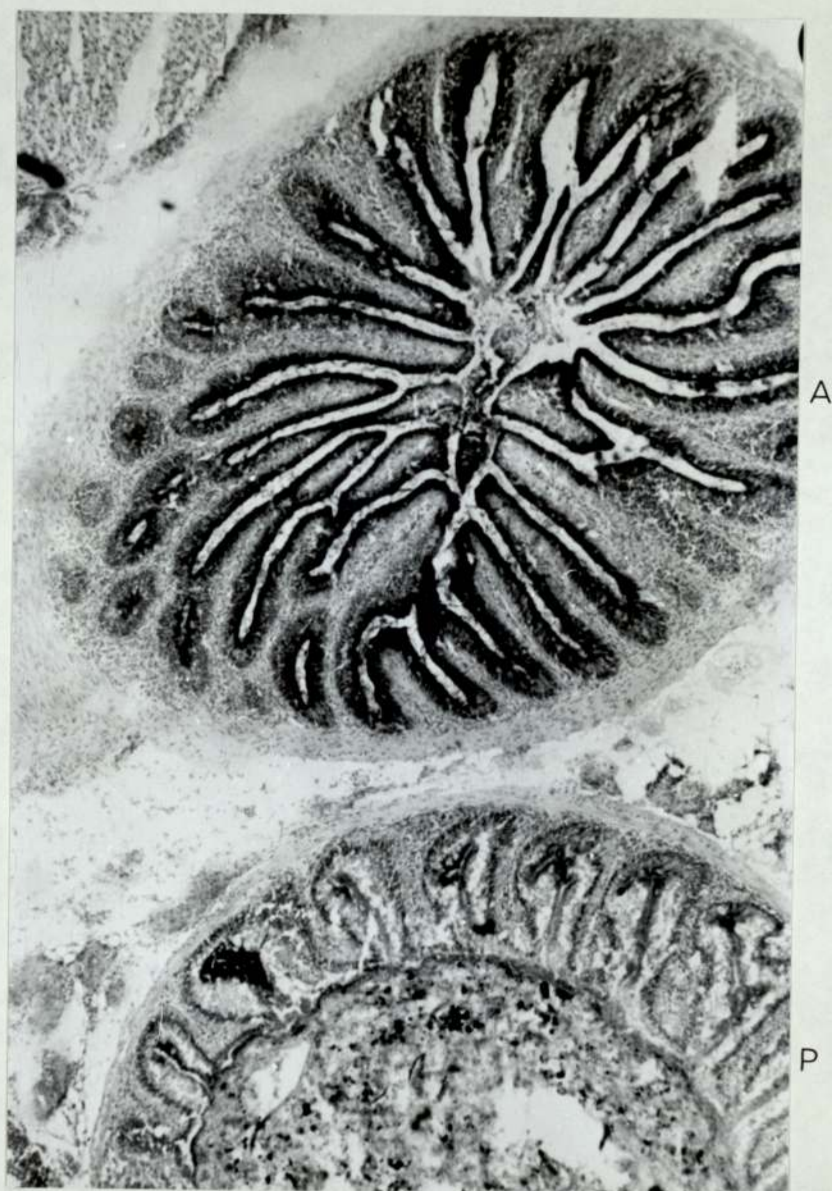


Fig. 25

5.3. BIOCHEMISTRY RESULTS

5.3.1. Protocol 1 - Quantitative Enzyme Experiments

Normal group of carp fed "Tetramin" diet at 17°C, for 4 weeks. This experiment showed quantitative levels of alkaline phosphatase and leucine aminopeptidase in homegenates of hepatopancreas plus intestine.

TABLE 9

Levels of alkaline phosphatase expressed in K.A. units (King-Armstrong) per mg/tissue

Sample No.	Wt. of Sample	Alkaline Phosphatase K.A. units	Units per mg/tissue
1	0.9306g	396	0.43
2	0.5447g	266	0.49
3	0.1731g	212	1.22
4	0.1020g	108	1.06
5	0.442g	322	0.73
6	0.6399g	404	0.63
Mean level of alkaline phosphatase measured per mg/tissue = 0.76			

TABLE 10

Levels of leucine aminopeptidase measured in G.R. units (Goldberg and Ratenburg) per mg/tissue

Sample No.	Wt. of Sample	Leucine Aminopeptidase G.R. units	Units per mg/tissue
1	0.9306g	2615	2.81
2	0.5447g	2365	4.34
3	0.1731g	1800	10.40
4	0.1020g	1235	12.11
5	0.4426g	1980	4.47
6	0.6399g	2715	4.25
Mean level of leucine aminopeptidase per mg/tissue = 6.40			

5.3.2. PROTOCOL 2 RESULTS

Quantitative levels of alkaline phosphatase and leucine aminopeptidase in samples of hepatopancreas and intestines from fish fed a high protein diet and from fish fed a low protein diet at 17°C for 56 days.

TABLE 11

Alkaline phosphatase levels in homogenates of hepatopancreas

Sample No.	Wt. of Sample	K.A. units	Units per mg/tissue
High protein			
1	1.036g	269	0.26
2	0.914g	106	0.12
3	1.062g	115	0.12
Low protein			
4	0.580g	192	0.33
5	0.113g	58	0.51
6	0.401g	144	0.36

TABLE 12

Alkaline phosphatase levels in homogenates of intestine

Sample No.	Wt. of Sample	K.A. units	Units per mg/tissue
High protein			
1	0.668g	691	1.033
2	2.359g	1728	0.73
3	1.218g	1574	1.29
Low protein			
4	1.885g	787	0.42
5	1.010g	1190	1.18
6	0.864g	691	0.80

TABLE 13

Leucine aminopeptidase levels in homogenates of hepatopancreas

Sample No.	Wt. of Sample	G.R. units	Units per mg/tissue
High protein			
1	1.036g	1225	1.18
2	0.914g	1275	1.39
3	1.062g	1120	1.05
Low protein			
4	0.580g	1215	2.09
5	0.113g	246	2.18
6	0.401g	795	1.98

TABLE 14

Leucine aminopeptidase levels in homogenates of intestine

Sample No.	Wt. of Sample	G.R. units	Units per mg/tissue
High protein			
1	0.668g	4590	8.22
2	2.359g	7900	3.35
3	1.218g	5050	4.15
Low protein			
4	1.885g	5760	3.06
5	1.010g	6950	6.88
6	0.864g	6060	7.01

5.3.3. PROTOCOL 3

Quantitative levels of alkaline phosphatase and leucine aminopeptidase in samples of hepatopancreas, intestine and sera from fish fed a high protein diet and from fish starved at 17°C for 84 days.

TABLE 15

Alkaline phosphatase levels in homogenates of hepatopancreas

A. High Protein Diet (17°C)			
Sample No.	Wt. of sample	K.A.Units	Units per mg/tissue
1	2.32g	106	0.41
2	1.16g	77	0.66
3	3.13g	96	0.30
4	0.86g	48	0.56
5	3.88g	106	0.27
6	4.97g	48	0.10
7	1.66g	77	0.46
8	2.50g	38	0.15
B. Starved fish			
1	3.15g	48	0.15
2	1.32g	96	0.72
3	0.86g	77	0.89
4	1.02g	48	0.47
5	0.95g	86	0.90
6	1.09g	48	0.44
7	1.78g	106	0.59
8	0.45g	48	0.07

TABLE 16

Alkaline phosphatase levels in homogenates of intestine. (17°C)

Sample No.	Wt. of Sample	K.A.Units	Units per mg/tissue
A.High protein			
1	3.20g	499	1.56
2	1.46g	461	3.16
3	2.29g	365	1.59
4	0.95g	288	3.03
5	2.08g	403	1.93
6	3.07g	461	1.50
7	0.63g	211	3.35
8	1.32g	211	1.59
B. Starved fish			
1	1.67g	288	1.72
2	3.44g	422	1.22
3	1.68g	480	2.85
4	0.85g	250	2.94
5	1.16g	384	3.31
6	0.55g	230	4.18
7	1.05g	134	1.27
8	1.24g	250	2.01

TABLE 17

Leucine aminopeptidase levels in homogenates of hepatopancreas (17°C)

Sample No.	Wt. of Sample	G.R. Units	Units per mg/tissue
A. High protein			
1	2.32g	400	1.72
2	1.16g	370	3.19
3	3.13g	315	1.01
4	0.86g	155	1.80
5	3.88g	250	0.64
6	4.97g	705	1.42
7	1.66g	525	3.16
8	2.50g	485	1.94
B. Starved Fish			
1	3.15g	595	1.89
2	1.32g	775	5.87
3	0.86g	375	9.36
4	1.02g	185	1.81
5	0.95g	660	6.94
6	1.09g	315	2.89
7	1.78g	440	2.47
8	0.45g	170	3.78

TABLE 18

Leucine aminopeptidase levels in homogenates of intestine (17°C)

Sample No.	Wt. of Sample	G.R.Units	Units per mg/tissue
A. High protein			
1	3.20g	1330	4.16
2	1.46g	1780	12.19
3	2.29g	1145	5.00
4	0.95g	1065	1.12
5	2.08g	2370	11.39
6	3.07g	3050	9.93
7	0.63g	1405	22.3
8	1.32g	1710	12.95
B. Starved fish			
1	1.67g	1200	7.18
2	3.44g	1860	5.40
3	1.68g	1320	7.86
4	0.85g	970	11.41
5	1.16g	1235	1.06
6	0.55g	785	14.27
7	1.05g	910	8.66
8	1.24g	2655	21.41

TABLE 19

Serum alkaline phosphatase levels from fishes fed high protein diet and starved at 17°C. (Units per 1 ml of serum)

A. High protein		B. Starved fish	
Sample No.	SAP.K.A.units	Sample No.	SAP.K.A.units
1	14	1	12
2	12	2	14
3	12	3	14
4	12	4	10
5	12	5	12
6	14	6	14
7	14	7	12
8	17	8	14

TABLE 20

Serum leucine aminopeptidase levels from fishes fed high protein diet and starved at 17°C. (Units per 1 ml serum)

A. High protein		B. Starved fish	
Sample No.	LAP G.R.units	Sample No.	LAP G.R.units
1	368	1	364
2	273	2	351
3	406	3	322
4	355	4	181
5	349	5	325
6	352	6	376
7	263	7	312
8	412	8	307

5.3.4. PROTOCOL 4

Quantitative levels of alkaline phosphatase and leucine aminopeptidase in homogenates of hepatopancreas, intestine and serum samples from fish fed a High Protein diet and from fish starved at 27°C for 84 days.

TABLE 21

Alkaline phosphatase levels in homogenates of hepatopancreas (27°C)

Sample No.	Wt. of sample	K.A. Units	Units per mg/tissue
A. High Protein			
1	2.92g	320	1.60
2	1.49g	370	0.94
3	1.99g	320	1.29
4	1.52g	460	0.92
5	2.00g	460	1.75
6	2.16g	505	0.99
7	1.26g	965	1.49
8	2.06g	490	1.64
B. Starved fish			
1	0.91g	575	7.31
2	1.12g	550	4.91
3	1.58g	185	1.17
4	1.14g	300	2.63
5	1.59g	140	0.88
6	0.88g	205	2.33
7	0.92g	205	2.23
8	1.09g	230	2.94

TABLE 22

Alkaline phosphatase levels in homogenates of intestine 27°C

Sample No.	Wt. of sample	K.A. Units	Units per mg/tissue
A. High Protein			
1	2.09g	1195	5.71
2	1.14g	370	3.24
3	1.43g	780	5.45
4	1.09g	595	5.46
5	1.45g	550	3.79
6	1.54g	1035	6.72
7	0.90g	825	9.17
8	1.47g	615	4.18
B. Starved fish			
1	1.00g	320	3.20
2	1.27g	390	3.20
3	1.72g	295	1.72
4	2.24g	255	1.14
5	1.74g	205	1.18
6	0.96g	275	2.86
7	1.00g	115	1.15
8	1.18g	185	1.57

TABLE 23

Leucine aminopeptidase levels in homogenates of hepatopancreas (27°C)

Sample No.	Wt. of Sample	G.R.Units	Units per mg/tissue
A. High Protein			
1	2.92g	580	1.99
2	1.49g	590	3.95
3	1.99g	495	2.48
4	1.52g	760	5.00
5	2.00g	525	2.63
6	2.16g	1105	5.12
7	1.26g	815	6.46
8	2.06g	580	2.81
B. Starved fish			
1	0.91g	1500	16.48
2	1.12g	1620	14.46
3	1.58g	1065	6.74
4	1.14g	1630	14.29
5	1.59g	555	3.49
6	0.88g	555	6.30
7	0.92g	650	7.06
8	1.09g	2190	20.09

TABLE 24

Leucine aminopeptidase levels in homogenates of intestine (27°C)

