THE SKIN AND MUCOUS SECRETION OF THE EUROPEAN EEL, *Anguilla anguilla* L.

by

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DECLARATION

This work has not been accepted in substance for any other degree, and is not concurrently being submitted for any other degree.

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This is to certify that the work here submitted was carried out by the candidate himself.

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ABSTRACT

The epidermis of the eel A. anguilla L. secretes a mucous layer mainly from its mucous cells although there may also be a contribution from surface epidermal cells. Under certain conditions, the epidermal cells may transform to a secretory cell type that resembles, but is structurally distinct from a mucous cell. This observation has not previously been reported in fish epidermis.

Specialised club cells, characteristic of the Anguillidae contain filamentous material that may be secreted beneath the epidermal surface. This previously unobserved phenomenon together with an increase in cell numbers around the lateral region may suggest an additional protective mechanism which may be of use, for example, during 'burrowing'.

The quantitative distribution of mucous cells over the body was also determined and the cellular contents of the main cell types characterised histochemically. Lymphocytes and previously unseen granular neutrophil-like cells may function in more specific protective mechanisms against pathogenic organisms.

The mucus has been examined biochemically and separated by chromatography and electrophoresis to reveal several components. Rheological study has established shear thinning properties of the mucus and functional groups essential to maintain the integrity of the mucous structure.

The dynamics of mucous secretion have been observed autoradiographically and a model for the mechanism of secretion proposed.

Attempts have been made to measure the rate of mucous secretion under a number of experimental conditions which are discussed in relation to the viscous nature of mucus and the proposed model for secretion.

The epidermis and its mucous secretion thus provide an effective lubricative barrier to the environment extremely important for locomotion and protection from dessication, osmotic stress and disease organisms.

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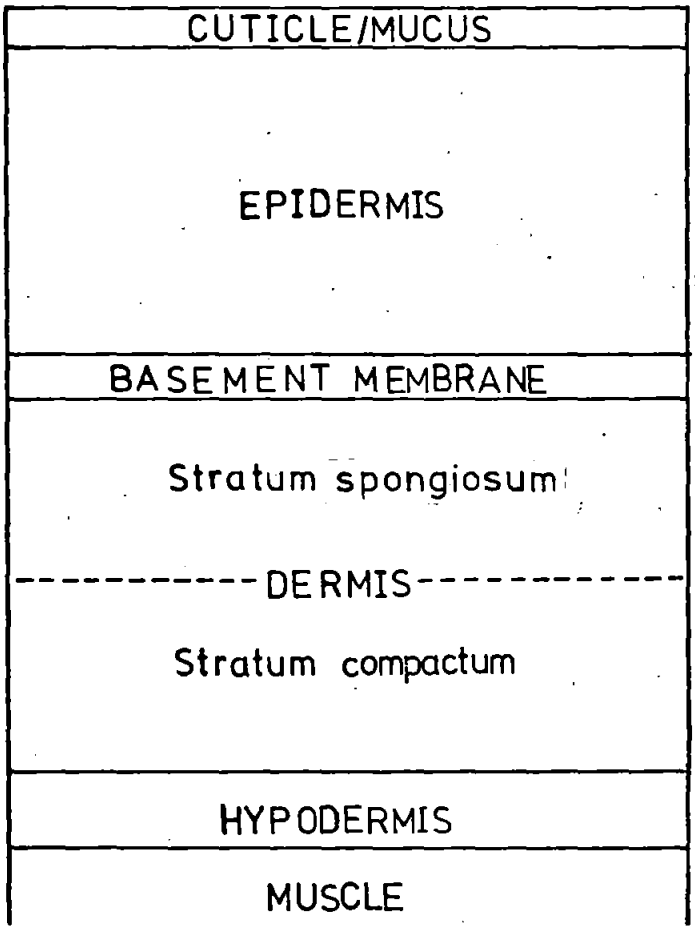
CONTENTS

INTRODUCTION

In fish, the barrier between the organism and the surrounding water is provided by a mucous-secreting epidermis. This barrier protects the fish from abrasion or other mechanical injury and also prevents the influx or efflux of body water and salts thus helping to maintain its osmotic and ionic integrity (Van Oosten, 1957). It may also protect the body from invasion and colonisation by pathogenic organisms (Pickering, 1974). In addition, the epidermis has to remain flexible and provides a lubricating surface between the fish and its environment which, in so doing allows greater mobility with the minimum cost in terms of energy. In heavily-scaled fish, it provides a lubricating surface between the overlapping scales as the body flexes during swimming. Furthermore in different species of fish a number of other diverse functions have evolved for the mucous secretion itself. These include the provision of a food source for young (Hildemann, 1959; 1964), the production of a temporary shelter (Winn, 1955) and also a more permanent protective cocoon during aestivation (Norman, 1975). The secretion may even be toxic (Thompson, 1969; Primor and Zlotkin, 1975).

The fish integument consists of two principle layers. The epidermis and the dermis (Fig. 1). The dermis may be subdivided into an upper layer, the stratum spongiosum and a lower region the stratum compactum. The stratum spongiosum consists of a loose network of collagen and reticulin fibres and contains chromatophores, scales, mononuclear cells, mast cells, osteoblasts (predominantly scleroblasts), blood

Fig.1 DIAGRAMMATIC REPRESENTATION OF FISH SKIN



and lymph vessels and nerve fibres. The stratum compactum consists of a variable number of layers of collagen fibres lying at right angles to each other thus providing mechanical strength to the integument. This region contains fibroblasts, mast cells, pigment cells and nerve fibres (Van Oosten, 1957; Bullock and Roberts, 1974).

The epidermis is separated from the dermis by a basement membrane and typically comprises filament containing cells, mucous cells, club cells, granular cells, macrophages, lymphocytes and occasionally melanin containing cells. Some sensory structures including neuromasts and chemical receptors are also present. Overlying the epidermis and derived from it is an amorphous, labile layer of sloughing cells, cellular debris and secretory material sometimes referred to as the 'cuticle' (Whitear, 1970; Bullock and Roberts, 1974).

The outermost covering of mucus, or rather protein polysaccharide molecules of the mucus, provides lubrication for locomotion by reducing the drag between the body and water (Rosen and Cornford, 1971). On secretion, this protein-polysaccharide complex takes up large amounts of water in a loose mesh-like structure and this chemical configuration is believed to assist in the protection against bacterial and fungal invasion (Pickering, 1974) and may well act as a waterproofing layer (Negus, 1963). Other additional protective substances may be present. Nigrelli and Breder (1934) and Nigrelli(1935) demonstrated the toxic properties of fish mucus to protozoa and to trematode worms. Furthermore, a bacteriocidal property of fish mucus has been related to the presence of lysozyme (Fletcher and White, 1973; Murray and Fletcher, 1976) and to the secretion of antibodies (O'Rourke, 1961; Bradshaw, Richard and Sigel, 1971). In many shoaling fish, a 'fright' reaction, in which the shoal disperses and individuals seek shelter, has been

shown to be initiated by material released into the mucus (Pfeiffer, 1962, 1963a,b; Pfeiffer and Pletcher, 1964).

Previous studies of the epidermis of fish skin have shown structural differences throughout the teleost group. Not only are there differences in the cell types present, the thickness of the epidermis and the physical consistency of its secretion, dependant on the species of fish and its particular habitat, but changes may also occur with season or sexual activity (Stoklosawa 1966, 1970; Yamada and Yokote, 1975; Pickering, 1976). In addition the biochemical properties of the mucous secretion may also be species specific (Barry and O'Rourke, 1959).

Further to these specific, structural and chemical differences in the epidermis and its secretions, is the use of different nomenclature by previous workers. The club cells, for example, have been termed Leydig cells (Rauther, 1907), columnar cells (Asakawa, 1970), clavate cells (Satô and Sannohe, 1967), goblet cells (Jakubowski, 1960a) and also 'giant cells' (Mittal and Munshi, 1970). Similarly epidermal cells have been termed polygonal cells (Rai and Mittal, 1976), principal cells (Bolognani-Fantin and Bolognani, 1966), filament-containing cells (Harris and Hunt, 1975a) and also Malpighian cells (Bullock and Roberts, 1974). Added confusion is brought about by conflicting evidence concerning the nature and presence of the cuticular structure overlying the epidermis (Whitear, 1970; Harris and Hunt, 1975a,b).

The nomenclature of protein-polysaccharide complexes is also confusing and the terms mucin, mucoid, mucosubstance, mucopolysaccharide and mucoprotein have all been used not only synonymously for mucous secretions but also more specifically to characterise the material in question. There is also inconsistency in the use of the term 'acid mucopolysaccharide' which histologists use to describe the contents of

mucous goblet cells which have particular staining reactions and also contain acidic carboxyl groups. Conversely a biochemist would use the term synonymously with glycosaminoglucuronoglycans, a group of protein-carbohydrate complexes characterised by the presence of uronic acids and usually found in connective and skeletal tissue. A biochemist would also describe the contents of mucous goblet cells as glycoprotein. A more detailed comparison between glycoproteins and proteoglycans (mucopolysaccharides) is presented in Appendix I.

The aim of the present study was to examine in detail both the skin and its mucous secretion from a single species of fish, the European eel, Anguilla anguilla L. Individual cell types within the epidermis were characterised histochemically at the light microscope level and their ultrastructure defined with a view to clarifying their roles in the production of the mucous secretion. Furthermore, the mucus was examined microscopically to determine its structural composition and examined biochemically to reveal its macromolecular composition. Viscosimetric analysis of eel mucus was used to study the components important in determining biochemical and physical properties.

Attempts were made to measure the rate of mucous secretion and the factors affecting mucous secretion have been examined. Autoradiographical studies to determine the rate of turnover of mucous cells were also conducted.

The European freshwater eel was chosen for this study because of previous confusion surrounding the nature of the club or clavate cells (Asakawa, 1970; Yamada and Yokote, 1975) and also because of its apparent copious mucous secretion. It is also widely available and relatively easily maintained under laboratory conditions.

LITERATURE REVIEW

This review of previous studies of the epidermis of fish and the nature and function of its mucous secretion is generally confined to teleost fish. However, where appropiate, other groups of fish are referred to but there is special reference and particular emphasis on previous work concerning the Anguillidae. The review is divided into two parts, the first part dealing with the structure and ultrastructure of the epidermis with a brief review of the dermis and the second with the nature of the mucous secretion.

General review articles on fish skin have been published almost regularly during the past seventy five years (Leydig, 1895; Dean, 1916, 1923; Biedermann, 1926; Rauther, 1927; Burgess, 1954; Van Oosten, 1957; Bertin, 1958; Jakowska, 1963; Bullock and Roberts, 1974) and recently an extensive bibliography of fish dermatology has also been published (Hatton, Roberts and Bullock, 1977).

Extensive histological studies at the light microscope level were carried out during the late nineteenth and early twentieth centuries (Leydig, 1851, 1879, 1892; Schulze, 1867; Reid, 1894; Studnicka, 1899, 1909; Wase, 1911; Grunelius, 1913; Gilchrist, 1920; Uhlich, 1937; Bhatti, 1935, 1938). More recently with the advent of the transmission and scanning electron microscopes detailed studies of the ultrastructure of the individual cell types comprising skin have been made (Henrikson and Matoltsy, 1968a,b,c; Kitzan and Sweeny, 1968; Yamada, 1968; Brown and Wellings, 1970; Downing and Novales, 1971a,b; Hawkes, 1974a,b; Lanzing and Wright, 1974; Harris and Hunt, 1975a,b).

The nature of the mucous secretion has also commanded considerable attention (Reid, 1893, 1894; Müller and Reinbach, 1914; Uhlich, 1937; Ferry, 1941; Randall, 1947; Chaikovskaya, 1954; Wessler and Werner, 1957; Enomoto, Nagao and Tomiyasu, 1960, 1961; Enomoto and Tomiyasu, 1960, 1961a, b, 1962; Enomoto, Nagatake and Tomiyasu, 1963; Enomoto, Nakagawa and Tomiyasu, 1964, 1966; Enomoto, Nakagawa, Matsuda and Tomiyasu, 1966; Harris and Hunt, 1973).

The Fish Integument

THE EPIDERMIS

Contrary to the situation in the epidermis of higher vertebrates where epidermal cell division occurs only at the basement membrane with the cells becoming gradually keratinised towards their uppermost layers, the epidermal cells of fish are capable of division at all levels (Henrickson, 1967; Roberts, Shearer, Elson and Munro, 1970; Bullock, Marks and Roberts, 1978a). In most species of fish the epidermis is not keratinised (Burgess, 1956) but histological and histochemical evidence of keratinisation has been obtained for a limited number of fish (Wiley and Collett, 1970; Fishelson, 1973; Spearman, 1973; Mittal and Banerjee, 1974a,b).