Sample No.	Wt. of tissue	G.R. Units	Units per mg/tissue
A. High Protein			
1	2.09g	4415	21.12
2	1.14g	3345	29.34
3	1.43g	3310	23.14
4	1.09g	3155	28.94
5	1.45g	4720	32.55
6	1.54g	6250	40.58
7	0.90g	4205	46.72
8	1.47g	4320	29.38
B. Starved fish			
1	1.00g	2810	28.10
2	1.22g	3565	29.22
3	1.72g	1860	10.81
4	2.24g	2885	12.88
5	1.74g	1730	9.94
6	0.96g	3385	35.26
7	1.00g	1005	10.05
8	1.18g	2100	17.80

TABLE 25

Serum alkaline phosphatase levels from fishes fed High Protein diet and starved at 27°C

A. High Protein		B. Starved Fish	
Sample No.	SAP.K.A.Units	Sample No.	SAP.K.A.Units
1	13	1	11
2	12	2	12
3	15	3	14
4	17	4	10
5	12	5	12
6	14	6	8
7	14	7	13
8	18	8	10

TABLE 26

Serum leucine aminopeptidase levels from fishes fed High Protein diet and starved at 27°C

A. High Protein		B. Starved Fish	
Sample No.	LAP.G.R.Units	Sample No.	LAP.G.R.Units
1	381	1	341
2	309	2	201
3	231	3	326
4	274	4	171
5	330	5	187
6	270	6	205
7	389	7	362
8	310	8	198

5.3.5. Statistical analysis from the quantitative enzyme results

The results from the various quantitative studies on activities of alkaline phosphatase and leucine aminopeptidase were submitted for statistical analysis of variance (The Kruskal-Wallis test) and the results are summarised below; (complete results are included in appendices 2)

Diets

i) There was significance at the 5% level of more activities of both enzymes from homogenates of hepatopancreas from carp fed the low protein diet, than carp fed the high protein diet. (Protocol 2).

ii) 17°C temperature. There was significance at the 5% level of more activities of both enzymes from homogenates of carp starved than carp fed at 17°C.

iii) 27°C temperature.

a) Alkaline phosphatase activity in homogenates of hepatopancreas from carp starved at 27°C was significantly increased at the 5% level.

b) Alkaline phosphatase activity in homogenates of intestine and serum samples from carp fed at 27°C was significantly increased at the 1% and 5% levels respectively.

c) Leucine aminopeptidase activity in homogenates of hepatopancreas from carp starved at 27°C was significantly increased at the 1% level.

d) Leucine aminopeptidase activity in homogenates of intestine and serum samples from carp fed at 27°C was significantly increased at the 5% level.

To gain more information from the quantitative enzyme estimations, a comparison was made between the results obtained from the tests made at 17°C, and the results obtained from the tests made at 27°C, and tabulated below.

TABLE 27

Significant levels of variance of enzyme levels between the two temperature ranges.

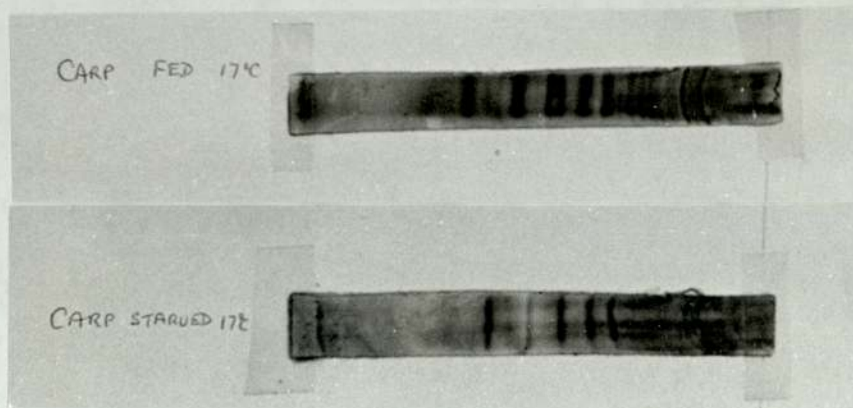
Alkaline Phosphatase				Leucine aminopeptidase			
Hepatopancreas		Intestine		Hepatopancreas		Intestine	
Fed	Starved	Fed	Starved	Fed	Starved	Fed	Starved
***	**	**	-	*	*	**	*

***	=	0.1% significant level of variance	From fish held at 27°C than at 17°C
**	=	1% significant level of variance	
*	=	5% significant level of variance	
-	=	No significant change.	

5.3.6. PROTOCOL 5

Serum Protein Examination - Electrophoresis

i) The samples examined using polyacrylamid gel (LKB-PAG plate) gave inconsistent results, and although there was separation of protein bands, it was not possible to differentiate them into albumin and globulins. (Fig. 26)



Serum proteins from carp fed and starved (17°C).

Coomassie brilliant blue.

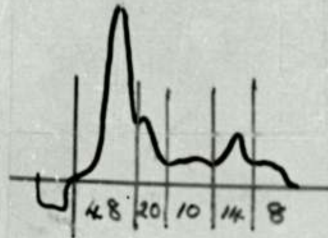
Fig. 26

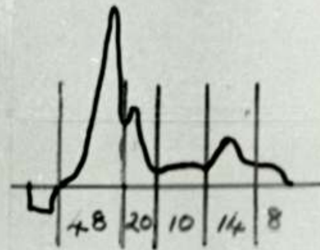
ii) The samples examined on the cellulose acetate film (Millipore Phoroslide strip) showed poor separation of the protein bands, the ponceau S stain would not bind itself to the albumin band, as it did in the control dog and rat samples. A quantitative analysis was made, but the results were inconclusive. (Fig. 27)

iii) The samples examined on the agarose gel (Corning-Eel) showed fairly good separation of the protein bands, with good binding of the amido black stain. The albumin bands are distinguishable, but not as clear as in the dog and rat control samples. Quantitative analysis was not possible because of equipment difficulties. (Fig. 28)

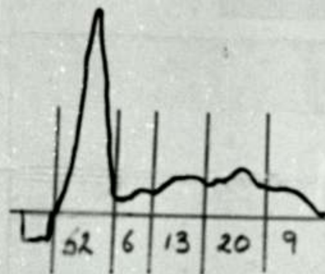
Fig 27. Electrophoresis using cellulose acetate film.
Albumin not demonstrated in carp samples.
Reading left to right - Albumin, α_1 Globulin,
α_2 Globulin, β Globulin and γ Globulin.
Ponceau S.

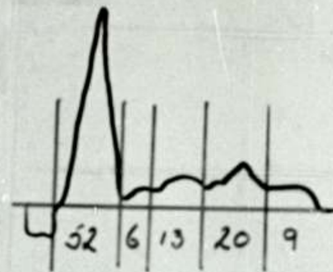
RAT





DOG



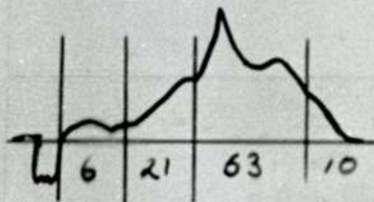


carp

A

H. PROT. 17°

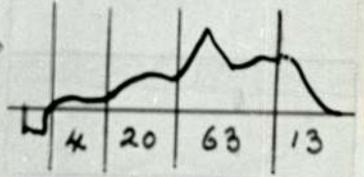
TOTAL PROTEIN 2.2 g%



D

H. PROT. 27°

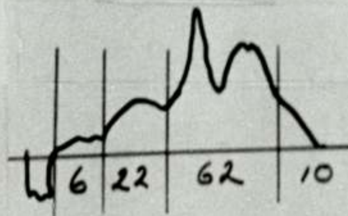
TOTAL PROTEIN 2.0 g%



C

L. PROT. 17°

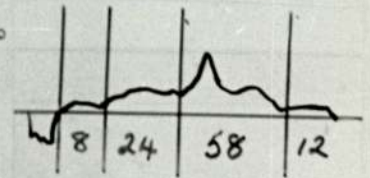
TOTAL PROTEIN 2.1 g%



F

L. PROT. 27°

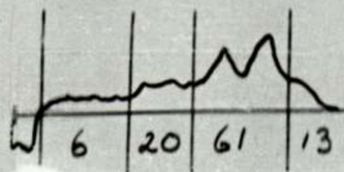
TOTAL PROTEIN 3.2 g%



B

STARVED 17°

TOTAL PROTEIN, INSUFFICIENT.



E

STARVED 27°

TOTAL PROTEIN 3.0 g%

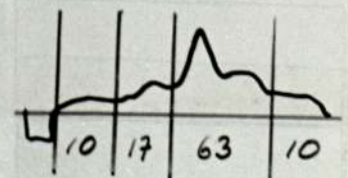


Fig. 27

Fig. 28 Electrophoresis using agarose gel strips. Reading left to right - separation of albumin and globulin bands are seen, particularly at 27°C. The lower right strip is an anomaly. Amido black.

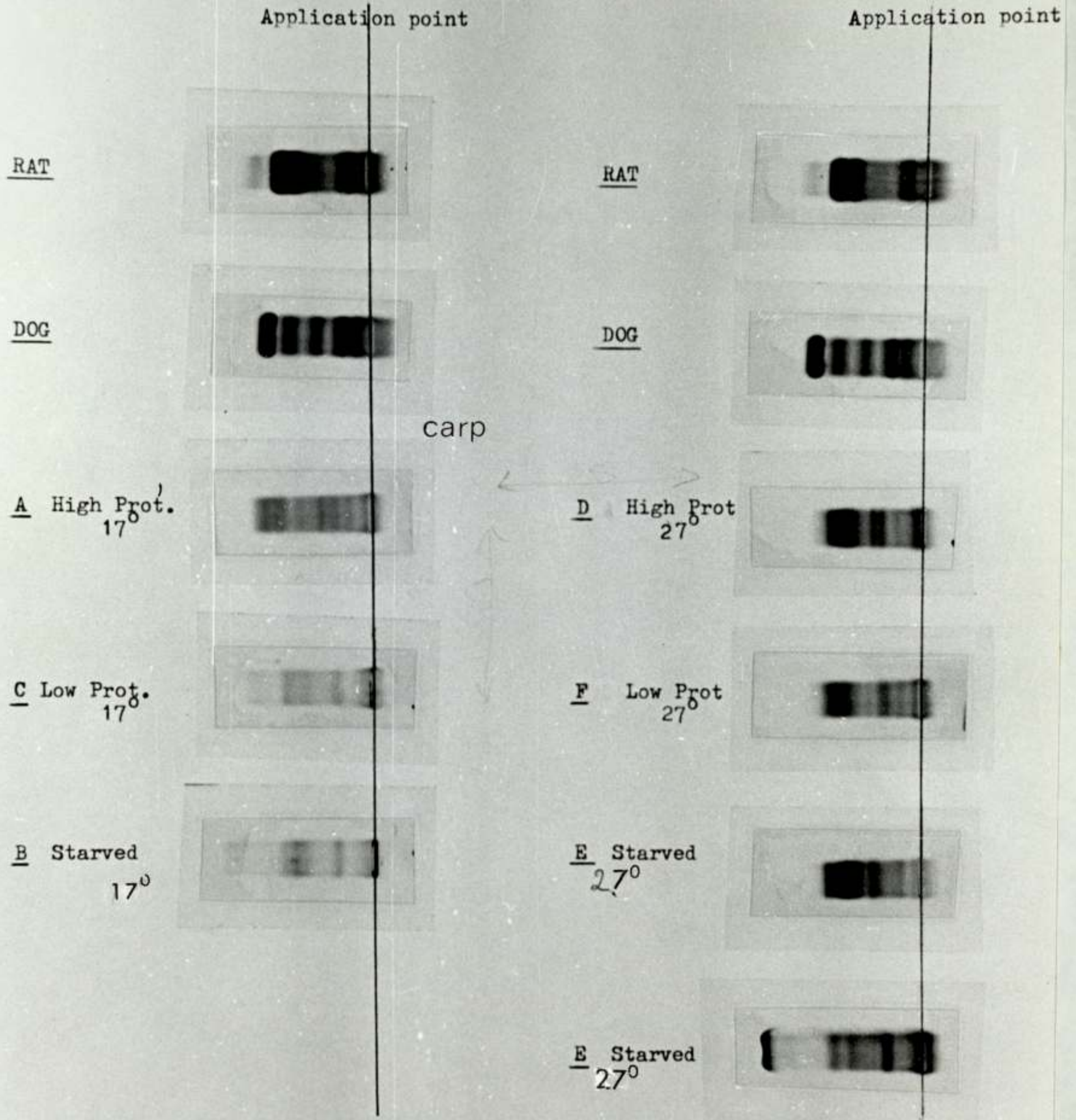


Fig. 28

6. DISCUSSION.

6. DISCUSSION

6.1. Histology

6.1.1. Protocol 2. Carp fed high and low proteins diets at 17°C

The object of this experiment had been to examine the histological details of the digestive tract and hepatopancreas for any changes which may have occurred when carp were fed either a high or a low protein diet. There appeared to be a paucity of work on the histological details of teleosts fed varied protein diets. The fact that significant changes could not be detected by histology or even the histochemical demonstration of the enzymes - alkaline phosphatase and aminopeptidase, indicated that these methods were not sensitive enough to show changes caused by differing protein contents in the diets.