The thickness of the epidermal layer varies considerably between species. Non-scaled fish or fish with very small scales, for example, generally have a thicker epidermis than those with larger scales (Van Oosten, 1957). There is also variation in epidermal thickness dependent on the position of the body which has been shown by Jakubowski (1958, 1959, 1960a,b) in his studies of the skin of the stone loach, Nemachilus barbatulus L., burbot, Lota lota L., eel, Anguilla anguilla L., blenny, Zoarces viviparous L., leather carp, Cyprinus carpio L. var. nuda and flounder, Pleuronectes flesus luscus Pall. and also by Pickering (1974) for the brown trout,

Salmo trutta L. and char, Salvelinus alpinus L. Stoklosowa (1966, 1970) has also revealed differences in the epidermis between juvenile and adult, male and female, sea trout, Salmo trutta trutta L. an observation confirmed by Pickering (1977). Benthic fish often have a thickened epidermis on the ventral surface (Bullock and Roberts, 1974), whereas in pelagic fish the dorsal epidermis may be thicker. In addition, variation of the epidermis with seasonal changes has been observed in the Japanese eel, Anguilla japonica Temminck et Schlegel, by Yamada and Yokote (1975), who found a general thickening of the epidermis during the winter months.

Epidermal Cells

In common with all other vertebrates, the epidermal cell of the teleost fish is the fundamental structural unit of the epidermis. These have been referred to as principle cells in the eel, Anguilla vulgaris F. (Bolognani-Fantin and Bolognani, 1964), polygonal cells in the murrel, Channa striata Bloch (Mittal and Munshi, 1975) filament-containing cells in salmonids (Harris and Hunt, 1975a) and have been generally referred to by Bullock and Roberts (1974) as Malpighian cells. This cell type will for the purpose of this study, be referred to as the epidermal cell. The epidermal cells are consistently present in all types of fish epidermis, where they are usually found to be the most numerous of the cell types present. They are characterised by large numbers of filaments around the periphery of the cell, with the majority of other organelles occurring in the perinuclear region (Harris and Hunt, 1975a). Where the epidermis is thick, the cells are usually vertically aligned and have extensive interdigitations between

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A. vulgaris F. is an older specific name given by some authors to the European eel (A. anguilla).

adjacent cells. Desmosomal junctions are present predominantly in the basal and mid-layers of the epidermis, although in Anguilla spp. they are present in the outermost layers (Henrickson and Matolsty, 1968a).

In the outermost layers of the epidermis, the epidermal cells become flattened and are frequently electron-dense. Most of the organelles become indistinct and the mitochondria appear swollen with reduced cristae (Henrickson and Matolsty, 1968a). At the surface, a series of microridges are formed, thus giving rise to finger print-like patterns. These patterns have been shown to be species specific (Yamada, 1966, 1968; Hawkes, 1974). Immediately before the cells are sloughed off they become very electron-lucent, with most of the cytoplasmic elements disappearing.

Bereiter-Hahn (1971) suggests that the filaments are aligned parallel to the microridges. These microridges are also capable of spontaneous movement and contractions which may be induced by change in the concentration of Ca^{++} and adenosine triphosphate (ATP). More recently he has demonstrated these contractile filaments to contain actin and these are distinct from the filaments that are non-contractile (sometimes termed tonofilaments) and occur in the other regions of the cell. Furthermore, the cell boundary was also found to be rich in actin containing contractile filaments (Bereiter-Hahn, 1978 pers. comm.). He suggests also that the epidermal cells are important in primary wound closure and Bullock, Marks and Roberts (1978b) substantiate this claim.

The surface epidermal cells of many fish also show secretory activity (Whitear, 1970; Bremer, 1972; Carmignani and Zaccone, 1974; Mittal and Banerjee, 1974; Bullock and Roberts, 1974) although it is not mentioned by Van Oosten (1957) or found in the Atlantic salmon,

Salmo salar L. and brown trout, S. trutta (Harris and Hunt, 1975a,b). Similarly no mention of secretory activity in epidermal cells was made in ultrastructural studies on Anguilla spp. by Henrickson and Matolsty (1968a) and Leonard and Summers (1976). Whitear (1970) suggests that it is the epidermal cell secretion that gives rise to the external layer of 'cuticle' which is distinct from the mucous cell secretory material.

Mucous cells

Mucous cells in the form of goblet cells are found in the epidermis of nearly all teleost fish but there is great variation, in both size and distribution, of these cells with age, species and area of the body. Pickering (1974) found a greater number of mucous cells in the epidermis on the anterior regions of the salmonids, Salmo trutta and Salvelinus alpinus. A variation in mucous cell concentration has also been observed during the spawning period of sea trout, S. trutta trutta (Stoklosowa, 1970) and the brown trout, S. trutta (Pickering, 1977). More mucous cells are apparent in the female and this may provide added protection during redd digging. In the male there are considerably fewer mucous cells and the epidermis is much thinner than in the female and frequently lost or 'moulted' (Stoklosowa, 1970). In hatchery reared brown trout, however, the epidermis of the male is significantly thicker than the female throughout the spawning season (Pickering, 1977).

The mucous cells are generally first distinguishable in the basal region of the epidermis. In fish with a thin epidermis, the mucous cells may have their base on the basement membrane (Bullock and Roberts, 1974). The mucous cells develop peripherally increasing in size until they reach the surface of the epidermis where their contents are discharged. In the epidermis of Esox americanus Gmelin, Merrilees

(1974) was able to identify differentiation of mucous cells between the claviform portions of the basal cells. Harris and Hunt (1975b) examined mucous cells of S. salar and S. trutta and were unable to recognise mucous cells until they had reached the central area of the epidermis. At this point they observed that the nuclei of the presumptive mucous cells had fewer indentations than the nuclei of epidermal cells. They also noticed relatively few desmosomes between mucous and epidermal cells and significantly more endoplasmic reticulum in the mucous cells.

Immature mucous cells are rounded in shape with a centrally positioned nucleus. Rough endoplasmic reticulum and the Golgi apparatus are relatively undeveloped at this stage but by the intermediate and final stages of development they, together with produced mucous vesicles displace the nucleus and other cell organelles to the base or periphery of the cell.

The mucus appears to be formed in membrane-bound vesicles closely associated with the Golgi apparatus (Henrickson and Matolsty, 1968b; Brown and Wellings, 1969; Merrilees, 1974; Harris and Hunt, 1975b). In mammalian colonic goblet cells (Freeman, 1962; Neutra and Leblond, 1966a,b) it has been suggested that the protein moiety of the mucus is synthesised by the rough-surfaced endoplasmic reticulum and coupled with the carbohydrate portion at the Golgi apparatus.

The nearer the periphery of the epidermis, the more enlarged the cell becomes and on reaching the surface the cell membrane ruptures to release the contents of the now goblet-shaped cell. Generally individual mucous vesicles coalesce once the cell membrane has ruptured but individual vesicles may be released directly on to the epidermal surface (Harris and Hunt, 1975b).

There can be little doubt that the contents of the mucous cells constitute the mucous layer and are of a protein-polysaccharide nature. Whitear (1970), however, believes that the contents of the mucous goblet cells are primarily for emergency lubrication and are secreted as a result of sudden stress. Contrary to this Pickering (1976) shows evidence of continuous turnover of mucus from the mucous cells and Pickering and Macey (1977) could detect no changes in the size or number of superficial mucous cells in the char, S. alpinus immediately after handling, as might be expected if their contents were released as a result of stress.

The mucous cells may also vary in size between species and there may be more than one type present (Bullock and Roberts, 1974). In gadoids there are two types of mucous cell, one small which produces a very intensely staining, viscid, secretion found mainly on fins and the other, a larger cell secreting a more fluid mucus generally confined to the trunk (Bullock, Roberts and Gordon, 1976). Two types of mucous cell distinguishable ultrastructurally are also found in E. americanus (Merrilees, 1974). Mittal and Munshi (1971) also showed two types of mucous cell in some air breathing fishes and three distinct types of cell were observed in Protopterus annectens Owen by Kitzan and Sweeny (1968).

In the Anguillidae mucous production follows the general pattern indicated above (Henrickson and Matolsty, 1968b) with only one type of mucous cell found (Reid, 1894). This is also confirmed by Leonard and Summers (1976) who reported only a single type of mucous cell present in all stages of the life cycle of the American eel, Angilla rostrata Le Sueur.

Club Cells

Much confusion has arisen over both the presence and the functions of the club cells and the term has been used to describe any large non-mucous cell in the epidermis. Consequently there is inconsistency in both structure and function of so called 'club cells' between many species of fish.

The club cells are generally larger than other cell types in the epidermis and are usually rounded or oval in shape with, in some species a protoplasmic foot extending into the basal region of the epidermis thus appearing 'club-shaped'. In Ostariophysi, the club cells are round and release an alarm substance when damaged. This secretion alerts the whole shoal of impending danger and produces the characteristic 'alarm reaction' (loss of shoaling, the fish seeking shelter and refusing to feed) (Von Frisch, 1941; Pfieffer, 1962, 1963a,b; Lebedeva, Malyukina and Kasumyan, 1974).

Fish of the Ostraciidae (puffer fishes) produce a powerful neurotoxin (ostratoxin) in their club cells (Thompson, 1969) and in the sun fish Mola mola L., Logan and Odense (1974) found eosinophilic cells of a secretory type but which were nevertheless distinct from mucous cells.

In studies of the club cells of Rita rita L., Mittal and Munshi (1970) proposed the name 'giant cells' because of their size and the presence of two nuclei. The clavate (club) cells of the loach, Misguranus anguillicaudatus Cantor, are also large (several times the size of the epidermal cells) and round or oval in shape (Satô and Sannohe, 1968). These authors also report that the club cells are invariably surrounded by epidermal cells with complex interdigitations and desmosomal junctions between both club cells and epidermal cells.

The nucleus may be polymorphic and is located in the centre of the cell. The cell organelles are found within the perinuclear region and in osmium fixed preparations large numbers of small vesicles between the perinuclear region and the cell membrane are seen although the identity of their contents has not been determined (Satô and Sannohe, 1968).

In the Anguillidae, there is no evidence that the club cells mediate a fright reaction (Pfeiffer, 1962). The cells appear as in the loach, with a centrally placed nucleus surrounded by the cell organelles. Some club cells have a foot extending into the basal layers of the epidermis. The remainder of the cell is packed with a large number of fine circular or oval structures which sometimes appear U-shaped or as helically-coiled filaments (Leonard and Summers, 1976). These are thought to give rise to filamentous elements in the mucus although their release is seldom seen in sectioned material (Henrickson and Matolsty, 1968c). A large central vacuole is also present in mature cells and its electron dense contents sometimes appear granular (Henrickson and Matolsty, 1968c; Leonard and Summers, 1976). The function of the club cells in the Anguillidae is unknown. Club cells similar in structure to those described in the loach and eel have also been reported for Pimelodus maculatus Lacépède (Ferri, Stipp, Sesso and Correa, 1977).

Granule Cells

The granule cells may be found in a variety of fish (Rauther, 1907) although their function is unknown. The granule cells of the catfish, Corydorus spp. are of similar size as the club cell (Henrickson and Matolsty, 1968c). The cell contents consisted of loosely packed granules, not membrane-bound, and membrane-bound vesicles within the peripheral cytoplasm. Pfeiffer (1962) and Pfeiffer and Pletcher (1964) believe the club and granular cells of the lamprey are the same cell type. They

believe that the granular type contains a non-mucous secretion which is distasteful to other fish. Downing and Novales (1971), however, suggest the cells are separate and that the granular cells may be involved in the release of mucus whilst burrowing.

Granule cells have also been reported in Salmonids (Roberts, Young and Milne, 1972) and in a clupeoid fish, Gadasia chapra (Kapoor, 1966). The granule cell has not been observed in the Anguillidae although the central vacuole of the club cell often contains considerable granular material, especially when near to the epidermal surface layers. In contrast, the granule cells of the Pleuronectidae appear as rounded cells, containing eosinophilic granules and occur often in large numbers between the basal epidermal cells (Bullock and Roberts, 1974).

Other Cell Types

Lymphocytes may often be seen in the basal layers of fish epidermis and it has been suggested that they are responsible for antibodies found in the mucous layer (Bullock and Roberts, 1974). Lymphocytes are seen in the epidermis of all stages of A. rostrata except the leptocephalus (Leonard and Summers, 1976).

Macrophage cells may be seen in the epidermis of many fishes. They frequently contain numerous melanin granules that appear to originate from melanophores in the dermis (Roberts, 1975) and they have also been reported in the epidermis of the eel (Leonard and Summers, 1976).

Large, round cells similar to mucous cells but with different histochemical staining characteristics have recently been found in the epidermis of salmonids (Bullock and Roberts, 1974 ; Pickering and Macey, 1977). These cells are strongly eosinophilic and generally appear in the epidermis after the fish has been subjected to stress (Pickering

and Macey, 1977) although their function is at present unknown. Eosinophilic granule cells found in Tetradon fluviatilis Hamilton-Buchanan (Mittal and Banerjee, 1976), appeared identical to chloride cells of the gills and buccal epithelium (Keys and Willmer, 1932; Burns and Copland, 1950).

Chemosensory and taste bud cells have been shown in the epidermis of minnow, Phoxinus phoxinus L., gurnard, Trigla lucerna L. and goby, Pomatoschistus minutus Pall. (Whitear, 1952). Associated nerve fibres supplying these sensory cells and also those of the lateralis system and also those thought to terminate in free endings for tactile and temperature perception have been demonstrated (Whitear, 1971).

The Epidermal 'Cuticle'

The presence of an external 'cuticle' completely overlying the fish and separate from the mucous secretion has aroused much interest. It was first reported by Schulze (1867, 1869) and has since been described in several Teleosts (Gaitel, 1888; Wolff, 1889; Jourdon, 1890; Rauther, 1919; Uhlich, 1937; Becker, 1941).