The study of nutritional deficiencies in intensively cultured catfish has been made by Lovell (1975) but no histological data was given. Judd and Cross (1966) again working with catfish, fed a high protein diet to a batch of fish, and compared the histological details with 'wild' fish taken straight from a river. Although this was not a comparison of the effect of different measured proteins, and other factors had to be considered, it was interesting to note that these authors saw hepatocyte degeneration with larger, highly-vacuolated hepatocytes in the fish which had been fed high protein diets, yet hepatocytes from the 'wild' fish were not vacuolated. It has already been stated in the general histological discussion (Ashley, 1975) that pale, vacuolated hepatocytes indicated the presence of glycogen, and in this experiment, there was not enough fish to form any statistical comment on the amounts of glycogen, except it 'appeared' that there

was slightly less liver glycogen demonstrated in the fish fed a low protein diet.

The problems with feeding fish diets containing different protein levels in enclosed tanks had to be considered, obviously, fish held like this were not expending energy, apart from when they were disturbed, and probably only metabolised sufficient protein to sustain themselves. Hepher and Chervinski (1965) suggested that wild carp diet contained 50% protein dry weight, and this had been suggested previously by Mann (1961) and Schäperclaus (1963). Hepher and Chervinski (1965, 1971) suggested that the carp's demand for protein in experimental ponds was small. Therefore, an experiment ultilising dietary proteins made in large cages described by Shiloh and Viola (1973) might be worthwhile.

It is considered that this experiment, as far as detecting histological changes, was considered to be negative, because of this, other factors which might have influenced histological changes were considered, such as feeding and starvation, plus changes in temperature, and that was the reason for abandoning the experiment. Also there was a technical hitch in the preparation of one of the diets, therefore other pathological changes may have occurred which might have been unconnected with protein levels.

6.1.2. Protocols 3 - 4

Histology of the digestive tract of carp fed and starved at 17°C and 27°C

This was an exercise in differentiating changes in the histological structure of the intestine and hepatopancreas from carp fed and starved at different temperatures, and comparing the results with the 'normal' histological structure of intestine and hepatopancreas previously described in 4.1.

Snieszko (1972) in his review on nutritional fish disease said that starvation was a common condition amongst wild freshwater fishes, and freshwater pond fishes in particular were often exposed to starvation in areas where temperature differences between summer and winter were marked. With these facts known, the temperatures chosen for these experiments were not selected to simulate summer and winter conditions which would occur in the normal environmental conditions of a carp living in the Northern Hemisphere. The idea of choosing a temperature of 17°C was that carp, fed normally in natural conditions at this temperature, and the higher temperature of 27°C was towards the upper optimum feeding temperature (Mann, 1961). Therefore, if fish were starved at these temperatures, pathological changes should be detected, although Steffens (1964) said that carp starved under unfavourable winter conditions showed pathological changes in the gut, noteably ulceration and necrotic epithelium, often associated with bacterial infections. This subject has been investigated by Bucke et al (1974) when similar observations were made in cases of suspected erythrodermatitis.

Fish fed and held at 17°C temperature had histopathological changes which were minimal and were similar to those already mentioned. The minimal changes observed, included an increase in mononuclear cells, associated with vascular elements and exocrine

pancreatic tissue in the hepatopancreas, also an increase in mononuclear cells in the intestinal mucosa. The reason for this increase in cellular activity may have just been a stress response to the fishes being kept in captivity, or more likely, a reponse to some form of infectious agent. Protozoan parasites were noticed in a few specimens, and possibly were present in most, if not all fish. The life history of many protozoans enables them to transfer from fish to fish through the water (Rogers and Gaines, 1975). Walker (1975) showed that granulomatous lesions occurred at sites of bacterial or parasitic infections and often phagocytic cells were associated with such lesions in the current experiments. The presence of eosinophilic material in the intestinal columnar epithelial cells in feeding fish has been described by Gauthier and Landis (1972) in C. auratus. These authors called this material supranuclear material, and considered it had entered the absorptive or epithelial cells by pinocytosis where it was acted upon by hydrolytic enzymes.

When fish were undergoing the "overwintering" period, i.e. in low temperatures, the intestines were at a resting stage and atrophied (Cheng, 1931) this state was not seen in starved carp held at 17°C, and this indicated that in fish starved at low temperatures, the activity of the cells was at a minimum, whereas at 17°C the cells should have been in an active state.

The only significant histological findings in fish starved at 17°C for 80 days were the further increase in mononuclear cells in the hepatopancreas and intestinal mucosa and an increase in mucous secreting cells in the posterior intestinal mucosal epithelium.

Cuillo (1975) made his investigations on the effects of starvation on Fundulus heteroclitus, (the common mummichog) a fish which was used extensively in research in the New World. His tests

were short term, lasting only for periods of 2-8 days, and the fish were kept at 12°C. Most of his significant findings were allied to lipid metabolism. However, he did show a reduction in alkaline phosphatase activity in the posterior to anal regions of fish after 2 days starvation. Itwas considered in this study that the histochemical examination of alkaline phosphatase and leucine aminopeptidase fluctuated so much in these samples, that the results were invalidated.

Ciullo, did report an increase in goblet cells in fish starved for 8 days, and made a point that the increase occurred in the anterior intestinal mucosa, a somewhat similar result was seen in the current results, and this was shown up on the histogram (Table 7). This author mentioned the presence of 'rodlet' cells in Fundulus, but did not indicate whether there was an increase or decrease due to starvation. The granular cell was also discussed, and Ciullo observed these cells in starved fish to be most abundant towards the rectal end of the intestine.

It has been quoted by several authors (Sundaraj et al 1966; Kamra, 1966, Inui and Egusa, 1967; and Nagai and Ikeda, 1971a) that liver glycogen was altered after starvation. Sundaraj et al, (1966) detected variable amounts of glycogen in the liver, but they were only starving their fish for periods of 24-72 hours. Kamra (1966) reported that in Gadus morhua the liver glycogen was reduced after prolonged fasting, yet the fish was able to sustain a liver glycogen store by breaking down fat deposits in other tissues and metabolising this fat into glycogen in the liver. In the current experiment there was only one fish of eight which showed depleted hepatic glycogen, but in the rest, moderate amounts were observed. Inui and Egusa (1967) reported that after 30 days starvation in the glass eel, approximately 25 of 50 fish showed a certain amount of hepatic

glycogen.

Nagai and Ikeda (1971a) made a biochemical estimation of hepatic glycogen in carp starved for 22 days and could scarcely detect any decrease.

The increase in temperature (27°C) revealed more histological changes, than in the previous experiment. The hepatocytes of the fed fish contained eosinophilic cytoplasm, but the cell membranes were distinct, yet in the starved fish, hepatocytes showed a compacted appearance, with indistinguishable cell membranes. The mononuclear cells were increased in numbers, most of all in the starved fish, particularly with pigmented phagocytic cells. The other significant change was the increased amount of glycogen detected in the livers of the fed fish, and an almost absence in the livers from the starved fish.

The eosinophilic cytoplasm of the hepatocytes was probably due to an increase in proteinaceous material and nucleic acids which had accumulated in the hepatocytes, probably because of an increase in the metabolic activity of the fish at the higher temperature. Haschemeyer (1973) studied this subject of protein synthesis control in acclimatisation to temperature changes, using the toadfish as the animal model. When she raised the temperature to 30°C it was seen that protein synthesis was more rapid and in greater amounts than at 20°C or 10°C. In mammalian experiments, Lagerstedt (1953) showed a disappearance of protein and nucleic acids from the hepatocytes, and it would be reasonable to consider that this would happen in teleost livers at low temperatures.

The increase in phagocytic cells containing pigment were probably due to this more rapid metabolic activity of the cells at the higher temperature, and it did not matter

whether the fish was feeding or not, the metabolism of the cells still functioned. Wachstein (1963) recorded the presence of similar pigment in human liver, after ethionine administration associated with certain diseases, accumulating in the reticulo-endothelial Kupffer cells. This author called the substances nonglycogenic polysaccharide reactions.

The compact appearance of the hepatocytes from the starved fish in this experiment was most likely due to the depletion of glycogen. Inui and Egusa (1967) recorded after starvation in the glass eel, a reduction in hepatocyte size, which gave the sections the appearance of concentrated nuclei.

In the fed fish at 27°C temperature, there was one fish which had a liver with 'foamy' cytoplasm of the hepatocytes, this could possibly be attributed to post-mortem autolysis, or according to Walter and Israel (1970) this situation could develop in the living animal subjected to adverse conditions such as hypoxia and the effects of poisons. It is difficult to actually identify a definite cause for this unexpected pathological change in one specimen, and any one of these conditions could have occurred.

The increase in alkaline phosphatase activity seen in the fish held at the higher temperature in the hepatopancreas was not surprising, particularly as two of the functions of this enzyme were dephosphorylation of hexose phosphatase in the formation of glycogen, and in the absorption of glucose, (Srivastava, 1966).

The pathological changes in the intestine were mainly found in the mucosal epithelium. The most striking were the moderate increase of mononuclear cells in the tissues from fed carp, and the marked increase of mononuclear cells in the tissues from starved carp. The intestinal epithelial cells from the fed fish

contained a distinct layer of PAS positive granular material, on the apical edge, and this was most distinct in the posterior intestine but not seen in the starved fish. To take the latter point first, Cuillo (1975) made a similar discovery in *F. heteroclitus*, when examining his fed fish. Gauthier and Landis (1972), and Western (1971) considered this material to be proteinaceous, the latter author considered it was broken down by proteolylic enzymes by the pinocytic activity of the epithelial cells, particularly in the posterior intestine, this was interesting, because aminopeptidase activity was present in marked amounts in this area of intestine, also alkaline phosphatase was seen at its highest activity. This enzyme was demonstrated all along the intestine, and not restricted to the posterior end only.

Occasionally, basophilic material was evident in the intestinal absorptive cells from the starved carp, and this emphasized again the fact that although the fish were starved, they were kept at 27°C which is a temperature of optimum metabolic activity for the carp, (Mann, 1961). It was likely, therefore, that there was some cell breakdown, by the reverse action of enzymes, (autolysis) and this could explain the reason for the occasional areas of degeneration seen in the intestinal mucosa. The epithelium of the intestine was certainly undergoing some change, because half of the fish showed desquamation of the epithelial lining.

The amounts of mononuclear cells present, particularly in the starved carp would indicate that a considerable amount of activity was taking place in the intestine, and these cells were reacting and absorbing the breakdown components of tissues or parasites (Roberts, 1975).

The amounts of granulomatous tissue in the hepatopancreas,

and pancreas appeared to be more abundant in the fish held at the higher temperature, and again it was not easy to find a definite reason for this. However, it was possible that the increased temperature caused an exacerbation of an endemic infection.

The increase in mucous secreting cells from the posterior intestinal mucosal epithelium of the starved fish was evident in the microscopical examination. In a previous experiment on the mucous secreting cells of pike (Bucke, 1971) there was an overall increase of mucous cells in the anterior intestine which was only significantly evident after quantitative analysis. Therefore, a quantitative analysis of mucous cells from the anterior and posterior sections of the intestine was made on fish from both temperature ranges.

The method of quantifying the mucous secreting cells was by counting the overall numbers of actual mucous cells, and applying a count of the total area of mucosubstances present in the two levels of intestine. Healey (1971) made an appraisal of the different methods of quantitative histopathology by image analysis, and pointed out that the results may be arbitary, but providing there was a control and test situation, and everything was constant in the technique, there would be comparative, but not absolute data.

The quantitative analysis on the previous study (Bucke, 1971) was made on an original Quantimet B image analyser, a much simpler machine than the Quantimet 720 image analyser which was used in this study, and in view of this the many errors such as incorrect focusing and machine manipulation were eliminated. A mean result of the mucous counts was made only, because of the shortage of time and relevant importance.

The sections were stained alcian blue/PAS to demonstrate the mucosubstance and give the differential contrast that was

necessary for the machine to elucidate a quantitative analysis (Mawdesley-Thomas and Healey, 1969).

The main feature of the results indicated that the amount of goblet cells, and the total areas of mucin followed a similar pattern, which was obvious in the teleost as there were no salivary glands (Andrew, 1959) and the only way mucous secretion could occur was through the goblet cells.

The other feature was that there was more mucosubstance in the posterior intestine, than in the anterior intestine, and many other workers have described a greater abundance of goblet cells in the posterior intestine of carp, than in the other regions (Pictet, 1909; Curry, 1939; Al-Hussaini, 1949a; Chaicharn and Bullock, 1967), giving their function mainly to faecal elimination.

At the low temperature (17°C) mucus secretion was very similar between fed and starved fish, yet the higher temperature (27°C) there was an overall increase in mucin production than at the lower temperature. The overall amount of mucus was greater in the starved fish at 27°C than in the fed fish at this temperature.