Van Oosten (1957), however, makes no mention of the cuticle in his review neither do Jones, Holliday and Dunn (1966) in their study of the herring, Clupea harengus L. Wellings, Chuinard and Cooper (1967) and Henrickson and Matolsty (1968a) note the presence of a 'fuzz' over the microridges of surface epidermal cells and Brown and Wellings (1970) and Fishelson (1971) suggest that this could be the cuticle. The structure and properties of a cell surface coat has been extensively reviewed by Luft (1976). This coat is sometimes referred to as a 'fuzz' is found surrounding most animal cells.

The cell surface coat is however, seriously affected by different fixation techniques which may alter its appearence. Luft (1976)

further mentions the staining reaction of the cell surface coat with ruthenium red which was also seen to stain the surface layer of the epidermis of Tilapia spp. (Lanzing and Wright, 1974) although they could find no positive reaction to this stain in either the mucous cells or the epidermal cells.

Whitear (1970) believes the cuticle to be both the 'fuzz' layer and also an overlying layer secreted by the epidermal cells and distinct from the mucous cell secretion which she believes is secreted for emergency lubrication only. In contrast, Roberts and Shearer (1970) and Harris and Hunt (1975b) could find no evidence for the presence of a cuticle in salmonids and Harris and Hunt (1975b) found the layer overlying the epidermis to have similar histochemical characteristics to the secretion within the mucous cells. Furthermore, they demonstrate the mucous cell secretion to comprise the major constituent of epithelial mucus and demonstrate this by histochemical analysis (Harris and Hunt, 1973; Harris, Watson and Hunt, 1973).

Bullock and Roberts (1974) believe the cuticle to be a complex of cell protoplasm, sloughed cells and goblet cell mucus with the goblet cell secretion consisting only a minor component compared with the contribution from the epidermal cells. Mittal and Banerjee (1976) have also investigated the cuticle and in agreement with Whitear (1971) and Bremer (1972) conclude that it is derived from epidermal cells.

In the Anguillidae the presence of a cuticle has been reported by Wolff (1889), Studnicka (1909) and Aust (1936). There is, however, no mention of a cuticular structure in the classical comprehensive histological study of the epidermis of A. anguilla (Reid, 1894). Furthermore ultrastructural studies have also failed to demonstrate a

cuticle at any stage during the life cycle of the eel (Henrickson and Matolsty, 1968a,b, c; Leonard and Summers, 1976).

THE DERMIS

The dermis is divided into two layers, the upper layer, the stratum spongiosum comprises a loose network of collagen and reticulin fibres. Within this network are pigmented cells (melanophores, lipophores, leucophores and iridophores), mononuclear cells, mast cells, scale and scale-forming cells. Blood capillaries and nerve fibres also occur in this layer. The lower layer, or stratum compactum, is a region of densely packed layers of collagen fibres lying at right angles to each other. These collagen fibres together with the calcareous scales provide the mechanical strength of the fish integument (Bullock and Roberts, 1974).

The hypodermis or subcutis is a fine network of loose connective tissue containing lipid cells and chromatophores and is believed to act as a flexible buffering layer between the stratum compactum and underlying muscle (Bullock and Roberts, 1974).

Vascularisation of the dermis varies between species (Jakubowski, 1959, 1960a,b). In the eel, the skin was thought to act as an accessory respiratory surface especially when the fish was out of water (Krough, 1904; Berg and Steen, 1965). More recently, however, Kirsch and Nonnotte (1977) have demonstrated the skin itself to have a high oxygen demand and they conclude that the skin does not act as a respiratory epithelium for the benefit of other organs. They have, in addition, shown increased dermal vascularisation in both native sea water eels and eels adapted to live in sea water (Nonnotte and Nonnotte, 1978 pers. comm.).

FISH MUCUS

Fish mucus comprises the secretions of the different secretory cells of the epidermis and the contents and cell membranes of the living and dead cells which slough off the epidermis. It may also contain components that are secreted from the lower levels of the skin through the epidermis (e.g. proteolytic enzymes, immunoglobulins). To elucidate the nature of fish mucus two major approaches have been previously employed. Histological and histochemical investigations have provided considerable insight into the nature of the secretion both inside the cells and on the epidermal surface. More recently, attention has been diverted to the chemical analysis of the secreted products.

Histology and Histochemistry

Harris et al. (1973) found the presence of both neutral and acidic mucosubstances in the mucous cells of S. trutta. Bremer (1972) investigating a number of teleost fish also reported the presence of both neutral and acidic mucosubstances together with a protein fraction not associated with carbohydrate.

Characterisation and localization of different lipids in the skin of a catfish, Heteropneustes fossilis Bloch (Mittal, Rai, Banerjee and Agarwal, 1976) showed the surface epidermal cells to be rich in phospholipid and also contain small amounts of cholesterol and its esters. Larger quantities of cholesterol, its ester and phospholipid were found in the basal cells and phospholipid was also found in the mucous cells. Lipid material and sulphated mucosubstances have also been found in epidermal cells of C. striata (Mittal and Banerjee, 1975) and Clarias batrochus L. (Banerjee and Mittal, 1975) together with a high succinic dehydrogenase activity. The granular cells present in C. striata were renamed sacciform granular cells and their contents were shown to be basic proteins.

The epidermal and club cells of the electric fish, Malopteurus electricus contained proteinaceous material (Carmignani and Zaccone, 1974). The mucous cells although not containing hyaluronic acid contained an acid mucopolysaccharide (Appendix I) with phosphate and carboxyl groups and also some sulphated mucopolysaccharide. In Torpedo acellata Raffinesque both mucous and epidermal cells were found to contain a neutral mucopolysaccharide, chondroitin sulphate B, mucopolysaccharides containg carboxyl and phosphate groups and sialic acid containing glycoproteins (Carmignani and Zaccone, 1974).

In the eel there are three main types of cell found in the epidermis viz. epidermal cells, club cells and mucous cells (Bolognani-Fantin and Bolognani, 1966). The epidermal cells are PAS positive in certain layers only. The club cells do not contain carbohydrate (PAS negative) but give positive reactions for proteins only. Whilst the mucous cells contain either a neutral polysaccharide only or one containing both neutral and non-sulphated polysaccharide of which one component is sialic acid.

In a study of the epidermis of the Japanese eel, Asakawa (1970) found the mucous cells to contain a sialic acid-containing glycoprotein and the columnar (club) cells contained a sulphated mucopolysaccharide. Yamada and Yokote (1972, 1975), however, dispute this suggestion and claim that the mucous cells contain a neuraminic (sialic) acid muco-substance and the clavate (club) cells contain a glycoprotein. Neither mention any possible role of the epidermal cells in mucous secretion.

The Biochemistry of Fish Mucus

Information on the biochemistry of fish mucus is by no means complete and the precise nature of the protein-carbohydrate complexes involved is largely unknown.

The chemical composition of the mucus from several species of fish was studied by Wessler and Werner (1957). They reported the presence of a simple protein and also varying amounts of nucleic acids and glycoproteins. The glycoproteins contained hexosamine, galactose, fucose and sialic acid.

In a primary chemical study of mucus from the loach, M. anguillicaudatus Turumi and Saito (1953) found the presence of glucosamine and galactose by paper chromatography. Enomoto and Tomiyasu (1960) also studying loach mucus identified sixteen amino acids and one unidentified amino acid. They also found ribose, arabinose, glucose, glucosamine and chrondrosamine and concluded that the polysaccharide portion of the mucus was composed of hexose and hexosamine in equimolar ratio. On re-examination of this mucus, Enomoto and Tomiyasu (1961a) could not detect the presence of arabinose but a hexuronic acid, throught to be glucuronic acid, was detected.

Enomoto, Nagao and Tomiyasu (1961) also identified sixteen amino acids by chromatography to be consistently present in eleven species of fish. Low levels of cysteine, methionine, tyrosine and one other unidentified component were noticable. Mucus from the conger eel, Astroconger myriaster Brevoort, was found to have similar amino acid composition, although in the carbohydrate portion of the molecule there was no glucose the presence of fucose could be shown.

Qualitative (Enomoto and Tomiyasu, 1961b) and quantitative (Enomoto, Nagatake and Tomiyasu, 1963) analyses of neutral, basic and acidic sugars revealed that galactose, fucose, ribose, glucosamine, galactosamine and hexuronic acid were consistently present in seven species of fish. In the mucus of the Japanese eel, A. japonica, galactosamine was found in far greater concentrations than

glucosamine. Hexuronic acid was found in relatively low concentrations. Neutral sugar composition from the mucus of six species of fish, including the Japanese eel (Enomoto and Tomiyasu, 1962) showed marine species to contain more fucose than the freshwater species.

The sialic acid, N-acetyl neuraminic acid (N.A.N.A.) has been identifed in the mucus of A. japonica (Enomoto et al., 1966). It was also found in both S. trutta and Salvelinus alpinus by Pickering (1974) who also identified N.A.N.A. as being the only sialic acid present in the mucus of A. anguilla.

Glycoproteins in the mucus of Salvelinus alpinus have also been separated by gel chromatography and their amino acid and carbohydrate fractions analysed (Wold and Selset, 1977). Their results were consistent with those of Harris and Hunt (1973) who examined the biochemical nature of the mucus of Atlantic salmon. Both studies revealed low concentrations of cysteine (an amino acid that can form disulphide linkages) in the separated components. The amino acid fractions of mucus of several species of fish have also commanded attention from Chaikovskaya (1954), Uskova, Chaikovskaya, Ustinovich and Davidenko (1970) and Uskova and Chaikovskaya (1973).

The lipid content of fish mucus was studied by Lewis (1970) who discovered large amounts of phospholipid which he suggested may link neutral lipids to the glycoproteins. Jelenko and Ginsberg (1971), however, could find no lipid in the skin of the oyster toad fish, Opsanus tau Rafinesque.

The Dynamics of Mucous Secretion

Greater concentrations of mucous cells in the epidermis covering the head regions in S. trutta led Pickering (1974) to suggest the possibility that mucus flows over the body from head to tail as the fish swims along. In contrast, Lemoine and Olivereau (1971) showed

an increase in the concentration of N.A.N.A. (a measure of 'mucification' of the skin) in the caudal regions of A. anguilla.

An increase in N.A.N.A. was also shown with progressive transfer of A. anguilla from freshwater to seawater but this increase was not correlated to prolactin cell activity (Lemoine and Olivereau, 1971). Prolactin was thought to be implicated in maintaining osmotic balance by affecting mucous production, however, no positive correlation was found after hypophysectomy or with ovine prolactin stimulation in A. anguilla (Olivereau and Lemoine, 1971, 1972) or Pacific staghorn sculpin, Leptocottus armatus Girard (Marshall, 1976).

In contrast, the activity of prolactin-secretory cells of the adenohypophysis or injection of ovine prolactin has shown good correlation with increased numbers of mucous cells in the goldfish, Carasius auratus (Ogawa and Johanssen, 1967; Ogawa, 1970), the three-spined stickleback, Gasterosteus aculeatus L. (Leatherland and Lam, 1968) and in the cave-fish, Anoptichthys jordani Hubbs and Innes (Mattheij and Sprangers, 1969). Furthermore, cell proliferation that occurs with the production of 'discus milk' in the discus fish, Symphysodon discus Heckel has also been correlated with prolactin activity (Egami and Ishii, 1962).

The syntheses of N.A.N.A. in the epidermis of S. trutta has been followed by Pickering (1976) who noticed the appearence of labelled N.A.N.A. in the mucus less than ten hours after the injection of C^{14} glucose, a precursor of N.A.N.A. There was also an indication that the turnover time of mucous cells within the epidermis was of the order of several days. Further evidence supporting this claim has been shown by Bullock, Marks and Roberts (1978a) in an autoradiographical study of the epidermis of the plaice, Pleuronectes platessa L.

Increased mucous secretion from fish under pH stress has been reported by Ellis (1937), Westfall (1945), Plonka and Neff (1969) and Daye and Garside (1975) who observed this phenomenon to be due to hypertrophy of the mucous cells. Increased secretion of mucus is also seen in fish subjected to heavy metals (Ellis, 1937; Jones, 1938; Ashley, 1970; McKone, Young, Bache and Lisk, 1971; Eisler and Gardner, 1973) and also to a pesticide (TFM) (Christie and Battle, 1963).

The Functions of Mucus

The mucus covering of fish is greatly hydrated and in the pike forms a layer approximately 40 - 45 μm thick (Fedak, Koval and Prokopenko, 1973). The mucous layer reduces efflux or influx of water in either hypertonic or hypotonic environments, removal of the mucus of a freshwater eel, for example, leads to an increase in water uptake which may result in death (Negus, 1963). The mucus may act as a waterproofing layer by holding an 'unstirred' layer close to the body surface (Levin, 1969). The mucus may also provide a lubricative layer protecting the body from mechanical abrasion (Van Oosten, 1957). Furthermore, continual replacement of mucus may discourage adhesion of parasites and other disease organisms (Pickering, 1974; Willoughby and Pickering, 1977).

In addition to non-specific protection by mucus, a number of more specific mechanisms have been established. Nigrelli and Breder (1934) discovered an immunity in the mucus against monogenetic trematodes. Later, Hines and Spira (1974) showed the presence of immobilising antibodies to Ichthyophthirus multifilis in the mucus of C. carpio. Chromatography indicates that each fish species has its own characteristic mucus (Barry and O'Rourke, 1959) and further studies by O'Rourke (1961b) indicated that serum proteins could be detected in the mucus of bass.

Immunoglobulins of the IgM class have been demonstrated in plaice, P. platessa and garfish, Lepisosteus platyrhinus Lacépède (Fletcher and Grant, 1969; Bradshaw, Richard and Sigel, 1971; Harrel, Ellinger and Hodgins, 1976). Natural agglutinin reactions between mucus and red blood cells have been shown in a number of fish (Di Conza, 1970; Di Conza and Halliday, 1971; Smith, 1977) and also in the Pacific hagfish, Eptatretus stoutii (Spitzer, Downing, Koch and Kaplan, 1976). Natural antibiotic and also toxic activity is also seen in the mucus of Pacific golden striped bass, Grammistes sexlineatus Thunberg (Lingouri, Ruggieri, Baslow, Stewpien and Nigrelli, 1963). Studies by Hildemann (1959) on Symphysodon discus showed the transfer of immunity from parents to young via the mucous secretion upon which the young feed and this was considered to be non-antibody mediated.

Specific antibodies are not the only mechanisms of defense against invasion by pathogens. Fletcher and White (1973) showed the presence of lysozyme by its lytic action on Micrococcus lysodiekiticus. This enzyme, effective against Gram positive bacteria, was found in both serum and mucus of P. platessa. The sites containing lysozyme were later located by Murray and Fletcher (1975) and it was noted that its distribution in the skin and mucus was affected by stress.

In an analysis of the lipid content of mucus from mullet, Mugil cephalus L., flathead, Planiprora fusca L. and catfish, Plotus anguillarius Bloch, Lewis (1970) found free fatty acids, which he suggests may also form part of a chemical defense mechanism against pathogens in a manner similar to that of higher vertebrates.

Contrary to the above findings, Hattingh and Van Warmello (1975) could find no antibiotic, bacteriostatic, mycostatic or mycocidal effects from the mucus of mudfish, Labeo umbratus L. and Labeo capensis L., yellowfish, Barbus holubi and carp, C. carpio.

Rheological Properties of Mucus

The rheological properties of respiratory, gastric and cervical mucus together with the synovial fluid of the joints has commanded much attention in recent years (Litt, 1971; Snary, Allen and Pain, 1971; Eliezer, 1974; Marriot and Irons, 1974; Purchelle and Zahm, 1974; Davies, Scobie and Inglis, 1975; Gilboa and Silberberg, 1976; Meyer, 1976; Litt, Khan and Wolff, 1976) and although there appears to be no literature specifically concerned with the viscosity of fish mucus a number of references to the viscous nature and lubricant properties of the mucus have been made.

Rosen and Cornford (1971) found, the natural 'slime' of fish had the ability, for example, to greatly decrease the fluid friction of water. They proposed that whilst the fish were swimming slowly, the layers of water very close to the body are not turbulent and the 'reluctance' of the slime (i.e. its ability to remain separate when added to still water) prevents the loss of the slime coat. During rapid swimming, the sub-layers of water break into turbulent flow, the 'reluctance' of the slime is overcome and some goes into solution. The dilute slime then dampens turbulence and lowers friction on the body. They also calculate a reduction in friction up to 65% in the barracuda, Sphyraena argentea L. with the maximum effect occurring with as little as a 5% mucous solution.

Lewis (1970, 1976) suggested that the retention of mucus on the skin of fishes is largely a function of its viscosity. He also measured the phospholipid content of mucus from different fish having high, moderate and low viscosity. He concluded that the interaction between phospholipid content and glycoprotein fraction determined the viscosity of the mucus and suggested that the phospholipid is derived

from the 'globule' membrane i.e. the membrane surrounding mucous 'pockets' within the mucous cell.

There are marked differences in the consistency of the mucus from individual species of fish. Generally those that swim slowly have a viscous mucus whilst the mucus of fast swimmers has relatively 'thin' consistency. In addition, the mucous coating tends to be thicker on naked or sparsely coated fish than those that are heavily scaled (Lewis, 1970).

Chaikovskaya (1954) found the presence of one more amino acid in the protein fraction of mucus from a fast swimming fish than in the mucus of a slow swimmer. Uskova, et al. (1970) and Uskova and Chaikovskaya (1975) found significantly lower amounts of protein in the mucus of slow-moving fish than in fast swimming fish and concluded it is the concentration of protein, especially proteins above 50, 000 daltons, that determins the hydrodynamic properties. The carbohydrate portion, they suggest, has little function in determination of hydrodynamic properties of the mucus.

Breder (1976) proposed, that in shoaling fish, the friction-reducing properties of dissolved mucus could benefit the entire shoal. The continued changing of front swimmers that occurs in a shoal would therefore allow all fish to share the effects of mucus, enabling faster and more economical movement than that of a single fish.

Structural alterations in fish mucus due to lead and mercury have been observed by Varanasi, Robisch and Malins (1975). They noticed significant non-reversible changes in the electron spin resonance (ESR) spectra of mucus in the presence of low concentrations of lead and mercury and proposed the possibility of a change occurring in

the rheological properties of mucus. The levels of the metals in the mucus were reduced after 24 hours, by approximately half in the case of lead, and they concluded that this was the result of the 'sloughing off' of the metal-complexed mucus which was replaced by metal-free mucus.

MATERIALS AND METHODS

Materials

Immature yellow eels, Anguilla anguilla (size range 30 - 300 g) were obtained from Pudleigh Mill fish farm, Somerset. Fish were also obtained from the Tamar Lake, North Devon by courtesy of South West Water Authority and from Slapton Ley, South Devon. All fish were transported in a minimal amount of water and held in 200 ℓ polythene tanks at 11 - 12 °C. Additional aeration was supplied from a compressor and fish were allowed at least two weeks acclimatisation after transportation before experimentation. Fish were starved for a period of at least one week prior to all experiments.

All reagents used in the study were (unless otherwise stated) obtained from either B.D.H. Ltd., Poole, Dorset or Sigma Ltd., London. Salmonella H antigen $\bar{a}$ was obtained from Difco Ltd., Detroit.

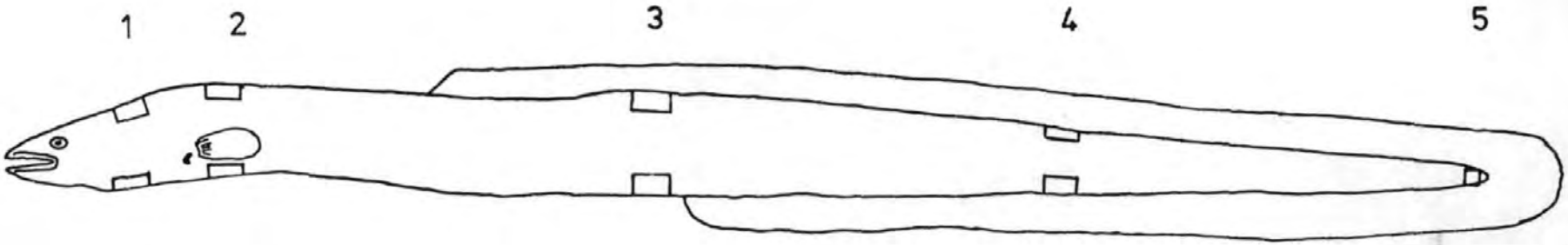
Methods

STRUCTURAL STUDIES

Light Microscopy

Samples of skin (both epidermis and underlying dermis) were removed from all regions of the body after decapitation or anaesthesia using 0.025% t-chlorbutol (Williams Ltd., London) dissolved in a few millilitres of ethanol prior to addition to the water. Routine tissue samples were removed from the dorso-lateral region just anterior to the dorsal fin. Underlying muscle fibres were removed from the block of tissue after at least 24 h fixation. Fixatives employed were either

A) Surface Staining



B) Sectioning

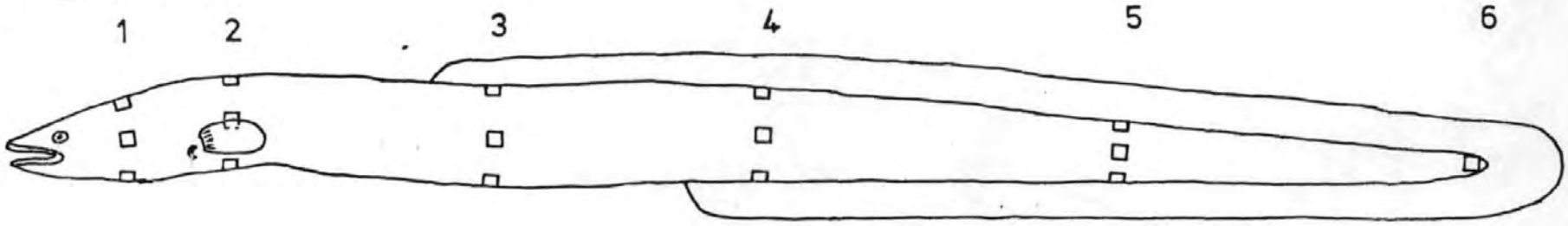


Fig. 2 DISTRIBUTION OF MUCUS & CLUB CELLS (Sampling regions)

modified Bouin's fixative (Harris et al., 1973) or Baker's formol calcium (Pearse, 1968). The samples were dehydrated in graded alcohols and embedded in paraffin wax (melting pt. 56 °C). For routine work, a rotary microtome was used to cut 7 µm sections.

Frozen sections of formalin fixed (10% solution) and unfixed tissue were cut at 15 µm on a cryostat (Slee Cold Pierce standing cryostat).

Scanning Electron Microscopy

Samples of skin removed from the dorso-lateral region just anterior to the dorsal fin were fixed for three days in 3% glutaraldehyde fixative. After trimming to approximately 2 mm x 2 mm, samples were dehydrated in a graded series of alcohols and critical point dried using carbon dioxide as the transitional fluid (Polaron E 3000 critical point drier, used by courtesy of The Marine Biological Association of the United Kingdom, Plymouth). After mounting on stubs and coating with gold, the samples were examined with a Philips SEM 500 (by courtesy of Pye Unicam Ltd., Cambridge).

Transmission Electron Microscopy

Samples of skin (approx. 5 mm square) were removed from all regions of the eel after decapitation and fixed in either,

a) 4% glutaraldehyde and 2% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.4) for 4 h at 4 °C followed by 2% osmium tetroxide in 0.1 M cacodylate buffer (pH 7.4) also for 4 h at 4 °C.

or b) 2% glutaraldehyde and 2% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.4) for 4 h at 4 °C.

The tissue samples were trimmed of any muscle fibres after 1 h in either fixative, cut to approximately 2 mm x 1 mm and then transferred to fresh fixative for the remaining fixation period.

En bloc staining with Ruthenium red, Alcian blue and Mercuric bromophenol blue was performed on representative samples according to Hayat (1972).

Dehydration was effected in a graded series of ethanol at 4 °C and the samples brought to room temperature before transfer to acetone and embedding in Spurr's low viscosity resin (Spurr, 1974).

Sections showing gold to silver interference colours (approx. 60 - 100 nm thick) were cut with glass knives using a Porter-Blum MT2B microtome and mounted on uncoated grids. After staining with saturated aqueous uranyl acetate and lead citrate according to Reynolds (1963) or Saito (1967), or phosphotungstic acid (PTA) according to Marinozzi (1967) they were examined in a Philips EM 300 transmission electron microscope.

Distribution of Mucous Cells

To examine the distribution of mucous cells over the skin surface two methods of counting mucous cells were employed; a) samples of skin were taken from the decapitated fish, at various regions of the body as drawn in Fig. 2A. The samples were then rinsed in distilled water to remove mucus and placed for 2 min in 1% Alcian blue (pH 2.5). Pickering (1974) claims that this staining procedure selectively stains the open surface mucous cells, this was confirmed in the present investigation by serially sectioning a stained skin sample. After a brief rinse in distilled water to remove excess stain, photographs of the stained skin were taken with a Tessovar photomicroscope and enlargements (approx. x 7) made of the prints. A similar enlargement was also made of a 1 mm square of graph paper. Surface staimed mucous cells within the 1 mm square were then counted. b) Samples of skin approx 1 mm square were removed from similar regions of the body (Fig. 2b) and

fixed, dehydrated and embedded in wax as previously described. Sections cut at 7 µm were stained with Alcian blue (pH 2.5) (Steedman, 1950) to detect mucous cells and then in mercuric bromophenol blue (Pearse, 1968) to reveal club cells. Estimations of the concentrations of mucous and club cells were then obtained for the different regions of the body (Fig. 2) in the following manner. From a total of approximately 150 sections from each area, the cells of every 6th section were counted (20 in all). This procedure avoided repeated counting of the same cells.

Preparation of Mucous Smears

Smears of mucous material were obtained by resting a glass slide on the dorsal surface of a fish and with the aid of a compressed air jet, blowing the mucus and other secreted material onto the slide. The smear was allowed to dry and then stained in either 1% Alcian blue pH 2.5 for mucosubstances or 1% Coomassie blue or mercuric bromophenol blue for protein (Pearse, 1968).