The reasons for this increase in mucin in the higher temperature group could be caused by several factors. Smit (1957) had shown that gastric secretion was increased at a raised temperature, and it had been mentioned previously that there was an increase in metabolic rate at a higher temperature. Phillips et al (1957) showed that a capsule of food with added dye moved more rapidly along the intestine of fish in warmer water. However, Western (1971) reported that the movement of food in the intestine was associated with mucus secretion, and the mucus probably acted as a protective mechanism. This author did not detect mucin in the tract of unfed C. gobio and other fish which had been starved,

but he was looking at neutral mucopolysaccharide (NPMS) secreted by columnar epithelial cells in the stomach and not goblet cells which secrete APMS. It has already been stated that the carp does not have a stomach. In the human, mucus is secreted by glands of Brunner, such as pyloric glands in the stomach and this mucus contains a proteolytic enzyme activated by hydrochloric acid. In the intestine, mucus is secreted by goblet cells, and again contains important enzymes which break down protein into amino-acids, split carbohydrates and activated tripsinogen, (Maximow and Bloom, 1953). The carp does not have gastric glands (Al-Hussaini, 1949a) but does have goblet cells which do secrete enzymes (Al-Hussaini, 1949b) and it was feasible that with the increased temperature and therefore metabolic activity, more mucin was secreted.

The reason for the increased amount of mucin in the starved fish may be because although no food was present, the stimulating factor was still there. Allee and Frank (1948) made several experiments on the ingestion of colloidal material and water in goldfish, and their observations indicated that these fish swallowed water and any other substances that the water contained. In a further project, Allee and Frank, (1949) showed that goldfish starved less rapidly if they were allowed to swim in water which contained bacteria or algae. These experiments indicated that the process of mucin release was activated.

The research project discussed was made in a closed circuit system, and it was quite likely that in time the water would contain high concentrations of micro-organisms, which could have been ingested by starving fish, thus stimulating mucin production in the intestine.

6.2. Biochemistry

6.2.1. Enzyme studies

The biochemical analysis of alkaline phosphatase and leucine aminopeptidase activities in the digestive tract and serum samples were made to supply quantitative data to support the histological investigation of the described experiments. The biochemistry of fish tissues has been well investigated according to reviews by Barrington (1957) de Lingny (1969) and Love (1970), including enzyme and protein analysis.

The initial study (Protocol 1) was made to investigate techniques of quantitation. Amounts of alkaline phosphatase and leucine aminopeptidase activities were analysed from intestine and hepatopancreas homogenates. It was seen from the results that these was considerable variation between each specimen and as leucine aminopeptidase activity had not been indentified by histochemical technique in the hepatopancreas, the intestines and hepatopancreas were examined separately in the remaining experiments.

The second experiment (Protocol 2) was an attempt to differentiate changes in activities of the two enzyme systems on two small groups of fish that had either been fed a high protein diet or a low protein diet. From the histological and histochemical results there was no significant changes indicated, and it was interesting to note when statistical analysis of variance (Kruskal-Wallis Test) was applied there was a significance at the 5% level of greater amounts of activity of both alkaline phosphatase and leucine aminopeptidase in the hepatopancreas from the low protein fed fish. These results should be viewed with caution however, because of the small samples in each group. The reason for the low numbers of samples was because of an accident

which had occurred when the diet was formulated and mouldy wheat middlings had inadvertantly been used. (Watret, Personel communication). The experiment was stopped and all the remaining fish on test destroyed.

Fish (1960) made a comparative study of enzymes in the digestive tracts of a herbivorous fish and carnivorous fish. Although that experiment was not identical to the work reported here, and Fish only identified "alkaline protease", it was appropriate to mention that he did not find any significant alteration between the two species. Al-Hussaini (1949b) examined proteolytic enzymes from three cyprinids (C. carpio, R. rutilus and G. gobio) and he was unable to find any significant changes in amounts of enzyme activity, despite the differing feeding habits of the fish.

The third experiment (Protocol 3) was made to differentiate changes in activities of the same two enzymes in groups of fish that had been fed and starved at a temperature of 17°C. In this experiment, alkhaline phosphatase and leucine aminopeptidase activities were measured from serum samples as well as tissue homogenates.

Statistical analysis was applied to the results of this experiment and the only significant changes were an increase in both enzyme activities in the hepatopancreas of starved fish than fed fish. The statistical changes which were found form the results of experiment 4, where starvation and feeding was carried out at 27°C, gave more information than the results of experiment 3, where no real information had been gained.

In experiment 4 there were significantly greater amounts of activity of alkaline phosphatase in the intestines of fed fish than starved fish, yet the activity of this enzyme in the

hepatopancreas of starved fish was greater than in fed fish. However, when the results of the serum alkaline phosphatase activity were statistically measured, there was greater activity in the fed than the starved fish, which meant that the overall trend was to secrete more alkaline phosphatase enzyme in the fish fed at 27°C, than starved at 27°C.

The activity of leucine aminopeptidase was contradictory, as the activity in hepatopancreas of starved fish was considerably greater than in fed fish, yet in the intestine, the opposite occurred. There was no overall trend indicated in the results of the serum estimations of this enzyme. To gain more information from statistical analysis of the enzyme results, further analysis was made, this time to compare and contrast the results of the two experiments, 3 and 4. Although there were no significant differences indicated in the serum enzymes, there was certainly a significant increase in activities of both enzymes from tissue homogenates in both fed and starved fish, held at 27°C than at 17°C. This was particularly noticeable with alkaline phosphatase.

Schlottke (1939) found that variations in levels of activity of proteolytic enzymes in carp, were not clearly correlated with the nature of their diet, although he did find that artificial food stimulated production of enzymes more readily than natural food.

Alkaline phosphatase is an enzyme found in most tissues of vertebrates in a variety of forms (Moss, 1974), and particularly occurs at sites of neutral moccopolysaccharide (Moog and Wenger, 1952). The enzyme has been demonstrated in the intestine and hepatopancreas of carp in these experiments, significantly more in the fish fed and kept at the higher temperature. Makino (1963) demonstrated greater levels of activity of this enzyme in the

intestine of fed, than starved loach, but gave no reasons for this increase. Western (1971) demonstrated the presence of the enzyme histochemically, only when the microvilli of the absorptive cells were identified, these were in the sites of most active absorption. Moog and Wenger (1952) and Srivastava (1966) have concluded that neutral mucopolysaccharides occurred at sites of high alakline phosphatase activity, and in this experiment, the histologicial results showed an increase in PAS positive material at the edge of the absorptive cells in the intestine of fed fish held at 27°C which agrees with the increase in alkaline phosphatase levels measured biochemically. This is consistent with the function of alkaline phosphatase which is to aid protein hydrolysis, and activate the transport of water, amino-acids and carbohydrates from the intestine of the liver (Utida and Isono, 1967). The effect of the higher temperature appeared to have stimulated more enzyme activity, than in the experiment made at the lower temperature.

Western (1971) demonstrated leucine aminopeptidase in the intestine of Cottid fish, at similar sites to alkaline phosphatase. In the intestine of the carp, the activity of this enzyme was at its highest in the fed fish at the high temperature.

Leucine aminopeptidase is a complexed proteolytic enzyme, part of the Peptidase group of enzymes. It occurs in various tissues of the vertebrate body, and particularly in the intestine where it functions in protein absorption (Nachlas et al 1957). In this study only one action of leucine aminopeptidase was investigated and the substrate used was L-leucyl β naphthylamide.

This attempts to explain why it was feasible that an increase in activity of this enzyme could be measured in the intestine, but it did not explain why there should be an increase in activity of

the enzyme in the hepatopancreas of starved fish particularly at the higher temperature. Siebert et al (1964) concluded that large quantities of autolytic enzymes present in fish muscle were there in readiness for periods of depletion, presumably this effect could also occur in liver. Parker and Vanstone (1966) showed that there was a loss of lipid after starvation in salmonids, and protein was consumed later. It was possible, therefore, that the hepatopancreas of carp was depleted in lipid, (and it had already been seen from the histology results that there was glycogen depletion at the higher temperature) that there was an accumulation of protein in the hepatocytes, and coupled with this, an increased amount of leucine aminopeptidase for an imminent breakdown of protein or autolysis of tissue. Creach (1966) showed that many of the free amino acids of liver, kidney and spleen increased during starvation, and he measured a definite increase in leucine in carp liver after starvation, but a decrease in the intestine. His fish had been starved for a period of 8 months. Bouche and Vellas (1975) showed that after short term starvation in the carp, the function of the liver remained to supply large amounts of plasma proteins.

The effects that a higher temperature had on increasing the metabolic rate of carp has been mentioned previously in the discussion, and Love (1958) showed that depletion in starved cod occurred more rapidly at a higher temperature. Vellas (1965) showed that there was an increased metabolic rate of liver enzymes in carp held at 20°C compared with those held at 8°C. In fact many research workers have recorded an increase in metabolism in fish when the temperature was raised. Phillips et al (1957) observed a capsule of food placed in the intestine of Salvelinus fontinalis to move more rapidly in a fish that was

placed in warmer water, than one in cold water, and they considered this to be due to an increase in peristaltic movement.

Smit (1967) studied gastric secretion in the bullhead (Ictalurus nebulosus) and recorded a maximum increase in secretion of gastric juice at a temperature of 25°C in a fish that can survive between temperatures of 10 - 30°C. Rozin and Mayer (1961) made a study of the regulation of food intake in the goldfish and observed that the fish consumed three times the amount of food at 20°C than at 10°C. The latter temperature was probably low for a goldfish to feed. However, in an earlier study it was observed, in the perch, which does feed at low temperatures normally, three times the amount of food was consumed when the fish were placed at a higher temperature (Pearse and Achtenberg, 1917).

Therefore, the experiments showed that metabolism of certain fish was increased when the environmental temperature was raised, thus contributing to an increase in enzyme production.

6.2.2. Serum protein studies

The examination of serum protein taken from carp subjected to trials previously reported and discussed, was an extra study conceived after the previous data had been concluded. It was considered that this may be a suitable method for examining carp, or other fish whilst they are undergoing nutritional trials, and the method might be both qualitative and quantitative.

Electrophoretic investigations are indicators with considerable sensitivity for protein composition of the blood serum. The method can show changes that arise in the animal organism and are more frequently used in analytical, diagnostical and prognostical studies of higher vertrebates.

The method of using electrophoresis on polyacrylamid gel (LKB Ampholine PAG plates) was not considered successful because of the variabilities incurred due to the senstivity of the method. From this experience it appeared more feasible to examine a simpler system. A system which is in routine use in many laboratories, and which is part automated, using cellulose acetate Phoroslide strips was used. This system, also was not successful, because it appeared that carp albumin was not bound by the ponceau S dye, and the remainder of the results were inconclusive as there was little difference detected between samples from each group. The third method using agarose gel electrophoresis showed more possibilities, as the protein bands were more distinct, and in this preliminary study it was seen visually that there were greater amounts of protein present in the carp serum held at 27°C than those held at 17°C.

It has been found in previous work on teleosts (Sorvachev, 1959; Lysak and Wojcick, 1960; and Kosmina, 1966) that there is a decrease in albumin following starvation. Sorvachev concluded that first there was a decrease in albumin, the the α and β globulins but the γ globulins were increased, and after six months starvation the total protein levels dropped from 3.9% to 2.8%. Courdier et al (1957) found that there were considerable variabilities of the presence or even absence of fractions of the albumin band in cyprinids, and although these authors were considering the technique for differentiating different species of cyprinids, it seems feasible that variations in results could be caused by having a mixed stock of carp, this conclusions was also made by Fujiya (1961) and Thurston (1967). Meisner and Hickman (1962) suggested that variables in proteins could be attributed to fresh or frozen serum samples. These authors

including Poston (1965) also considered that the trout liver was where most of the synthesis of albumin and globulins occurred, there was metabolic overcompensation indicated at a lower temperature, thus giving an increase in albumins and globulins. It appeared that the reverse situation occurred in the carp, as seen by these current results, and agrees with Sorvachev (1959).

The results obtained in this preliminary study correlate reasonably well with results obtained by Liebmann et al (1960) and Riedmüller (1965) who studied the effects of starvation and feeding on carp serum proteins, and it was considered that a further study using larger carp would be more feasible.

6.3. Conclusion

It can be concluded that there were no significant differences in the digestive tracts of carp fed diets containing high and low proteins, nor were there significant differences detected in fish fed or starved at a temperature of 17°C. However, when the temperature was raised to 27°C, significant changes were observed and could be detected histologically and correlated with the biochemical studies on certain enzymes. The increase in temperature also raised the metabolic activity of the carp, thus making it more efficient in food conversion, yet if the fish were withheld food, at the increased temperature a rapid deterioration in cellular structure occurred. This was because of a hyper-activity of proteolytic enzymes which caused autolytic changes in the cells.

There was considerable evidence for further studies on serum protein levels in carp, when investigating changes in diets or water quality, as electrophoresis was readily adapted for samples from different animal species and could be fitted into a routine laboratory system.

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APPENDICES.

Appendix 1

Report on examination of water sample from Cromwell House, Huntingdon. The site of the experiments. 22.8.74.