Experimental Procedures

Sections of skin were removed from the dorso-lateral regions of the decapitated fish after the eels had been held under a number of experimental conditions to assess factors which may stimulate or reduce mucous secretion.

Effects of Atropine. Atropine prevents the action of acetylcholine by combining with muscle and gland receptors although it does not interfere with the release of acetylcholine from parasympathetic nerves (Keele and Neil, 1971). To examine the action of atropine on the mucous secretion of the eel, two fish (approx. 35 g) were each held in a tank containing 1 ℓ of distilled water (11 - 12 °C) containg 0.15% atropine. The fish were sacrificed after 24 h. Control fish were held under similar conditions but without inclusion of atropine.

Effect of Electrical Stimulation. To investigate the effect of electrical stimulation of the epidermis, fish were anaesthetised in 0.025% t-chlorbutol solution, removed from their water and briefly stimulated with a six-volt electrical supply with electrodes held 20 cm apart. After 10 such stimulations, skin samples from the dorso-lateral region just anterior to the dorsal fin were immediately removed, fixed and examined.

Effect of Chloroform. Chloroform is known to stimulate the release of mucus (Reid, 1894). To re-examine this phenomenon, fish (approx. 35 g) were anaesthetised in 0.025% t-chlorbutol solution and a swab previously soaked in chloroform held approximately 5 cm from the fish for 10 seconds. A skin sample from the dorso-lateral region was immediately removed, fixed and examined.

Effect of Antibiotic. Introduction of fish into water containing streptomycin sulphate initiates a stress behaviour pattern. This, however, is only temporary, lasting at most a couple of minutes. To investigate the longer term effects of streptomycin, three fish were each held in tanks containing 500 cm^3 distilled water (11 - 12 °C) and 0.4 mg/cm^3 streptomycin sulphate. The fish were sacrificed after 24 h. Control fish were held in distilled water without the inclusion of streptomycin sulphate.

Effect of pH Change. Recent literature (Daye and Garside, 1976) has suggested changes in mucous cells with pH stress. To investigate possible stimulation of club cells with pH stress three fish (acclimatised to 15 °C) were placed in 10 ℓ tanks containing freshwater (15 °C) adjusted to either pH 5.0 or pH 9.0 (with HCl or NaOH). After 1 h the fish were removed and sacrifeced. Control fish were kept in similar containers in freshwater at 15 °C.

All skin samples were fixed and embedded in wax, sectioned and stained with either Alcian blue pH 2.5 (for mucous cells) or mercuric bromophenol blue (for club cells) and compared with their respective controls.

HISTOCHEMISTRY

Sections (7 μm) of eel skin were dewaxed in xylene and subjected to staining procedures as listed in Table 1. Fresh frozen sections (15 μm) and formalin-fixed frozen sections were used where appropriate e.g. for lipid stains.

SPECTROSCOPIC ANALYSIS OF MUCUS

Ultra-Violet Spectrum

Mucus was removed from the fish with compressed air (Uskova and Chaikovskaya, 1975). Distilled water was then sprayed over the fish and a further sample was collected. The pooled mucus sample was then centrifuged at 24, 000 g (30 min at 4 °C) to remove all cellular debris. This was then checked microscopically. Ultra-Violet absorption spectrum (190 - 440 nm) was measured using a Perkin-Elmer Ultraviolet spectrophotometer (1 cm light path in quartz cells).

Infra-Red Spectrum

Approximately 10 cm^3 of centrifuged mucous solution was freeze dried (New Brunswick freeze drier) and 1 mg of dried material was ground and compacted with potassium bromide. The Infra-Red absorption spectrum (400 - 4000 nm) was measured on a Perkin-Elmer Infra-Red spectrophotometer.

TABLE 1. HISTOCHEMICAL STAINING REACTIONS OF EEL EPIDERMIS

Staining Procedure	Indicates	Reference
A. General Procedures		
Mallory' Triple	General stain	Putt (1972)
Masson's Triple	General stain	Putt (1972)
Haematoxylin & Eosin	Nuclei, cytoplasm	Putt (1972)
Methylene Blue Extinction	Level of basophilia	Pearse (1968)
B. Carbohydrate Procedures		
Periodic-acid Schiff (PAS)	Vicinal hydroxyl groups	Pearse (1968)
Amylase - PAS	Glycogen	Pearse (1968)
Alcian Blue (AB) pH 2.5	Acidic mucosubstances	Steedman (1950)
Alcian Blue (AB) pH 1.0	Sulphated muco-substances	Steedman (1950)
PAS - AB pH 2.5 PAS - AB pH 1.0	To distinguish hyaluronic and sialomucins from strongly acidic sulphated muco-substances	Mowry & Winkler (1956)
Neuraminidase - AB pH 2.5	Sialic acid	Spicer & Duvenci (1964)
Testicular hyaluron-idase - AB pH 2.5	Uronic acid	Leppi & Stoward (1965)
Mild Methylation (37 °C, 4 h) - AB pH 2.5	Alcianophilia of non-sulphated mucins inhibited	Spicer (1960)
Active Methylation (60 °C, 4 h) - AB pH 2.5 + Saponification	Alcianophilia of both non-sulphated and sulphated mucins inhibited. Restores alciano-philia	Spicer (1960)
Aldehyde Fuchsin (AF)	Sulphated mucosub-stances	Spicer & Meyer (1960)

TABLE 1. Continued

Staining Procedure	Indicates	Reference
AF - AB pH 2.5 AF - AB pH 1.0	Sulphated mucosubstances	Spicer & Meyer (1960)
Low Iron Diamine (LID) High Iron Diamine (HID)	Sulphated mucosubstances	Spicer (1965)
Periodic Acid Para-diamine (PAD)	Periodate reactive mucosubstances	Pearse (1968)
Toluidine Blue	Metachromatic substances	Pearse (1968)
C. Protein Procedures		
Methyl Green - Pyronin Y	RNA and DNA	Pearse (1968)
Alkaline Fast Green	Basic proteins and nuclei	Pearse (1968)
Mercuric Bromophenol Blue	Protein	Pearse (1968)
Millons Reagent	Tyrosine	Pearse (1968)
Tetrasonium Method	Sulphate groups	Pearse (1968)
Methyl Violet	Amyloid	Pearse (1968)
Orcinol Method	Elastic fibres	Pearse (1968)
D. Lipid Procedures		
Sudan Black B	Lipid	Pearse (1968)
Sudan Blue Sudan III	Neutral lipid	Pearse (1968)
Nile Blue Sulphate	Acidic lipid	Pearse (1968)
Oil Red O	Neutral lipid	Putt (1972)
Menschik Reaction	Phospholipid	Pearse (1968)

TABLE 1. Continued

Staining Procedure	Indicates	Reference
Pseudoplasmal Reaction	Phospholipid	Pearse (1968)
Luxol Blue (+ unmasking)	Phospholipid	Pearse (1968)
Performic-acid Schiff (PFAS)	Phospholipid (not specific)	Pearse (1968)
Modified Bruchner Reaction	Sugar-containing lipid	Pearse (1968)
Modified Molische Reaction	Sugar-containing lipid	Pearse (1968)
Schultz Method	Cholesterol and esters	Pearse (1968)
Okamoto Method	Cholesterol and esters	Pearse (1968)

GROSS BIOCHEMICAL ANALYSES OF MUCUS

Mucus was removed as described previously, centrifuged, placed into test tubes and freeze dried. Freeze dried mucous samples were incubated in an oven at approximately 50 °C until they were at constant weight. Samples were then subjected to a number of spectrophotometric analyses (Table 2) to determine the protein content and the carbohydrate composition of the mucus.

MEASUREMENT OF LYSOZYME IN MUCUS

Mucus was removed with compressed air, centrifuged (24,000 g, 4 °C, 30 min) and the supernatent concentrated by dialysis against 30% polyethylene glycol. After concentration an aliquot of mucus was freeze dried and stored at -20 °C. The pellet containing cellular debris after the initial centrifugation was also freeze dried and stored at -20 °C. A pooled sample of the freeze dried mucus (4.3 mg) from three fish and a pooled sample of the cellular debris were each reconstituted in 1 cm^3 of buffer solution (0.1% NaCl in 0.054 M phosphate buffer, pH 6.4) and ultrason icated for 5 min (Pye Unicam Ultrasonicator). Aliquots (0.5 cm^3) of the reconstituted samples and 0.5 cm^3 aliquots of the concentrated mucus were subjected to the Lysozyme assay as described by Litwack (1955). This procedure measures the percentage change in absorbance (at 550 nm) due to lytic action of the enzyme on killed Micrococcus lysodeikticus cells.

CHROMATOGRAPHIC AND ELECTROPHORETIC ANALYSES OF MUCUS

Gel Chromatography

Sephadex G.200 (Pharmacia, London) was allowed to swell overnight in Tris-HCl buffer (0.02 M, pH 7.4). Sepharose 6B and Sepharose 4B

TABLE 2. BIOCHEMICAL ANALYSES OF MUCUS

Assay	Method	Reference
Protein	Biuret	Snell & Ettre, (1974)
Protein	Modified Folin-Ciocalteau	Hartree, (1972)
Total Carbohydrate	Phenol-Sulphuric Acid	Dubois, Gilles, Hamilton Rebers & Smith, (1956)
Non-nitrogenous Sugar	Orcinol-Sulphuric Acid	Gottschalk, (1972)
Hexosamine	Elson-Morgan Reaction	Gottschalk, (1972)
Uronic Acid	Modifed Carbazole Reaction	Bitter & Ewins, (1961)
Fucose	Cysteine-Sulphuric Acid	Gottschalk, (1972)
Sialic Acid	Thiobarbituric Acid	Warren, (1958)

(Pharmacia, London) were obtained as pre-swollen gels and were washed in buffer prior to use. The gels were made into slurries with buffer and were packed into glass columns (100 cm x 1.2 cm i.d.) according to the manufacturers directions. Upward buffer flow from a peristaltic pump (Watson-Marlow) was effected with a flow rate of approximately 4 cm^3/h and equilibrated for 24 h at 4 °C. The concentrated mucous solution (prepared by centrifugation and dialysis, see above) was further dialysed (overnight) against distilled water, ultrasonicated and a 3 - 4 cm^3 aliquot applied to the column. Forty 4 cm^3 fractions were collected and analysed for protein by a modified Lowry technique (Hartree, 1972), for carbohydrate by the phenol-sulphuric acid method (Dubois, Gilles, Hamilton, Rebers and Smith, 1956) and for sialic acid by the thiobarbituric acid assay described by Warren (1958).

Ion-Exchange Chromatography

Pre-swollen ion-exchange gel (Whatman D.E.52) was washed in Tris-HCl buffer (0.01 M, pH 7.4), packed into glass columns (30 cm x 1.2 cm i.d.) and equilibrated with a downward flow of buffer for 2 h. Approximately 4 cm^3 of mucous solution (centrifuged, concentrated, dialysed and ultrasonicated as previously described) was loaded onto the column. After washing with one bed volume (determined by blue dextran) of buffer, increasing strengths of sodium chloride (0.1, 0.2, 0.5, 1.0 M) in 0.01 M Tris-HCl buffer were used to elute from the column. Fractions (4 cm^3) were collected and assayed for protein as above.

Polyacrylamide Gel Electrophoresis

Discontinuous polyacrylamide gel electrophoresis was carried out in 8 cm x 0.5 cm i.d. glass tubes as described by Davis (1964). A separating gel containing 3% acrylamide was used without a stacking gel. Urea (final concentration 6 M) was added to both discontinuous gel

buffer and electrode buffer. Concentrated and sonicated mucous samples (20 - 50 µl), applied to the gel in sucrose or glycerol solution, were separated by a constant current of 5 mA per gel until the bromophenol blue tracking dye (10 µl, 0.001% solution) added to the sample had migrated to within 1 cm of the bottom of the tube. The gels were removed from their tubes and stained in either 1% Coomassie blue in 7% acetic acid (Maurer, 1972), 1% Alcian blue (Caldwell and Pigman, 1965) or with PAS (Zaccharius, Zell, Morrison and Woodlock, 1969).

RHEOLOGY OF MUCUS

Information on the theory and measurement of the rheological properties of mucus appears in Appendix II. A pooled mucous sample from 6 - 10 eels (mean wt. 300 g) was collected and centrifiged (2,000 g, 4 °C, 15 min) to remove cellular debris. An aliquot (approx. 20 cm^3) was further centrifuged (24,000 g, 4 °C, 30 min) to remove all filamentous material. Both fractions were then allowed to warm to ambient temperature and the viscosity of samples (approx. 3 cm^3) from each fraction, at increasing shear rates was measured with a Weissenberg cone-plate rheogonimeter. The results are presented as a graph plotting the viscosity against shear rate. The data was then transformed logarithmically and the regression coefficients and intrinsic viscosities (viscosity where shear rate = 0, obtained by extrapolation of the regression line) then compared by Students t-test after the variance of each had been compared by analysis of variance (F-test) (Bailey, 1969).

The viscosities of a number of mucous solutions (approx. 5, 10, 15, 25, 40, 50, 75 and 100% in distilled water) were measured and samples of these concentrations analysed for protein content by the modified Lowry technique (Hartree, 1972). The intrinsic viscosities (shear rate = 0) were then plotted against mucous concentration.