Clinical Examination	Results in mg./L except where stated.
Appearance	Clear and bright.
Colour ° hazen.	Less than 5.
Turbility (Formazin units)	1.0
pH.	7.55
Electrical conductivity (μ mhos)	1,000.
Total solids	686.
Permanganate values (4 hrs)	1.97
Ammoniacal nitrogen	0.016
Albuminoid nitrogen	0.256.
Nitrite	nil.
Nitrate	5.04
Chloride	7.4
Sulphate	nil
Total hardness	236
Total alkalinity	200
Non carbonate hardness	3.6
Calcium hardness	176
Phosphate	0.062
Silica	8.0
Fluoride	0.352
Residual Chlorine free combined total	0.3

Metals	Results mg/L
Sodum	75.0
Potassium	12.0
Iron	0.05
Manganese	0.005
Copper	0.078
Lead	not detected
Zinc	0.029
Cadmium	not detected

Bacterial Examination

Total coliforms per 100 ml. = 0

Total E. coli per 100 ml. = 0

Plate count 37°C at 1 day = 0

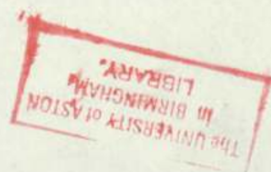
Plate count 37°C at 2 days = 5

Plate count 20°C at 3 days = 6

Appendix 2

Statistics

K R U S K A L - W A L L I S T E S T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EXPERIMENT II (A) ON: 11/06/76 AT: 14/02/32

PARAMETER : HEPATOPANCREAS

ALKALINE PHOSPHATASE (17°C)
GROUP 1 – HIGH PROTEIN DIET
GROUP 2 – LOW PROTEIN DIET

K R U S K A L - W A L L I S   T E S T

GROUP	PERCENTILES 2.5	97.5
1	0.12000	0.26000
2	0.33000	0.51000

GROUP	SIZE	MEAN RANK	MEAN OF OBSERVATIONS
1	3	2.0000	0.16667
2	3	5.0000	0.40000

KRUSKAL-WALLIS ANALYSIS OF VARIANCE
H-STATISTIC = 3.9706 WITH 1 DEGREES OF FREEDOM
THIS IS SIGNIFICANT AT THE 5% LEVEL (P=.05)
THE 5% SIGNIFICANCE POINT IS 3.84100

MULTIPLE COMPARISONS

GROUP COMPARISON	ABSOLUTE DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% "L.S.D.'S"	0.1% "L.S.D.'S"
1 , 2	3.0000	2.9939	3.9349	5.0271

COMPARISON WITH GROUP 1

GROUP	DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% %L.S.D.'S"	0.1% "L.S.D.'S"
2	-3.0000	2.9939	3.9349	5.0271

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EXPERIMENT II (A) ON: 11/06/76 AT: 14/02/48

PARAMETER : INTESTINE

ALKALINE PHOSPHATASE (17°C)

GROUP 1 - HIGH PROTEIN DIET

GROUP 2 - LOW PROTEIN DIET

K R U S K A L - W A L L I S T E S T

GROUP	PERCENTILES 2.5	97.5
1	0.73000	1.2900
2	0.42000	1.1800

GROUP	SIZE	MEAN RANK	MEAN OF OBSERVATIONS
1	3	4.0000	1.0177
2	3	3.0000	0.80000

KRUSKAL-WALLIS ANALYSIS OF VARIANCE
H-STATISTIC = 0.42857 WITH 1 DEGREES OF FREEDOM
THIS IS NOT SIGNIFICANT
THE 5% SIGNIFICANCE POINT IS 3.84100

MULTIPLE COMPARISONS

GROUP COMPARISON	ABSOLUTE DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% "L.S.D.'S"	0.1% "L.S.D.'S"
1 , 2	1.0000	2.9939	3.9349	5.0271

COMPARISON WITH GROUP 1

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GROUP	DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% %L.S.D.'S"	0.1% "L.S.D.'S"
2	1.0000	2.9939	3.9349	5.0271

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EXPERIMENT II (B) ON: 11/06/76 AT: 14/03/05

PARAMETER : HEPATOPANCREAS

LEUCINE AMINOPEPTIDASE (17°C)

GROUP 1 - HIGH PROTEIN DIET

GROUP 2 - LOW PROTEIN DIET

K R U S K A L - W A L L I S T E S T

GROUP	PERCENTILES 2.5	97.5
1	1.0500	1.3900
2	1.9800	2.1800

GROUP	SIZE	MEAN RANK	MEAN OF OBSERVATIONS
1	3	2.0000	1.2067
2	3	5.0000	2.0833

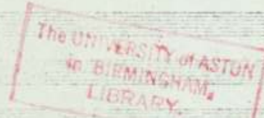
KRUSKAL-WALLIS ANALYSIS OF VARIANCE
H-STATISTIC = 3.8571 WITH 1 DEGREES OF FREEDOM
THIS IS SIGNIFICANT AT THE 5% LEVEL (P=.05)
THE 5% SIGNIFICANCE POINT IS 3.84100

MULTIPLE COMPARISONS

GROUP COMPARISON	ABSOLUTE DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% "L.S.D.'S"	0.1% "L.S.D.'S"
1 , 2	3.0000	2.9939	3.9349	5.0271

COMPARISON WITH GROUP 1

GROUP	DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% %L.S.D.'S"	0.1% "L.S.D.'S"
2	-3.0000	2.9939	3.9349	5.0271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EXPERIMENT II (B) ON: 11/06/76 AT: 14/03/22

PARAMETER : INTESTINE

LEUCINE AMINO PEPTIDASE (17°C)

GROUP 1 - HIGH PROTEIN DIET

GROUP 2 - LOW PROTEIN DIET

K R U S K A L - W A L L I S T E S T

GROUP	PERCENTILES 2.5	97.5
1	3.3500	8.2200
2	3.0600	7.0100

GROUP	SIZE	MEAN RANK	MEAN OF OBSERVATIONS
1	3	3.6667	5.2400
2	3	3.3333	5.6500

KRUSKAL-WALLIS ANALYSIS OF VARIANCE
H-STATISTIC = 0.47619E-01 WITH 1 DEGREES OF FREEDOM
THIS IS NOT SIGNIFICANT
THE 5% SIGNIFICANCE POINT IS 3.84100

MULTIPLE COMPARISONS

GROUP COMPARISON	ABSOLUTE DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% "L.S.D.'S"	0.1% "L.S.D.'S"
1 , 2	0.33333	2.9939	3.9349	5.0271

COMPARISON WITH GROUP 1

GROUP	DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% %L.S.D.'S"	0.1% "L.S.D.'S"
2	0.33333	2.9939	3.9349	5.0271

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EXPERIMENT III (A) ON: 11/06/76 AT: 14/03/39

PARAMETER : HEPATOPANCREAS

ALKALINE PHOSPHATASE (17°C)

GROUP 1 - FED

GROUP 2 - STARVED

K R U S K A L - W A L L I S T E S T

GROUP	PERCENTILES 2.5	97.5
1	0.10000	0.66000
2	0.15000	1.0700

GROUP	SIZE	MEAN RANK	MEAN OF OBSERVATIONS
1	8	5.8125	0.36375
2	6	11.187	0.68750

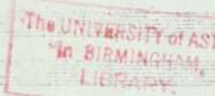
KRUSKAL-WALLIS ANALYSIS OF VARIANCE
H-STATISTIC = 5.1059 WITH 1 DEGREES OF FREEDOM
THIS IS SIGNIFICANT AT THE 5% LEVEL (P=.05)
THE 5% SIGNIFICANCE POINT IS 3.84100

MULTIPLE COMPARISONS

GROUP COMPARISON	ABSOLUTE DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% "L.S.D.'S"	0.1% "L.S.D.'S"
1 , 2	5.3750	4.6657	6.1321	7.8341

COMPARISON WITH GROUP 1

GROUP	DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% %L.S.D.'S"	0.1% "L.S.D.'S"
2	-5.3750	4.6657	6.1321	7.8341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EXPERIMENT III (A) ON: 11/06/76 AT: 14/03/56

PARAMETER : INTESTINE

ALKALINE PHOSPHATASE (17°C)

GROUP 1 - FED

GROUP 2 - STARVED

K R U S K A L - W A L L I S T E S T

GROUP	PERCENTILES 2.5	97.5
1	1.5000	3.3500
2	1.2200	4.1800

GROUP	SIZE	MEAN RANK	MEAN OF OBSERVATIONS
1	8	8.2500	2.2137
2	8	8.7500	2.4375

KRUSKAL-WALLIS ANALYSIS OF VARIANCE
H-STATISTIC = 0.44183E-01 WITH 1 DEGREES OF FREEDOM
THIS IS NOT SIGNIFICANT
THE 5% SIGNIFICANCE POINT IS 3.84100

MULTIPLE COMPARISONS

GROUP COMPARISON	ABSOLUTE DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% "L.S.D.'S"	0.1% "L.S.D.'S"
1 , 2	0.50000	4.6657	6.1321	7.8341

COMPARISON WITH GROUP 1

GROUP	DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% %L.S.D.'S"	0.1% "L.S.D.'S"
2	-0.50000	4.6657	6.1321	7.8341

EXPERIMENT III (B) ON: 11/06/76 AT: 14/04/13

PARAMETER : HEPATOPANCREAS

LEUCINE AMINOPEPTIDASE (17°C)

GROUP 1 - FED

GROUP 2 - STARVED

K R U S K A L - W A L L I S T E S T

GROUP	PERCENTILES 2.5	97.5
1	0.64000	3.1900
2	1.8100	9.3600

GROUP	SIZE	MEAN RANK	MEAN OF OBSERVATIONS
1	8	5.7500	1.8600
2	8	11.250	4.3762

KRUSKAL-WALLIS ANALYSIS OF VARIANCE
H-STATISTIC = 5.3382 WITH 1 DEGREES OF FREEDOM
THIS IS SIGNIFICANT AT THE 5% LEVEL (P=.05)
THE 5% SIGNIFICANCE POINT IS 3.84100

MULTIPLE COMPARISONS

GROUP COMPARISON	ABSOLUTE DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% "L.S.D.'S"	0.1% "L.S.D.'S"
1 , 2	5.5000	4.6657	6.1321	7.8341

COMPARISON WITH GROUP 1

GROUP	DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% %L.S.D.'S"	0.1% "L.S.D.'S"
2	-5.5000	4.6657	6.1321	7.8341

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EXPERIMENT III (B) ON: 11/06/76 AT: 14/04/30

PARAMETER : INTESTINE

LEUCINE AMINOPEPTIDASE (17°C)

GROUP 1 - FED

GROUP 2 - STARVED

K R U S K A L - W A L L I S T E S T

GROUP	PERCENTILES 2.5	PERCENTILES 97.5
1	1.1200	22.300
2	1.0600	21.410

GROUP	SIZE	MEAN RANK	MEAN OF OBSERVATIONS
1	8	8.6250	9.8800
2	8	8.3750	9.6562

KRUSKAL-WALLIS ANALYSIS OF VARIANCE
H-STATISTIC = 0.11029E-01 WITH 1 DEGREES OF FREEDOM
THIS IS NOT SIGNIFICANT
THE 5% SIGNIFICANCE POINT IS 3.84100

MULTIPLE COMPARISONS

GROUP COMPARISON	ABSOLUTE DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% "L.S.D.'S"	0.1% "L.S.D.'S"
1 , 2	0.25000	4.6657	6.1321	7.8341

COMPARISON WITH GROUP 1

GROUP	DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% %L.S.D.'S"	0.1% "L.S.D.'S"
2	0.25000	4.6657	6.1321	7.8341

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EXPERIMENT III (C) ON: 11/06/76 AT: 14/04/47

PARAMETER : ALKALINE PHOSPHATASE

K R U S K A L - W A L L I S T E S T

SERUM (17°C)

GROUP 1 - FED

GROUP 2 - STARVED

GROUP	PERCENTILES 2.5	97.5
1	12.000	17.000
2	10.000	14.000

GROUP	SIZE	MEAN RANK	MEAN OF OBSERVATIONS
1	8	9.0000	13.375
2	8	8.0000	12.750

KRUSKAL-WALLIS ANALYSIS OF VARIANCE

H-STATISTIC = 0.21127 WITH 1 DEGREES OF FREEDOM

THIS IS NOT SIGNIFICANT

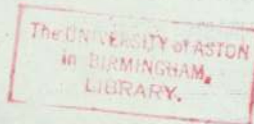
THE 5% SIGNIFICANCE POINT IS 3.84100

MULTIPLE COMPARISONS

GROUP COMPARISON	ABSOLUTE DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% "L.S.D.'S"	0.1% "L.S.D.'S"
1 , 2	1.0000	4.6657	6.1321	7.8341

COMPARISON WITH GROUP 1

GROUP	DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% %L.S.D.'S"	0.1% "L.S.D.'S"
2	1.0000	4.6657	6.1321	7.8341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EXPERIMENT III (C) ON: 11/06/76 AT: 14/05/05

PARAMETER : LEUCINE AMINOPEPTIDASE

SERUM (17°C)