Samples (0.5 cm^3) of materials (listed in Table 3) to be tested for their effects on the rheological properties of mucus were added to 9.5 cm^3 of centrifuged (24,000 g, 4 °C, 30 min) mucous solution. A control containing 0.5 cm^3 distilled water was also prepared. The viscosity of each sample was measured and the results presented as graphs plotting the viscosity against shear rate. Comparison between experimental and control mucous solutions were tested statistically on logarithmically transformed data by Students t-test after the variances had been compared by analysis of variance (F-test). In those cases where the variances were significantly different (at the 5% level) the Students t-test was employed but with 'f' degrees of freedom, where

$$f = \frac{1}{\frac{u^2}{n_1 - 2} + \frac{(1-u)^2}{n_2 - 2}}$$

$$u = \frac{\frac{s_1^2}{{}_1(x - \bar{x}_1)^2}}{\frac{s_1^2}{{}_1(x - \bar{x}_1)^2} + \frac{s_1^2}{{}_2(x - \bar{x}_2)^2}}$$

n = number of points on curve

s = variance

x = log. viscosity

$\bar{x}$ = mean

TABLE 3. RHEOLOGY OF EEL MUCUS

Material Added to Mucus	Final Concentration in Mucous Solution
NaCl	0.01 M
KCl	0.01 M
$CaCl_2$	0.01 M
$MgCl_2$	0.01 M
$PbCl_2$	0.01 M
$CdCl_2$	0.01 M
Tris HCl buffer	0.01 M
DDT	Saturated solution
β-Mercaptoethanol	0.01 M
Neuraminidase	100 IU
Pronase	100 IU
Hyaluronidase	100 IU
Chymotrypsin	100 IU
Glucuronidase	100 IU
Galactosidase	100 IU

IMMUNOLOGY

To examine the appearence of agglutinating antibodies in the mucus three eels (mean wt. 300 g) were held at 14 - 15 °C and injected intramuscularly with Salmonella H antigen a (1cm^3 injection with Freunds complete adjuvant 1:1 ratio). Three injections on successive days were followed by a further injection after seven days. Mucus was removed from the fish at ten day intervals for 80 days using compressed air, pooled and centrifuged to remove threads and cellular debris (24,000 g, 4 °C, 30 min). After concentration (by dialysis against Aquacide III) to approximately 25% of their original volume the samples were stored at -20 °C until used. Six-fold serial dilution series of each sample were prepared (i.e. 1:2, 1:4, 1:8, 1:16, 1:32 and 1:64) and together with an undiluted sample of mucus transferred to a disposable well plate (50 µl of sample per well). Salmonella H antigen a (50 ul) was added to each well and after incubation at room temperature for 30 min the plate was examined for agglutination.

DYNAMICS OF MUCOUS SECRETION

Determination of the Rate of Mucous Secretion

In an attempt to determine the rate of mucous secretion of fish held under experimental conditions, a method measuring total protein and carbohydrate in the surrounding water was employed. 500 cm^3 of clean, sterile water was added to separate, closed, siliconized plastic containers which had been previously sterilized by swabbing with ethanol. A single eel (body wt. 30 - 60 g) was placed into each container and six replicates were maintained at each of the following temperatures: 4 °C, 11 - 12 °C, 14 - 15 °C and 22 °C. Fish were allowed approximately one week gradual acclimatisation (2 °C change every 2 days) to both 4 °C and 22 °C before experimentation. To investigate the effects of

osmotic stress (at 11 - 12 °C) fish were transferred from freshwater to sterile, full strength, artificial sea water (Sea Aquarium Ltd., London). Some fish were also transferred to sea water in three stages (25 %, 50% and 75% saline) over a period of three weeks.

Bacterial growth, within the system, was monitored at 4 °C, 11 - 12 °C and 22 °C and experimentally controlled by the addition of 100 mg Streptomycin sulphate to each 500 cm^3 container.

The effect of atropine sulphate, which inhibits mucous secretion in mammals (Keele and Neil, 1971) was also investigated (at 11 - 12 °C). The influence of physical disturbance of the surrounding water using a magnetic stirrer was also examined.

Experimental Procedure

At regular intervals (for the first 50 h) 15 cm^3 samples of water were removed from each tank after gentle dispersion of any particulate material in the water. 15 cm^3 of clean sterile water, containing a suitable dilution of streptomycin or atropine where necessary, was added to each container after sampling. The pipettes used for sampling were siliconised to minimise adsorption of mucus and samples were stored at -20 °C.

Estimations of the total protein were made by means of a modified Lowry method (Hartree, 1972) and total carbohydrate was measured by the phenol-sulphuric acid method of Dubois et al. (1956).

The effects of repeated sampling were adjusted by applying a correction factor to the readings (after subtraction of blank values for streptomycin and atropine). The corrected value was expressed as

$$P = p_n + \frac{15}{500} \cdot P_{(n-1)}$$

P = corrected value ($\mu g/cm^3$)

p = observed value ($\mu g/cm^3$)

n = sample number (with first sample = 0).

After experimentation the fish were anaesthetised, weighed and measured and an estimate of the body surface area was obtained by covering the fish with aluminium foil. This was then trimmed to remove excess foil, removed from the fish, weighed and compared to the weight of a standard known area of foil.

The mean corrected values for the concentrations of protein and carbohydrate in the water were then plotted against sampling times. The regression coefficient representing the rate of appearance of mucus in the surrounding water ($\mu g/cm^3/h^{-1}$) was determined.

Estimation of Bacterial Numbers in the Experimental System

Six closed containers were carefully sterilized with ethanol. Sterile distilled water (500 cm^3) was added and a single eel (body wt. 30 - 60 g) introduced to each container. Streptomycin sulphate (100 mg) was added to three of the containers and they were all kept at a constant temperature (13 - 14 °C). Samples (10 cm^3) were removed from each container at 24 h intervals (for 4 days). A six fold series (10^{-1} - 10^{-6}) was made for each sample and a 1 cm^3 aliquot from each dilution was placed in a sterile petri dish. Nutrient agar was added to each dish to a depth of 0.5 cm and the dishes agitated in the standard pour-plate method. After incubation for 48 h at 20 °C bacterial colony counts were made on the most suitable plates of each series.

Protein analyses were performed on filtered (millipore 0.22 μm filter) and non-filtered water samples taken at 24 h and 48 h intervals, to determine the proportion of protein attributable to bacteria, other particulate material and soluble protein.

Autoradiography of Eel Skin

To measure the rate of development of the mucous cells in the epidermis, an autoradiographical method was employed. Ten eels (mean wt. approx. 35 g), held at 19 °C, were each injected intraperitoneally with 200 μCi of H^3 galactose. At intervals of 2, 4, 8, 12, 24, 36, 48, 72 and 96 h an eel was sacrificed and skin samples removed from the dorsal region just anterior to the dorsal fin. Tissue samples were fixed and embedded in wax, sectioned at 7 μm, collected on slides previously 'subbed' with gelatin solution and stained with Alcian blue (pH 2.5). The slides were then covered (in the dark) with photographic emulsion (Kodak AR 10) using the stripping film technique (Rogers, 1972). After six weeks exposure, at 4 °C, the slides were processed in developer (Kodak, D19) and observed at the light microscope level.

Both labelled (those with at least four silver grains per cell) and non-labelled mucous cells in the lower, mid- and upper regions of a 1 mm length of epidermis (for five sections) were counted for each sampling time. These were then plotted as percentage mucous cell labelled against time.

OBSERVATIONS AND RESULTS

HISTOLOGY OF THE EPIDERMIS

Light Microscopy

At the light microscope level three different major cell types may be easily recognised in the epidermis (Pls 1A,B). These are the epidermal cells, the club cells and the mucous cells. The epidermal cells are the smallest and the most numerous cell type seen. They are generally rounded to oval in shape (approx. 5 - 6 µm in diameter) in the lower and middle regions of the epidermis. The epidermal cells also appear to comprise the basal cell layer, just above the basement membrane, where they assume a more cuboid appearance and often possess a conical apex (up to 9 µm long). Towards the surface, the epidermal cells become more flattened (Pl. 1A).

Club cells may be either rounded or extended basally (Pl. 1A, 2A) to assume a 'club-shaped' appearance and they are often the largest cell type found in the epidermis (up to 15 x 40 µm). In the upper regions of the epidermis the club cells frequently become more rounded and there is also a significant change in appearance. The contents of the central vacuole appear to become granular (Pls 2A,B). At the light microscope level, under normal conditions, club cells were not observed at the epidermal surface and were not seen to release their contents directly into the mucous layer.

The mucous cells are generally rounded or ovoid in shape. They do not usually occur in the basal cell layer of the epidermis but may be recognised by their characteristic secretory vesicles, throughout

more peripheral regions of the epidermis (Pl.1B). Towards the upper regions of the epidermis and where they are open at the surface (Pl. 2B) they become slightly flattened laterally and the secretory vesicles seem to displace the nucleus towards the base of the cell (Pl. 2A). Mucous cells are usually 8 - 12 μm in diameter but exceptionally may measure up to 25 μm in diameter.

The numbers of both club and mucous cells may be seen to vary greatly (Pls 1A,B) depending on the area of the body from which the epidermal sections were taken. The large numbers of mucous cells seen in plate 1B are from a section taken from the ventral area whilst the lower numbers of mucous cells and higher numbers of club cells seen in plate 1A were taken from sections of the lateral regions of the fish.

A mucous secretion, which was frequently seen covering the superficial epidermal cells was presumed to be derived from the numerous cells that are open at the surface (Pl. 1B) because of the similarity in staining response between the mucous cell contents and the superficial secretion.

Scanning Electron Microscopy

When examined under the scanning electron microscope, surface epidermal cells are, as confirmed by sectioned skin samples, the only cells that are consistently observed. The surface of the epidermal cells possess a series of ridges which form a reticulate pattern (Pl. 3B). These ridges are approximately 0.15 - 0.2 μm broad (as measured in sections) and the distance between adjacent ridges may vary between 0.2 - 0.6 μm. The 'pattern' produced by the ridges appears to vary between individual cells and occasionally between different areas of a single cell (Pl. 3B). The cell boundaries are

clearly visible with this technique (Pl. 3B) and recognised by the close proximity of ridges of adjacent cells. Openings, which are probably those of the mucous cells, occur between epidermal cells (Pl. 3B). These openings may be 9 µm in diameter and a mucus-like material sometimes forms a sheet covering adjacent cells (Pl. 4A). The opening of a mucous cell is shown in more detail in plate 4B and again mucous material is seen at the edge of the open pore where it appears to extend across the top of the microridges. The microridges of surrounding epidermal cells also appear to extend down into the opening (Pl 4B). Occasionally, small dark round areas (approx. 0.21 µm in diameter) are seen on the surface of the epidermal cells and these may be the openings of secretory vesicles of epidermal cells (Pl. 4B cf Pl. 13A).

Club cells were not apparent at the epidermal surface, an observation supported by study of sectioned preparations.

Transmission Electron Microscopy

General Structure. Epidermal cells of the surface layers are seen in plate 5A (size approx. 7 x 3 µm). The microridges observed in scanning electron microscope preparations appear as 'finger-like' projections or microvilli approximately 0.15 - 0.20 µm across and 0.20 - 0.30 µm deep. Stretched across the apices of the ridges is fine fibrillar material which probably forms part of the mucous layer, and, at the inter-cellular junctions a raised 'trough' between two ridges is distinguishable. Tight junctions and desmosomal junctions occur between epidermal cells and vesicles, sometimes numerous, are seen within the cells particularly at the periphery.

Cuboid epidermal cells (approx. 7 x 10 µm wide) comprise the basal cell layer (Pl. 5B). Complex interdigitations occur between adjacent basal cells and they appear more electron dense than the epidermal

cells of higher layers. Lymphocytes and occasionally granular neutrophil-like cells, characterised by their small size (approx. 5 μm in diameter) and irregular shape, may be found between the apices of these cells (Pls 5B, 21C). A large inter-cellular space generally surrounds both white blood cell types and neither tight junctions nor desmosomal junctions are found between them and the surrounding epidermal cells.

The basement membrane is a thin layer (approx. 0.2 μm thick) directly beneath the basal cell layer separating the epidermis from the collagen fibres of the dermis (Pls 6A,B). The basement membrane generally appears as an electron-lucent layer above a deeper more electron-dense layer and is composed of a fine fibrous material. Hemidesmosomal junctions between the basement membrane and the epidermis are often seen and the basement membrane may also extend into the basal cell layer as a complex series of folds (Pl. 6B).

Epidermal cells at the surface of the epidermis, just before they are sloughed off, show an apparent disintegration of cellular components (Pl. 7A). The cells usually become electron-lucent and contain numerous lysosomes which are characteristic of dying cells. Beneath these cells there is frequently evidence of the formation of microridges on the upper surfaces of underlying epidermal cells (Pl. 7A arrowed).

The delicate nature of the mucous layer and/or the cuticular structure is confirmed by its absence in most sectioned preparations, however, when present it forms an overlying layer approximately 0.5 μm thick (Pl. 7B). It appears to be composed of fine filamentous threads and an amorphous material, stretched over the peaks of the microridges. Some of the material appears closely attached to the underlying epidermal cells but in this particular preparation, there is little evidence to suggest that the mucous layer is produced by the epidermal cells.