GROUP 1 - FED

GROUP 2 - STARVED

K R U S K A L - W A L L I S T E S T

GROUP	PERCENTILES 2.5	97.5
1	263.00	412.00
2	181.00	376.00

GROUP	SIZE	MEAN RANK	MEAN OF OBSERVATIONS
1	8	9.7500	347.25
2	8	7.2500	317.25

KRUSKAL-WALLIS ANALYSIS OF VARIANCE
H-STATISTIC = 1.1029 WITH 1 DEGREES OF FREEDOM
THIS IS NOT SIGNIFICANT
THE 5% SIGNIFICANCE POINT IS 3.84100

MULTIPLE COMPARISONS

GROUP COMPARISON	ABSOLUTE DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% "L.S.D.'S"	0.1% "L.S.D.'S"
1 , 2	2.5000	4.6657	6.1321	7.8341

COMPARISON WITH GROUP 1

GROUP	DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% %L.S.D.'S"	0.1% "L.S:D.'S"
2	2.5000	4.6657	6.1321	7.8341

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EXPERIMENT IV (A) ON: 11/06/76 AT: 14/05/22

PARAMETER : HEPATOPANCREAS

ALKALINE PHOSPHATASE (27°C)
GROUP 1 – FED
GROUP 2 – STARVED

K R U S K A L - W A L L I S T E S T

GROUP	PERCENTILES 2.5	97.5
1	0.92000	1.7500
2	0.88000	7.3100

GROUP	SIZE	MEAN RANK	MEAN OF OBSERVATIONS
1	8	6.1250	1.3275
2	8	10.875	3.0500

KRUSKAL-WALLIS ANALYSIS OF VARIANCE
H-STATISTIC = 3.9816 WITH 1 DEGREES OF FREEDOM
THIS IS SIGNIFICANT AT THE 5% LEVEL (P=.05)
THE 5% SIGNIFICANCE POINT IS 3.84100

MULTIPLE COMPARISONS

GROUP COMPARISON	ABSOLUTE DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% "L.S.D.'S"	0.1% "L.S.D.'S"
1 , 2	4.7500	4.6657	6.1321	7.8341

COMPARISON WITH GROUP 1

GROUP	DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% %L.S.D.'S"	0.1% "L.S.D.'S"
2	-4.7500	4.6657	6.1321	7.8341

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EXPERIMENT IV (A) ON: 11/06/76 AT: 14/05/39

PARAMETER : INTESTINE

ALKALINE PHOSPHATASE (27°C)

GROUP 1 - FED

GROUP 2 - STARVED

K R U S K A L - W A L L I S   T E S T

GROUP	PERCENTILES 2.5	97.5
1	3.2400	9.1700
2	1.1400	3.2000

GROUP	SIZE	MEAN RANK	MEAN OF OBSERVATIONS
1	8	12.500	5.4650
2	8	4.5000	2.0025

KRUSKAL-WALLIS ANALYSIS OF VARIANCE
H-STATISTIC = 11.311 WITH 1 DEGREES OF FREEDOM
THIS IS SIGNIFICANT AT THE 0.1% LEVEL (P=.001)
THE 0.1% SIGNIFICANCE POINT IS 10.8270

MULTIPLE COMPARISONS

GROUP COMPARISON	ABSOLUTE DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% "L.S.D.'S"	0.1% "L.S.D.'S"
1 , 2	8.0000	4.6657	6.1321	7.8341

COMPARISON WITH GROUP 1

GROUP	DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% %L.S.D.'S"	0.1% "L.S.D.'S"
2	8.0000	4.6657	6.1321	7.8341

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EXPERIMENT IV (B) ON: 11/06/76 AT: 14/05/56

PARAMETER : HEPATOPANCREAS

Leucine Aminopeptidase (27°C)
Group 1 - Fed
Group 2 - Starved

K R U S K A L - W A L L I S T E S T

GROUP	PERCENTILES 2.5	97.5
1	1.9900	6.4600
2	3.4900	20.090

GROUP	SIZE	MEAN RANK	MEAN OF OBSERVATIONS
1	8	5.1250	5.8050
2	8	11.875	11.114

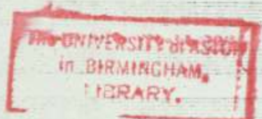
KRUSKAL-WALLIS ANALYSIS OF VARIANCE
H-STATISTIC = 8.0404 WITH 1 DEGREES OF FREEDOM
THIS IS SIGNIFICANT AT THE 1% LEVEL (P=.01)
THE 1% SIGNIFICANCE POINT IS 6.63500

MULTIPLE COMPARISONS

GROUP COMPARISON	ABSOLUTE DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% "L.S.D.'S"	0.1% "L.S.D.'S"
1 , 2	6.7500	4.6657	6.1321	7.8341

COMPARISON WITH GROUP 1

GROUP	DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% %L.S.D.'S"	0.1% "L.S.D.'S"
2	-6.7500	4.6657	6.1321	7.8341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EXPERIMENT IV (B) ON: 11/06/76 AT: 14/06/13

PARAMETER : INTESTINE

LEUCINE AMINOPEPTIDASE (27°C)

K R U S K A L - W A L L I S T E S T

GROUP 1 - FED

GROUP 2 - STARVED

GROUP	PERCENTILES 2.5	97.5
1	21.120	46.720
2	9.9400	55.260

GROUP	SIZE	MEAN RANK	MEAN OF OBSERVATIONS
1	8	11.125	31.471
2	8	5.8750	19.257

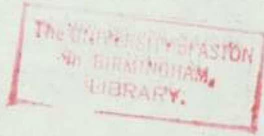
KRUSKAL-WALLIS ANALYSIS OF VARIANCE
H-STATISTIC = 4.8640 WITH 1 DEGREES OF FREEDOM
THIS IS SIGNIFICANT AT THE 5% LEVEL (P=.05)
THE 5% SIGNIFICANCE POINT IS 3.84100

MULTIPLE COMPARISONS

GROUP COMPARISON	ABSOLUTE DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% "L.S.D.'S"	0.1% "L.S.D.'S"
1 , 2	5.2500	4.6657	6.1321	7.8341

COMPARISON WITH GROUP 1

GROUP	DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% %L.S.D.'S"	0.1% "L.S.D.'S"
2	5.2500	4.6657	6.1321	7.8341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EXPERIMENT IV(C) ON: 11/06/76 AT: 14/06/30

PARAMETER : ALKALINE PHOSPHATASE

SERUM (27°C)

Group 1 - FED

Group 2 - STARVED

K R U S K A L - W A L L I S T E S T

GROUP	PERCENTILES 2.5	97.5
1	12.000	18.000
2	8.0000	14.000

GROUP	SIZE	MEAN RANK	MEAN OF OBSERVATIONS
1	8	11.437	14.375
2	8	5.5625	11.250

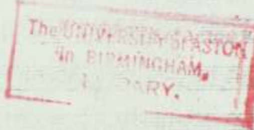
KRUSKAL-WALLIS ANALYSIS OF VARIANCE
H-STATISTIC = 6.2378 WITH 1 DEGREES OF FREEDOM
THIS IS SIGNIFICANT AT THE 5% LEVEL (P=.05)
THE 5% SIGNIFICANCE POINT IS 3.84100

MULTIPLE COMPARISONS

GROUP COMPARISON	ABSOLUTE DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% "L.S.D.'S"	0.1% "L.S.D.'S"
1 , 2	5.8750	4.6657	6.1321	7.8341

COMPARISON WITH GROUP 1

GROUP	DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% %L.S.D.'S"	0.1% "L.S.D.'S"
2	5.8750	4.6657	6.1321	7.8341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EXPERIMENT IV (C) ON: 11/06/76 AT: 14/07/17

PARAMETER : LEUCINE AMINOPEPTIDASE

SERUM (27°C)

GROUP 1 – FED

GROUP 2 – STARVED

K R U S K A L - W A L L I S   T E S T

GROUP	PERCENTILES 2.5	97.5
1	251.00	389.00
2	171.00	362.00

GROUP	SIZE	MEAN RANK	MEAN OF OBSERVATIONS
1	8	10.375	311.75
2	6	6.6250	248.67

KRUSKAL-WALLIS ANALYSIS OF VARIANCE
H-STATISTIC = 2.4816 WITH 1 DEGREES OF FREEDOM
THIS IS NOT SIGNIFICANT
THE 5% SIGNIFICANCE POINT IS 3.84100

MULTIPLE COMPARISONS

GROUP COMPARISON	ABSOLUTE DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% "L.S.D.'S"	0.1% "L.S.D.'S"
1 , 2	3.7500	4.6657	6.1321	7.8341

COMPARISON WITH GROUP 1

GROUP	DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% "L.S.D.'S"	0.1% "L.S.D.'S"
2	3.7500	4.6657	6.1321	7.8341

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EXPERIMENT IV (C) ON: 11/06/76 AT: 14/07/17

PARAMETER : LEUCINE AMINOPEPTIDASE

SERUM (27°C)

GROUP 1 - FED

GROUP 2 - STARVED

K R U S K A L - W A L L I S T E S T

GROUP	PERCENTILES 2.5	97.5
1	231.00	389.00
2	171.00	362.00

GROUP	SIZE	MEAN RANK	MEAN OF OBSERVATIONS
1	8	10.375	311.75
2	6	6.6250	248.67

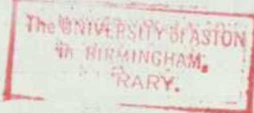
KRUSKAL-WALLIS ANALYSIS OF VARIANCE
H-STATISTIC = 2.4816 WITH 1 DEGREES OF FREEDOM
THIS IS NOT SIGNIFICANT
THE 5% SIGNIFICANCE POINT IS 3.84100

MULTIPLE COMPARISONS

GROUP COMPARISON	ABSOLUTE DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% "L.S.D.'S"	0.1% "L.S.D.'S"
1 , 2	3.7500	4.6657	6.1321	7.8341

COMPARISON WITH GROUP 1

GROUP	DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% %L.S.D.'S"	0.1% "L.S.D.'S"
2	3.7500	4.6657	6.1321	7.8341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ON: 16/07/76 AT: 09/41/35

PARAMETER :

K R U S K A L - W A L L I S   T E S T

ALKALINE PHOSPHATASE

STARVED CARP, INTESTINE

GROUP 1 = 17°C

GROUP 2 = 27°C

GROUP	PERCENTILES 2.5	97.5
1	1.2200	4.1800
2	1.1400	3.2000

GROUP	SIZE	MEAN RANK	MEAN OF OBSERVATIONS
1	8	9.8125	2.4375
2	8	7.1875	2.0025

KRUSKAL-WALLIS ANALYSIS OF VARIANCE
H-STATISTIC = 1.2196 WITH 1 DEGREES OF FREEDOM
THIS IS NOT SIGNIFICANT
THE 5% SIGNIFICANCE POINT IS 3.84100

MULTIPLE COMPARISONS

GROUP COMPARISON	ABSOLUTE DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% "L.S.D.'S"	0.1% "L.S.D.'S"
1 , 2	2.6250	4.6657	6.1321	7.8341

COMPARISON WITH GROUP 1

GROUP	DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% %L.S.D.'S"	0.1% "L.S.D.'S"
2	2.6250	4.6657	6.1321	7.8341

ON: 16/07/76 AT: 09/41/35

PARAMETER :

K R U S K A L - W A L L I S   T E S T

ALKALINE PHOSPHATASE
STARVED CARP, INTESTINE
GROUP 1 = 17°C
GROUP 2 = 27°C

GROUP	PERCENTILES 2.5	97.5
1	1.2200	4.1800
2	1.1400	3.2000

GROUP	SIZE	MEAN RANK	MEAN OF OBSERVATIONS
1	8	9.8125	2.4375
2	8	7.1875	2.0025

KRUSKAL-WALLIS ANALYSIS OF VARIANCE
H-STATISTIC = 1.2196 WITH 1 DEGREES OF FREEDOM
THIS IS NOT SIGNIFICANT
THE 5% SIGNIFICANCE POINT IS 3.84100

MULTIPLE COMPARISONS

GROUP COMPARISON	ABSOLUTE DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% "L.S.D.'S"	0.1% "L.S.D.'S"
1 , 2	2.6250	4.6657	6.1321	7.8341

COMPARISON WITH GROUP 1

GROUP	DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% XL.S.D.'S"	0.1% "L.S.D.'S"
2	2.6250	4.6657	6.1321	7.8341

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PARAMETER : INTESTINE

ALKALINE PHOSPHATASE

FED CARP

GROUP 1 = 17°C

GROUP 2 = 27°C

K R U S K A L - W A L L I S   T E S T

GROUP	PERCENTILES 2.5	97.5
1	1.5000	3.3500
2	3.2400	9.1700

GROUP	SIZE	MEAN RANK	MEAN OF OBSERVATIONS
1	8	4.6250	2.2137
2	8	12.375	5.4650

KRUSKAL-WALLIS ANALYSIS OF VARIANCE
H-STATISTIC = 10.615 WITH 1 DEGREES OF FREEDOM
THIS IS SIGNIFICANT AT THE 1% LEVEL (P=.01)
THE 1% SIGNIFICANCE POINT IS 6.63500