Club Cells. Club cells are large (15 x 40 µm in diameter), typically rounded or ovoid in shape and found in all regions of the epidermis except at the epidermal surface (Pl. 8A). The cell nucleus is generally central but not always rounded in shape (Pls 8A, 9C). A large electron-dense vacuole is usually situated in the supranuclear region although it sometimes occurs below the nucleus. Surrounding the nucleus and central vacuole is an irregular electron-dense area, which may also extend around the perimeter of the cell (Pls 8A, 9C). Generally only mitochondria and rough endoplasmic reticulum are recognisable and they are usually found in the electron-dense area around the nucleus. The lighter staining area between the peri-nuclear region and the cell periphery contains helically coiled filaments which appear, in cross section, as circular structures approximately 25 - 28 nm in diameter (Pls 9A,C). The filaments measure approximately 5 nm in diameter and there is an approximate 15 nm gap between each coil (Pl. 9B). Always adjacent to club cells are epidermal cells and their ajoining cell walls are often thrown into a complex series of interdigitations (Pl. 9A).

Towards the surface of the epidermis, the club cells frequently change in appearence to become larger and more rounded (Pls 2A,B). The central vacuole increases in size and its contents appear more granular (Pl. 10A). The electron-dense areas around the vacuole and the perimeter of the cell are still retained and they may join in places. The lighter staining area composed of the helically coiled filaments are also still discernible.

During the present investigation no evidence was obtained to indicate that the club cells actively secrete their contents on to the surface of the epidermis. They may be seen occasionally, however, releasing their contents below the epidermal surface thus causing the

overlying epidermal cells to lift from the epidermal surface layers (Pl. 8B). The rupture and release of club cell contents in the lower levels of the epidermis may cause the complete loss of its upper layers (Pl. 10B); the gross effect of this action is clearly seen in thick epoxy sections under the light microscope (Pls 11A,B). Long dark-staining threads arranged in parallel arrays form filamentous bundles within the lighter staining cytoplasm, after the breakdown of the cell wall (Pls 10B, 12A,B,C). The lighter staining cytoplasm contains circular structures (filaments in cross-section) similar to those that are characteristic of club cells. These circular structures are not always complete and thus indicate that the filaments are not aligned in any one direction.

Epidermal Cells. The epidermal cells can be observed in all regions of the epidermis. At the epidermal surface they become flattened (Pl. 5A) whilst those forming the basal cell layer are cuboid in shape (Pl. 5B). The epidermal cells are generally smaller (approx. 5 - 6 μm in diameter) than the other main cell types, viz. the club cells and the mucous cells, and are usually characterised by bundles of small filaments (Pls 14A,B) measuring approximately 8 nm in diameter. The presence of these filaments has led to the epidermal cells sometimes being referred to as filament or filament-containing cells. Near the epidermal surface they may often contain small (0.25 μm in diameter) vesicles which open to the surface (Pl. 13A) and fine filamentous material can be seen to be exuded. From their reaction with PTA it would appear that the vesicles contain a mucopolysaccharide-like material (Pl. 13B) (for terminology see Appendix I), although not all vesicles take up the stain. Melanin granules (approx. 0.45 μm in diameter) are also occasionally found within epidermal cells (Pl. 13A) although

they are more commonly found within melanin-containing macrophages (Pl. 23B).

After mechanical removal of surface mucus by compressed air, the epidermal cells may become active secretory cells not unlike but nevertheless distinct from goblet-type mucous cells (Pls 14A,B, 15A,B, 16A,B). This appears to be a reaction to the removal of mucus as the conversion of epidermal cells to secretory cells was not observed under any other circumstances. Epidermal cells in the process of becoming secretory cells were found mainly in the middle and upper regions of the epidermis but were not evident at the epidermal surface secreting their contents. This may indicate that the short experimental period chosen (fish were sampled at 24 h intervals up to 72 h after removal of mucus) was not sufficient time for the complete conversion to an actively secreting cell type. It was noted, however, that the transformation occurring within epidermal cells was not totally synchronised between cells and more than one stage was apparent amongst the epidermal cells of any one section.

The process is characterised by an increase in numbers of mitochondria and the appearence of secretory droplets associated with the Golgi apparatus (Pl. 14A). These droplets coalesce to form vesicles (approx. 0.25 µm in diamter) that appear similar to vesicles frequently observed in the surface epidermal cells (Pl. 13A). The characteristic bundles of 8 nm filaments are retained in the cell around its periphery (Pl. 15A) and the numbers of vesicles increase to almost fill the cell (Pl. 15B). Further coalescence now occurs and the cell becomes similar to a mucous cell (Pl. 16A). A number of features, however, distinguish the transformed cell from a true mucous cell. Firstly transformed cells may contain melanin granules (approx. 0.45 µm in diameter) (Pl. 16A); these were only observed in epidermal cells and never found in mucous

cells (see also Pl. 13A). Secondly, the numerous small vesicles (0.28 μm in diameter) as found in transforming cells (Pl. 16A) were not observed in mucous cells in which mucin material coalesced close to the Golgi apparatus to produce mucin 'packets' (approx. 0.8 - 1.2 μm in diameter). The fully formed 'packets' of secretory material found in transforming epidermal cells (Pl. 16A) are, however, of similar size to the mucin 'packets' found in mucous cells. Furthermore, bundles of 8 nm filaments were not seen in mucous cells but were found within epidermal cells containing secretory material (Pl. 16B). During the early stages of the transformation into the secretory phase (Pls 17A,B) rough endoplasmic reticulum and free ribosomes are associated with increased numbers of mitochondria. Small droplets, forming characteristic 0.25 μm vesicles, are found closely associated with the Golgi apparatus (c.f. production of mucin 'packets' in a goblet type mucous cell, Pls 20A,B) before coalescence into larger 'packets' of secretory material.

Mucous Cells. Mucous cells may be observed in all layers of the epidermis except the basal cell layer, however, in the immature stages they are frequently found between the apices of the basal cell layer. These immature mucous cells are rounded in shape (Pl. 18A), with a centrally positioned nucleus and only a few membrane-bound 'packets' of mucin. The mucous cells appear much larger in the middle and upper regions of the epidermis and near the surface they assume the characteristic 'goblet' shape (Pl. 18B) (they are sometimes referred to as 'goblet' cells). As the cell becomes swollen with the 'packets' of mucin, the nucleus and other cell organelles become displaced basally and are confined to the lower peripheral regions of the cell.

The mature mucous cells open, via their apex, on to the epidermal surface and their contents released to cover the epidermal surface (Pls 1B, 4A). The rupturing of the mucin 'packets' may occur either prior to, or just after, release from the mucous cell. Occasionally, individual mucin 'packets' may be seen on the epidermal surface. There is evidence that mucous cells open at the surface may still be actively synthesizing mucus as immature mucin 'packets' may be found associated with rough endoplasmic reticulum in the perinuclear region (Pl. 19A). The mucin 'packets' stain densely for mucopolysaccharide (see Appendix I) as shown by the PTA method (Pl. 19B) and in common with sections stained with lead citrate and uranyl acetate (Pl. 18B) there is some variation in the staining density of the 'packets'.

In the production of mucus (Pls 20A,B) (c.f. epidermal cell secretory activity, Pls 17A,B). The mitochondria are surrounded by rough endoplasmic reticulum and small secretory droplets are associated with and thought to be derived from the Golgi apparatus (Pl. 20B). The larger membrane-bound 'packets' of mucin appear to be formed by coalescence of vesicles from the Golgi apparatus.

Other Cell Types. Small irregular cells (approx. 3 µm in diameter) containing round or ovoid electron-dense granules (approx. 0.4 µm in diameter) (Pls 21A,B,C) are occasionally seen between the apices of the basal cell layer. The nucleus of these cells is large and frequently appears lobed. Other structures, possibly tubular in nature, may also be seen and are approximately 0.13 x 0.04 µm in size (Pl. 21B). The intercellular space around this cell type is generally large and the cells appear similar to polymorphonuclear neutrophil cells seen in mammals (Rodin, 1972).

Lymphocytes are occasionally found in the lower regions of the epidermis, between the basal cell apices and are sometimes present nearer the surface layers (Pls 5A, 22A). These are characterised by their small size (approx. 2.5 μm), irregular shape, large nucleus and a cytoplasm without the spherical or ovoid granules of the neutrophil cells. In common with the neutrophil, a large intercellular space is usually found around the cell and there are no tight junctions between these and adjacent cells.

Larger cells (Pl. 22B) (approx. 8.0 μm in diameter), believed to be wandering macrophages are sometimes found in the upper regions of the epidermis. They are characterised by numerous membrane-bound vesicles, probably secondary lysosomes formed as a result of the ingestion of cellular debris. The lysosomal contents appear as either membraneous whorls or multivesicular bodies. There are also large intercellular spaces.

More commonly found throughout the epidermis are melanin-containing macrophage cells that are almost entirely packed with melanin granules (Pl. 23B). These granules are presumed to be derived from melanophores contained within the pigmented areas of the dermis as the cells are sometimes seen across the basement membrane. The macrophage contents are released at the surface of the epidermis into the mucous layer as the cell is sloughed off.

Nerve Fibres and Blood Vessels. Non-myelinated nerve fibres (approx. 0.7 μm in diameter) are frequently observed between the other celltypes. Myelinated nerve fibres are more rarely seen (Pl. 23A) and are not normally found within the epidermis of teleost fish. The presence of blood capillaries in the epidermis is also rare but they do appear in the eel (Pl. 23A). The endothelial lining the capillary lumen contains the pinocytotic vesicles characteristically found in other animal species (Rhodin, 1972).

PLATE 1

A. Light micrograph of wax embedded, lateral region of eel epidermis. Haematoxylin and Eosin (x 600).

BL Basal layer

C Club cell

D Dermis

E Epidermis

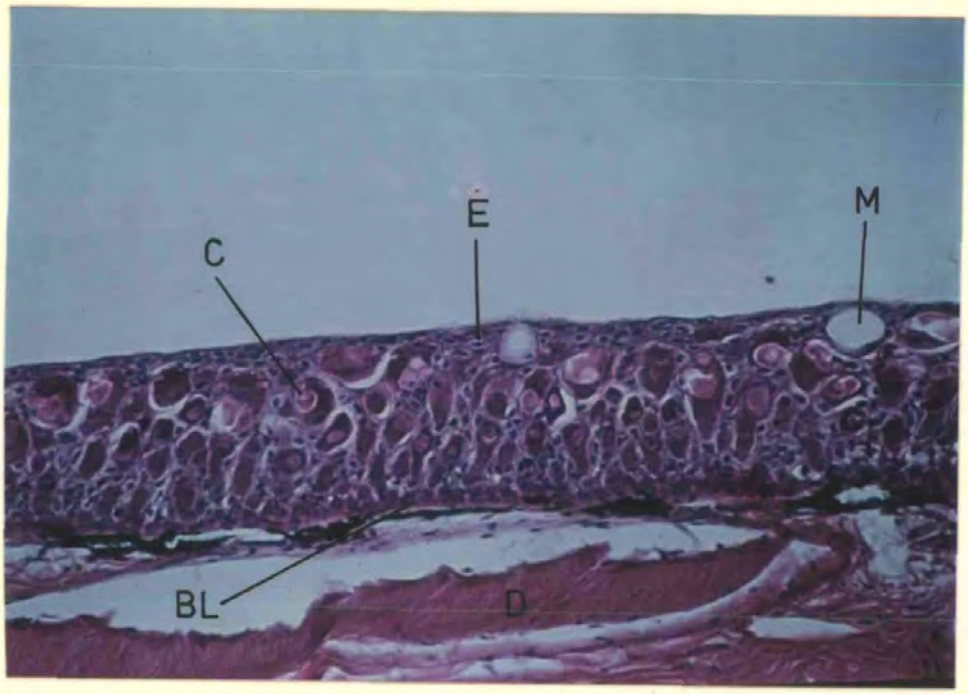
M Mucous cell

B. Light micrograph of wax embedded, ventral region of eel epidermis with comparatively large numbers of mucous cells and some granular club cells. Massons triple stain (x 1, 280).