MULTIPLE COMPARISONS

GROUP COMPARISON	ABSOLUTE DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% "L.S.D.'S"	0.1% "L.S.D.'S"
1 , 2	7.7500	4.6657	6.1321	7.8341

COMPARISON WITH GROUP 1

GROUP	DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% %L.S.D.'S"	0.1% "L.S.D.'S"
2	-7.7500	4.6657	6.1321	7.8341

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PARAMETER : INTESTINE

ALKALINE PHOSPHATASE
FED CARP
GROUP 1 = 17°C
GROUP 2 = 27°C

K R U S K A L - W A L L I S   T E S T

GROUP	PERCENTILES 2.5	97.5
1	1.5000	3.3500
2	3.2400	9.1700

GROUP	SIZE	MEAN RANK	MEAN OF OBSERVATIONS
1	8	4.6250	2.2137
2	8	12.375	5.4650

KRUSKAL-WALLIS ANALYSIS OF VARIANCE
H-STATISTIC = 10.615 WITH 1 DEGREES OF FREEDOM
THIS IS SIGNIFICANT AT THE 1% LEVEL (P=.01)
THE 1% SIGNIFICANCE POINT IS 6.63500

MULTIPLE COMPARISONS

GROUP COMPARISON	ABSOLUTE DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% "L.S.D.'S"	0.1% "L.S.D.'S"
1 , 2	7.7500	4.6657	6.1321	7.8341

COMPARISON WITH GROUP 1

GROUP	DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% %L.S.D.'S"	0.1% "L.S.D.'S"
2	-7.7500	4.6657	6.1321	7.8341

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PARAMETER :

Alkaline Phosphatase
Hepatopancreas

Carp fed

Group 1 = 17°C

Group 2 = 27°C

K R U S K A L - W A L L I S   T E S T

GROUP	PERCENTILES 2.5	97.5
1	0.10000	0.66000
2	0.92000	1.7500

GROUP	SIZE	MEAN RANK	MEAN OF OBSERVATIONS
1	8	4.5000	0.36375
2	8	12.500	1.3275

KRUSKAL-WALLIS ANALYSIS OF VARIANCE
H-STATISTIC = 11.294 WITH 1 DEGREES OF FREEDOM
THIS IS SIGNIFICANT AT THE 0.1% LEVEL (P=.001)
THE 0.1% SIGNIFICANCE POINT IS 10.8270

MULTIPLE COMPARISONS

GROUP COMPARISON	ABSOLUTE DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% "L.S.D.'S"	0.1% "L.S.D.'S"
1 , 2	8.0000	4.6657	6.1321	7.8341

COMPARISON WITH GROUP 1

GROUP	DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% %L.S.D.'S"	0.1% "L.S.D.'S"
2	-8.0000	4.6657	6.1321	7.8341

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ON: 16/07/76 AT: 09/42/11

PARAMETER :

ALKALINE PHOSPHATASE
HEPATOPANCREAS

CARP FED

GROUP 1 = 17°C

GROUP 2 = 27°C

K R U S K A L - W A L L I S   T E S T

GROUP	PERCENTILES 2.5	97.5
1	0.10000	0.66000
2	0.92000	1.7500

GROUP	SIZE	MEAN RANK	MEAN OF OBSERVATIONS
1	8	4.5000	0.36375
2	8	12.500	1.3275

KRUSKAL-WALLIS ANALYSIS OF VARIANCE
H-STATISTIC = 11.294 WITH 1 DEGREES OF FREEDOM
THIS IS SIGNIFICANT AT THE 0.1% LEVEL (P=.001)
THE 0.1% SIGNIFICANCE POINT IS 10.8270

MULTIPLE COMPARISONS

GROUP COMPARISON	ABSOLUTE DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% "L.S.D.'S"	0.1% "L.S.D.'S"
1 , 2	8.0000	4.6657	6.1321	7.8341

COMPARISON WITH GROUP 1

GROUP	DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% %L.S.D.'S"	0.1% "L.S.D.'S"
2	-8.0000	4.6657	6.1321	7.8341

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ON: 16/07/78 AT: 09/41/53

PARAMETER : ALKALINE PHOSPHATASE

HEPATOPANCREAS
CARP STARVED
GROUP 1 = 17°C
GROUP 2 = 27°C

K R U S K A L - W A L L I S   T E S T

GROUP	PERCENTILES 2.5	97.5
1	0.15000	1.0700
2	0.88000	7.3100

GROUP	SIZE	MEAN RANK	MEAN OF OBSERVATIONS
1	8	4.8750	0.68750
2	8	12.125	3.0500

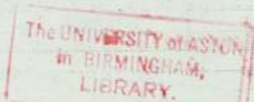
KRUSKAL-WALLIS ANALYSIS OF VARIANCE
H-STATISTIC = 9.2757 WITH 1 DEGREES OF FREEDOM
THIS IS SIGNIFICANT AT THE 1% LEVEL (P=.01)
THE 1% SIGNIFICANCE POINT IS 6.63500

MULTIPLE COMPARISONS

GROUP COMPARISON	ABSOLUTE DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% "L.S.D.'S"	0.1% "L.S.D.'S"
1 , 2	7.2500	4.6657	6.1321	7.8341

COMPARISON WITH GROUP 1

GROUP	DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% %L.S.D.'S"	0.1% "L.S.D.'S"
2	-7.2500	4.6657	6.1321	7.8341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PARAMETER : ALKALINE PHOSPHATASE

HEPATOPANCREAS
CARP STARVED
GROUP 1 = 17°C
GROUP 2 = 27°C

K R U S K A L - W A L L I S   T E S T

GROUP	PERCENTILES 2.5	97.5
1	0.15000	1.0700
2	0.88000	7.3100

GROUP	SIZE	MEAN RANK	MEAN OF OBSERVATIONS
1	8	4.8750	0.68750
2	8	12.125	3.0500

KRUSKAL-WALLIS ANALYSIS OF VARIANCE
H-STATISTIC = 9.2757 WITH 1 DEGREES OF FREEDOM
THIS IS SIGNIFICANT AT THE 1% LEVEL (P=.01)
THE 1% SIGNIFICANCE POINT IS 6.63500

MULTIPLE COMPARISONS

GROUP COMPARISON	ABSOLUTE DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% "L.S.D.'S"	0.1% "L.S.D.'S"
1 , 2	7.2500	4.6657	6.1321	7.8341

COMPARISON WITH GROUP 1

GROUP	DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% %L.S.D.'S"	0.1% "L.S.D.'S"
2	-7.2500	4.6657	6.1321	7.8341

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ON: 16/07/76 AT: 09/39/45

PARAMETER :

SERUM ALKALINE PHOSPHATASE

CARP FED

Group 1 = 17°C

Group 2 = 27°C

K R U S K A L - W A L L I S T E S T

GROUP	PERCENTILES 2.5	97.5
1	12.000	17.000
2	12.000	18.000

GROUP	SIZE	MEAN RANK	MEAN OF OBSERVATIONS
1	8	7.3125	13.375
2	8	9.6875	14.375

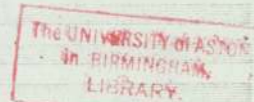
KRUSKAL-WALLIS ANALYSIS OF VARIANCE
H-STATISTIC = 1.0847 WITH 1 DEGREES OF FREEDOM
THIS IS NOT SIGNIFICANT
THE 5% SIGNIFICANCE POINT IS 3.84100

MULTIPLE COMPARISONS

GROUP COMPARISON	ABSOLUTE DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% "L.S.D.'S"	0.1% "L.S.D.'S"
1 , 2	2.3750	4.6657	6.1321	7.8341

COMPARISON WITH GROUP 1

GROUP	DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% %L.S.D.'S"	0.1% "L.S.D.'S"
2	-2.3750	4.6657	6.1321	7.8341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ON: 16/07/76 AT: 09/39/45

PARAMETER :

SERUM ALKALINE PHOSPHATASE

CARP FED

Group 1 = 17°C

Group 2 = 27°C

K R U S K A L - W A L L I S T E S T

GROUP	PERCENTILES 2.5	97.5
1	12.000	17.000
2	12.000	18.000

GROUP	SIZE	MEAN RANK	MEAN OF OBSERVATIONS
1	8	7.3125	13.375
2	8	9.6875	14.375

KRUSKAL-WALLIS ANALYSIS OF VARIANCE
H-STATISTIC = 1.0847 WITH 1 DEGREES OF FREEDOM
THIS IS NOT SIGNIFICANT
THE 5% SIGNIFICANCE POINT IS 3.84100

MULTIPLE COMPARISONS

GROUP COMPARISON	ABSOLUTE DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% "L.S.D.'S"	0.1% "L.S.D.'S"
1 , 2	2.3750	4.6657	6.1321	7.8341

COMPARISON WITH GROUP 1

GROUP	DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% %L.S.D.'S"	0.1% "L.S.D.'S"
2	-2.3750	4.6657	6.1321	7.8341

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PARAMETER : SERUM ALKALINE PHOSPHATASE

CARP STARVED

GROUP 1 = 17°C

GROUP 2 = 27°C

K R U S K A L - W A L L I S T E S T

GROUP	PERCENTILES 2.5	97.5
1	10.000	14.000
2	8.0000	14.000

GROUP	SIZE	MEAN RANK	MEAN OF OBSERVATIONS
1	8	10.375	12.750
2	8	6.6250	11.250

KRUSKAL-WALLIS ANALYSIS OF VARIANCE
H-STATISTIC = 2.6533 WITH 1 DEGREES OF FREEDOM
THIS IS NOT SIGNIFICANT
THE 5% SIGNIFICANCE POINT IS 3.84100

MULTIPLE COMPARISONS

GROUP COMPARISON	ABSOLUTE DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% "L.S.D.'S"	0.1% "L.S.D.'S"
1 , 2	3.7500	4.6657	6.1321	7.8341

COMPARISON WITH GROUP 1

GROUP	DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% %L.S.D.'S"	0.1% "L.S.D.'S"
2	3.7500	4.6657	6.1321	7.8341

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PARAMETER : SERUM ALKALINE PHOSPHATASE

CARP STARVED

GROUP 1 = 17°C

GROUP 2 = 27°C

K R U S K A L - W A L L I S   T E S T

GROUP	PERCENTILES 2.5	97.5
1	10.000	14.000
2	8.0000	14.000

GROUP	SIZE	MEAN RANK	MEAN OF OBSERVATIONS
1	8	10.375	12.750
2	8	6.6250	11.250

KRUSKAL-WALLIS ANALYSIS OF VARIANCE
H-STATISTIC = 2.6533 WITH 1 DEGREES OF FREEDOM
THIS IS NOT SIGNIFICANT
THE 5% SIGNIFICANCE POINT IS 3.84100

MULTIPLE COMPARISONS

GROUP COMPARISON	ABSOLUTE DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% "L.S.D.'S"	0.1% "L.S.D.'S"
1 , 2	3.7500	4.6657	6.1321	7.8341

COMPARISON WITH GROUP 1

GROUP	DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% %L.S.D.'S"	0.1% "L.S.D.'S"
2	3.7500	4.6657	6.1321	7.8341

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REFERENCE ONLY

ON: 16/07/76 AT: 09/39/09

PARAMETER : SERUM LEUCINE AMINOPEPTIDASE

CARP FED

GROUP 1 = 17°C

GROUP 2 = 27°C

K R U S K A L - W A L L I S   T E S T

GROUP	PERCENTILES 2.5	97.5
1	263.00	412.00
2	231.00	389.00

GROUP	SIZE	MEAN RANK	MEAN OF OBSERVATIONS
1	8	9.8750	347.25
2	8	7.1250	311.75

KRUSKAL-WALLIS ANALYSIS OF VARIANCE
H-STATISTIC = 1.3346 WITH 1 DEGREES OF FREEDOM
THIS IS NOT SIGNIFICANT
THE 5% SIGNIFICANCE POINT IS 3.84100

MULTIPLE COMPARISONS

GROUP COMPARISON	ABSOLUTE DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% "L.S.D.'S"	0.1% "L.S.D.'S"
1 , 2	2.7500	4.6657	6.1321	7.8341

COMPARISON WITH GROUP 1

GROUP	DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% %L.S.D.'S"	0.1% "L.S.D.'S"
2	2.7500	4.6657	6.1321	7.8341

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ON: 16/07/76 AT: 09/39/09

PARAMETER : SERUM LEUCINE AMINOPEPTIDASE

CARP FED

GROUP 1 = 17°C

GROUP 2 = 27°C

K R U S K A L - W A L L I S T E S T

GROUP	PERCENTILES 2.5	97.5
1	263.00	412.00
2	231.00	389.00

GROUP	SIZE	MEAN RANK	MEAN OF OBSERVATIONS
1	8	9.8750	347.25
2	8	7.1250	311.75

KRUSKAL-WALLIS ANALYSIS OF VARIANCE
H-STATISTIC = 1.3346 WITH 1 DEGREES OF FREEDOM
THIS IS NOT SIGNIFICANT
THE 5% SIGNIFICANCE POINT IS 3.84100

MULTIPLE COMPARISONS

GROUP COMPARISON	ABSOLUTE DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% "L.S.D.'S"	0.1% "L.S.D.'S"
1 , 2	2.7500	4.6657	6.1321	7.8341

COMPARISON WITH GROUP 1

GROUP	DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% %L.S.D.'S"	0.1% "L.S.D.'S"
2	2.7500	4.6657	6.1321	7.8341

The UNIVERSITY of ASTON In BIRMINGHAM, LIBRARY.

ON: 16/07/76 AT: 09/38/51

PARAMETER : SERUM LEUCINE AMINOPEPTIDASE

CARP FED

GROUP 1 = 17°C

GROUP 2 = 27°C

K R U S K A L - W A L L I S T E S T

PERCENTILES

GROUP	2.5	97.5
1	181.00	376.00
2	171.00	362.00

GROUP	SIZE	MEAN RANK	MEAN OF OBSERVATIONS
1	8	10.000	317.25
2	8	7.0000	248.87

KRUSKAL-WALLIS ANALYSIS OF VARIANCE
H-STATISTIC = 1.5882 WITH 1 DEGREES OF FREEDOM
THIS IS NOT SIGNIFICANT
THE 5% SIGNIFICANCE POINT IS 3.84100

MULTIPLE COMPARISONS

GROUP COMPARISON	ABSOLUTE DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% "L.S.D.'S"	0.1% "L.S.D.'S"
1 , 2	3.0000	4.6657	6.1321	7.8341

COMPARISON WITH GROUP 1

GROUP	DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% %L.S.D.'S"	0.1% "L.S.D.'S"
2	3.0000	4.6657	6.1321	7.8341

The UNIVERSITY of ASTON in BIRMINGHAM, LIBRARY.

ON: 16/07/76 AT: 09/38/51

PARAMETER :
SERUM
[illegible]

CARB FED

GROUP 1 = 17°C

GROUP 2 = 27°C

K R U S K A L - W A L L I S   T E S T

GROUP	PERCENTILES 2.5	97.5
1	181.00	376.00
2	171.00	362.00

GROUP	SIZE	MEAN RANK	MEAN OF OBSERVATIONS
1	8	10.000	317.25
2	8	7.0000	248.87

KRUSKAL-WALLIS ANALYSIS OF VARIANCE
H-STATISTIC = 1.5882 WITH 1 DEGREES OF FREEDOM
THIS IS NOT SIGNIFICANT
THE 5% SIGNIFICANCE POINT IS 3.84100

MULTIPLE COMPARISONS

GROUP COMPARISON	ABSOLUTE DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% "L.S.D.'S"	0.1% "L.S.D.'S"
1 , 2	3.0000	4.6657	6.1321	7.8341

COMPARISON WITH GROUP 1

GROUP	DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% %L.S.D.'S"	0.1% "L.S.D.'S"
2	3.0000	4.6657	6.1321	7.8341

ON: 16/07/76 AT: 09/40/03

PARAMETER :

Leucine aminopeptidase

INTESTINE

CARP STARVED

Group 1 = 17°C

Group 2 = 27°C

K R U S K A L - W A L L I S T E S T

GROUP	PERCENTILES 2.5	97.5
1	1.0600	21.410
2	9.9400	35.260

GROUP	SIZE	MEAN RANK	MEAN OF OBSERVATIONS
1	8	6.0000	9.6562
2	8	11.000	19.257

KRUSKAL-WALLIS ANALYSIS OF VARIANCE
H-STATISTIC = 4.4118 WITH 1 DEGREES OF FREEDOM
THIS IS SIGNIFICANT AT THE 5% LEVEL (P=.05)
THE 5% SIGNIFICANCE POINT IS 3.84100

MULTIPLE COMPARISONS

GROUP COMPARISON	ABSOLUTE DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% "L.S.D.'S"	0.1% "L.S.D.'S"
1 , 2	5.0000	4.6657	6.1321	7.8341

COMPARISON WITH GROUP 1

GROUP	DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% %L.S.D.'S"	0.1% "L.S.D.'S"
2	-5.0000	4.6657	6.1321	7.8341

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ON: 16/07/76 AT: 09/40/03

PARAMETER :

LEUCINE AMINOPEPTIDASE - INTESTINE

CARP STARVED

Group 1 = 17°C

Group 2 = 27°C

K R U S K A L - W A L L I S T E S T

GROUP	PERCENTILES 2.5	97.5
1	1.0600	21.410
2	9.9400	35.260

GROUP	SIZE	MEAN RANK	MEAN OF OBSERVATIONS
1	8	6.0000	9.6562
2	8	11.000	19.257

KRUSKAL-WALLIS ANALYSIS OF VARIANCE
H-STATISTIC = 4.4118 WITH 1 DEGREES OF FREEDOM
THIS IS SIGNIFICANT AT THE 5% LEVEL (P=.05)
THE 5% SIGNIFICANCE POINT IS 3.84100

MULTIPLE COMPARISONS

GROUP COMPARISON	ABSOLUTE DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% "L.S.D.'S"	0.1% "L.S.D.'S"
1 , 2	5.0000	4.6657	6.1321	7.8341

COMPARISON WITH GROUP 1

GROUP	DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% %L.S.D.'S"	0.1% "L.S.D.'S"
2	-5.0000	4.6657	6.1321	7.8341

The UNIVERSITY of ASTON in BIRMINGHAM, LIBRARY.

ON: 16/07/76 AT: 09/40/40

PARAMETER : LEUCINE AMINOPEPTIDASE

INTESTINE

CARP FED

GROUP 1 = 17°C

GROUP 2 = 27°C

K R U S K A L - W A L L I S T E S T

GROUP	PERCENTILES 2.5	97.5
1	1.1200	22.300
2	21.120	46.720

GROUP	SIZE	MEAN RANK	MEAN OF OBSERVATIONS
1	8	4.6250	9.8800
2	8	12.375	31.471

KRUSKAL-WALLIS ANALYSIS OF VARIANCE
H-STATISTIC = 10.599 WITH 1 DEGREES OF FREEDOM
THIS IS SIGNIFICANT AT THE 1% LEVEL (P=.01)
THE 1% SIGNIFICANCE POINT IS 6.63500

MULTIPLE COMPARISONS

GROUP COMPARISON	ABSOLUTE DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% "L.S.D.'S"	0.1% "L.S.D.'S"
1 , 2	7.7500	4.6657	6.1321	7.8341

COMPARISON WITH GROUP 1

GROUP	DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% %L.S.D.'S"	0.1% "L.S.D.'S"
2	-7.7500	4.6657	6.1321	7.8341

The UNIVERSITY of ASTON in BIRMINGHAM. LIBRARY.

ON: 16/07/76 AT: 09/40/40

PARAMETER : LEUCINE AMINOPEPTIDASE

INTESTINE

CARP FED

Group 1 = 17°C

Group 2 = 27°C

K R U S K A L - W A L L I S T E S T

GROUP	PERCENTILES 2.5	97.5
1	1.1200	22.300
2	21.120	46.720

GROUP	SIZE	MEAN RANK	MEAN OF OBSERVATIONS
1	8	4.6250	9.8800
2	8	12.375	31.471

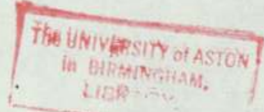
KRUSKAL-WALLIS ANALYSIS OF VARIANCE
H-STATISTIC = 10.599 WITH 1 DEGREES OF FREEDOM
THIS IS SIGNIFICANT AT THE 1% LEVEL (P=.01)
THE 1% SIGNIFICANCE POINT IS 6.63500

MULTIPLE COMPARISONS

GROUP COMPARISON	ABSOLUTE DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% "L.S.D.'S"	0.1% "L.S.D.'S"
1 , 2	7.7500	4.6657	6.1321	7.8341

COMPARISON WITH GROUP 1

GROUP	DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% %L.S.D.'S"	0.1% "L.S.D.'S"
2	-7.7500	4.6657	6.1321	7.8341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EXPERIMENT IV (A) ON: 16/07/76 AT: 09/41/17

PARAMETER : HEPATOPANCREAS

CARP STARVED

Group 1 = 17°C

Group 2 = 27°C

K R U S K A L - W A L L I S T E S T

GROUP	PERCENTILES 2.5	97.5
1	0.64000	3.1900
2	1.9900	6.4600

GROUP	SIZE	MEAN RANK	MEAN OF OBSERVATIONS
1	8	5.5000	1.8600
2	8	11.500	3.8050

KRUSKAL-WALLIS ANALYSIS OF VARIANCE
H-STATISTIC = 6.3529 WITH 1 DEGREES OF FREEDOM
THIS IS SIGNIFICANT AT THE 5% LEVEL (P=.05)
THE 5% SIGNIFICANCE POINT IS 3.84100

MULTIPLE COMPARISONS

GROUP COMPARISON	ABSOLUTE DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% "L.S.D.'S"	0.1% "L.S.D.'S"
1 , 2	6.0000	4.6657	6.1321	7.8341

COMPARISON WITH GROUP 1

GROUP	DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% %L.S.D.'S"	0.1% "L.S.D.'S"
2	-6.0000	4.6657	6.1321	7.8341

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EXPERIMENT IV (A) ON: 16/07/76 AT: 09/41/17

PARAMETER : HEPATOPANCREAS LEUCINE AMINOPEPTIDASE

CARP STARVED

GROUP 1 = 17°C

GROUP 2 = 27°C

K R U S K A L - W A L L I S   T E S T

GROUP	PERCENTILES 2.5	97.5
1	0.64000	3.1900
2	1.9900	6.4600

GROUP	SIZE	MEAN RANK	MEAN OF OBSERVATIONS
1	8	5.5000	1.8600
2	8	11.500	3.8050

KRUSKAL-WALLIS ANALYSIS OF VARIANCE
H-STATISTIC = 6.3529 WITH 1 DEGREES OF FREEDOM
THIS IS SIGNIFICANT AT THE 5% LEVEL (P=.05)
THE 5% SIGNIFICANCE POINT IS 3.84100

MULTIPLE COMPARISONS

GROUP COMPARISON	ABSOLUTE DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% "L.S.D.'S"	0.1% "L.S.D.'S"
1 , 2	6.0000	4.6657	6.1321	7.8341

COMPARISON WITH GROUP 1

GROUP	DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% XL.S.D.'S"	0.1% "L.S.D.'S"
2	-6.0000	4.6657	6.1321	7.8341

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ON: 16/07/76 AT: 09/40/58

PARAMETER : HEPATOPANCREAS

LEUCINE AMINOPEPTIDASE

CARP STARVED

GROUP 1 = 17°C

GROUP 2 = 27°C

K R U S K A L - W A L L I S   T E S T

GROUP	PERCENTILES 2.5	97.5
1	1.8100	9.3600
2	3.4900	20.090

GROUP	SIZE	MEAN RANK	MEAN OF OBSERVATIONS
1	8	5.6250	4.3762
2	8	11.375	11.114

KRUSKAL-WALLIS ANALYSIS OF VARIANCE
H-STATISTIC = 5.8346 WITH 1 DEGREES OF FREEDOM
THIS IS SIGNIFICANT AT THE 5% LEVEL (P=.05)
THE 5% SIGNIFICANCE POINT IS 3.84100

MULTIPLE COMPARISONS

GROUP COMPARISON	ABSOLUTE DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% "L.S.D.'S"	0.1% "L.S.D.'S"
1 , 2	5.7500	4.6657	6.1321	7.8341

COMPARISON WITH GROUP 1

GROUP	DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% %L.S.D.'S"	0.1% "L.S.D.'S"
2	-5.7500	4.6657	6.1321	7.8341

ON: 16/07/76 AT: 09/40/58

PARAMETER : HEPATOPANCREAS

[illegible]

CARP STARVED

GROUP 1 = 17°C

GROUP 2 = 27°C

K R U S K A L - W A L L I S   T E S T

GROUP	PERCENTILES 2.5	97.5
1	1.8100	9.3600
2	3.4900	20.090

GROUP	SIZE	MEAN RANK	MEAN OF OBSERVATIONS
1	8	5.6250	4.3762
2	8	11.375	11.114

KRUSKAL-WALLIS ANALYSIS OF VARIANCE
H-STATISTIC = 5.8346 WITH 1 DEGREES OF FREEDOM
THIS IS SIGNIFICANT AT THE 5% LEVEL (P=.05)
THE 5% SIGNIFICANCE POINT IS 3.84100

MULTIPLE COMPARISONS

GROUP COMPARISON	ABSOLUTE DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% "L.S.D.'S"	0.1% "L.S.D.'S"
1 , 2	5.7500	4.6657	6.1321	7.8341

COMPARISON WITH GROUP 1

GROUP	DIFFERENCES IN MEAN RANK	5% "L.S.D.'S"	1% XL.S.D.'S"	0.1% "L.S.D.'S"
2	-5.7500	4.6657	6.1321	7.8341