GC Granular club cell

M Mucous cell

ML Mucous layer


C
E
M
BL
D

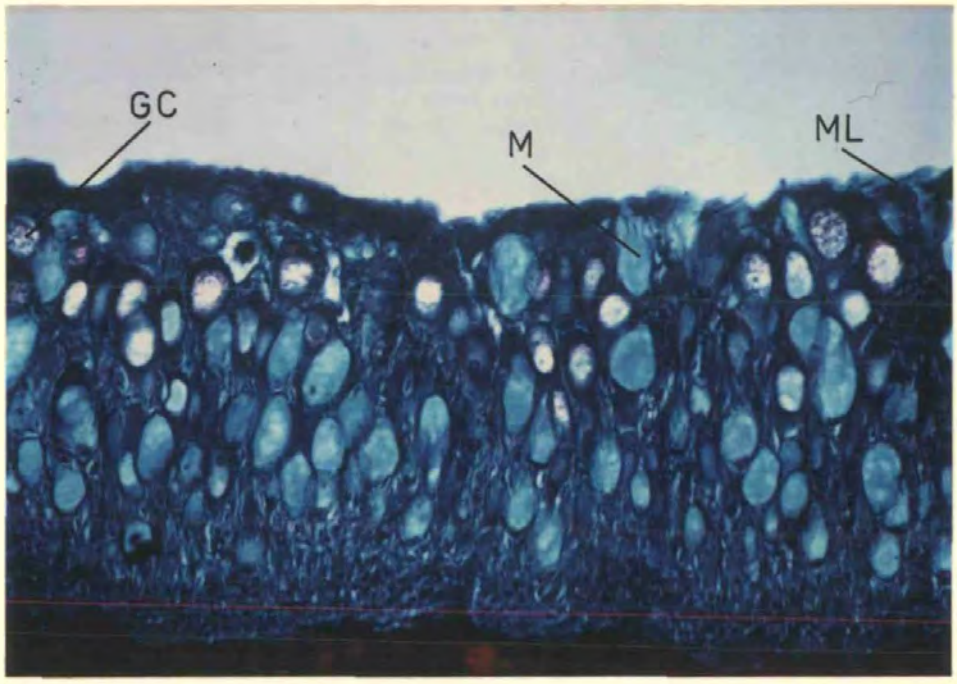

GC
M
ML

PLATE 2

A. Light micrograph, epoxy embedded, dorsal section of eel epidermis. Granular club cells are seen close to the periphery of the epidermis, whilst club cells with an extended 'foot' are seen further down. Developing mucous cells are seen in both lower and upper regions of the epidermis. Acridine orange and methylene blue (x 1,280).

C Club cell

CV Club cell vacuole

GC Granular club cell

IM Immature mucous cell

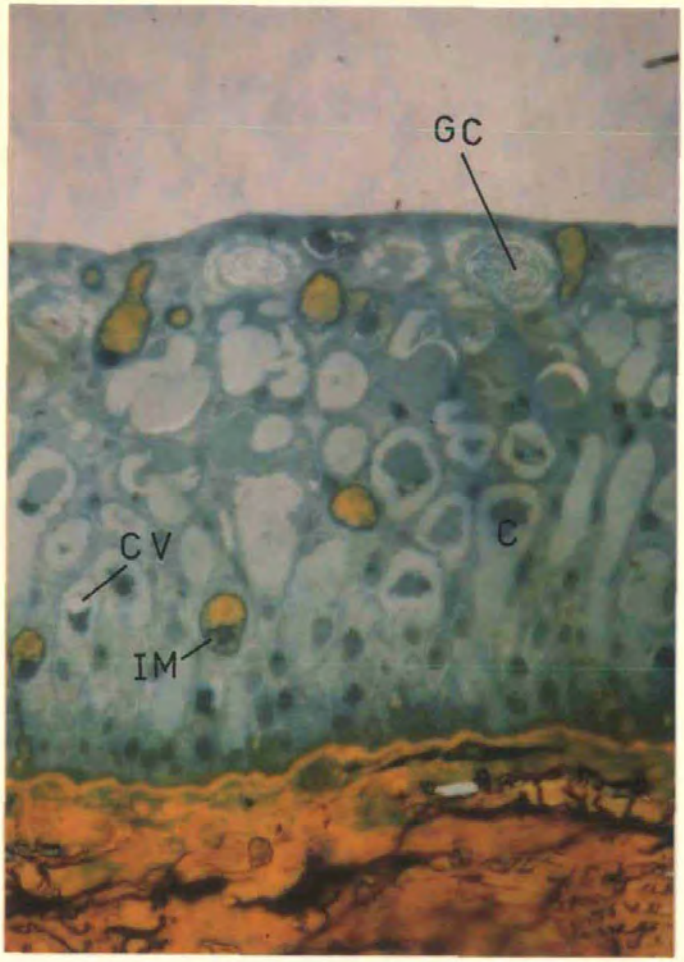
B. Light micrograph, epoxy embedded, dorsal section of eel epidermis. Dividing nuclei are seen in both basal cell layer and in lower regions of the epidermis. Differences in the stain intensity of mucous cells are also apparent. Basic fuchsin and methylene blue (x 800).

C Club cell

GC Granular club cell

DN dividing nuclei (epidermal cell)

M Mucous cell


GC
CV
C
IM

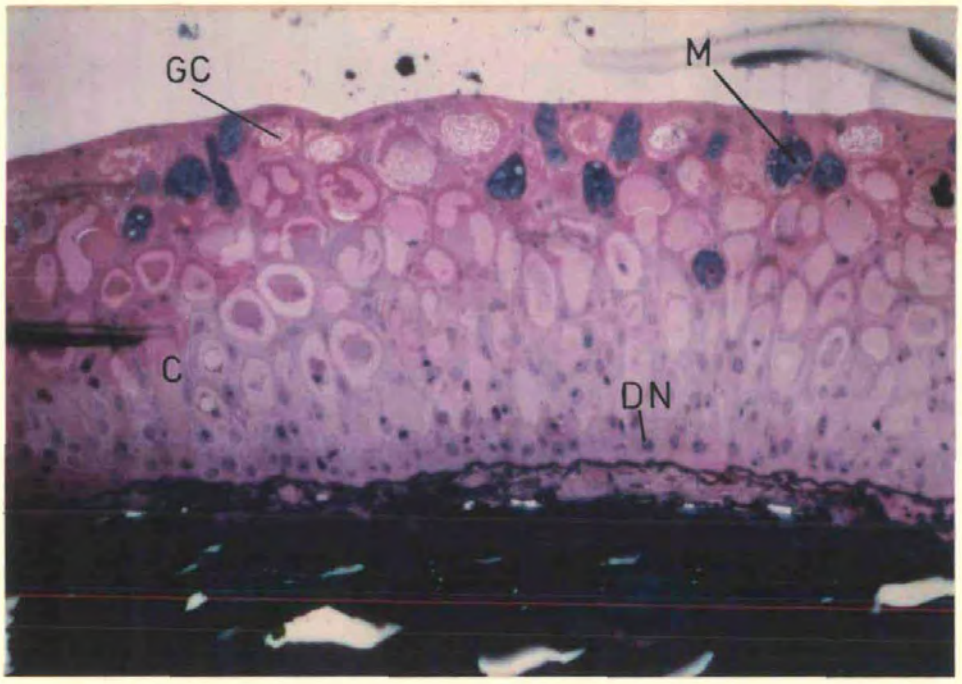

GC
M
C
DN

PLATE 3

A. Scanning electronmicrograph of skin surface from dorsal region. Cell boundaries are just visible and numerous mucous cells open onto the surface. There is evidence of the remains of sloughed cells (x 453).

SCR Sloughed cellular remains

B. Scanning electronmicrograph of skin surface from dorsal region showing reticulate pattern of microridges. Mucous cell openings occur between epidermal cells and a raised double ridge is present at the cell wall boundary (x 915).

ECB Epidermal cell boundary

MO Mucous cell opening

Mr Microridges of epidermal cell


SCR

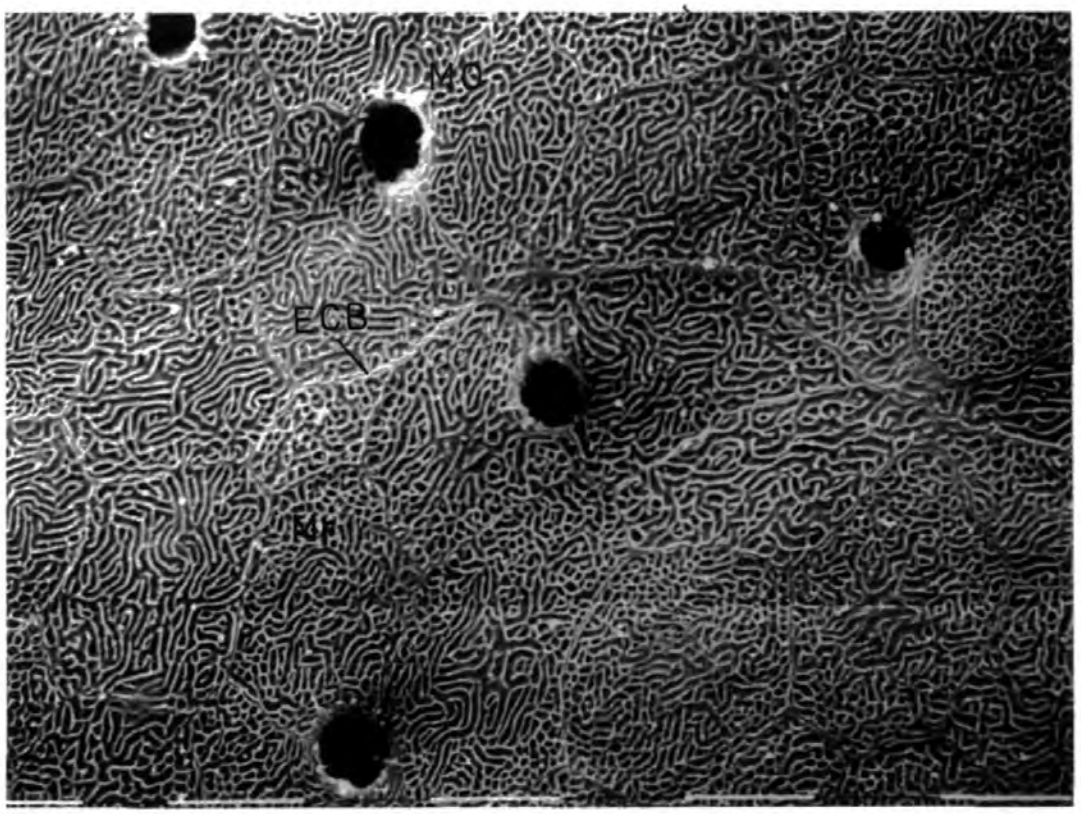

MG
ECB
MF

PLATE 4

A. Scanning electronmicrograph of skin surface from dorsal region. Mucous covering can be seen around the mucous cell opening (x 3,480).

ML Mucous layer

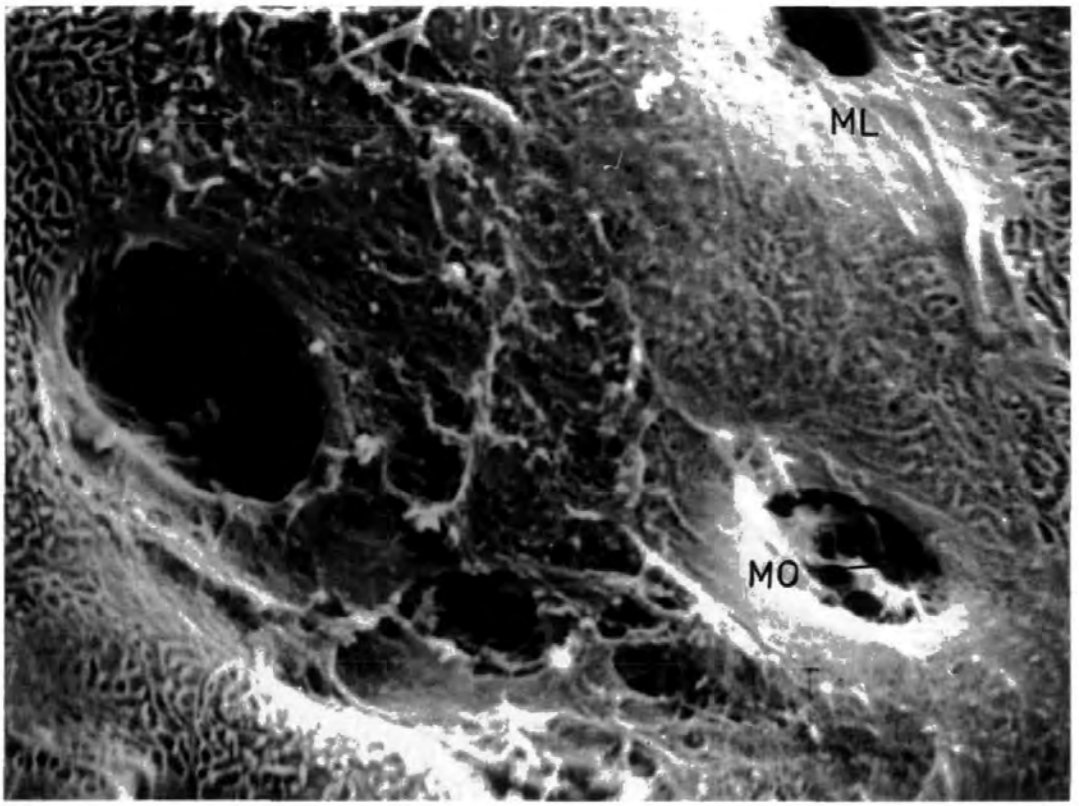
MO Mucous cell opening

B. Scanning electronmicrograph of skin surface from dorsal region showing opening of mucous cell. The internal structure of the mucous cell is not easily distinguishable. The microridges of the surrounding epidermal cells appear to extend into the mucous cell opening and a mucous covering appears to extend across the apices of the microridges. Small openings (approx. 0.2 µm in diameter) are seen in places between the ridges of surrounding epidermal cells (x 13,920).

ESV Epidermal secretory vesicle

ML Mucous layer

Mr Microridges


ML
MO

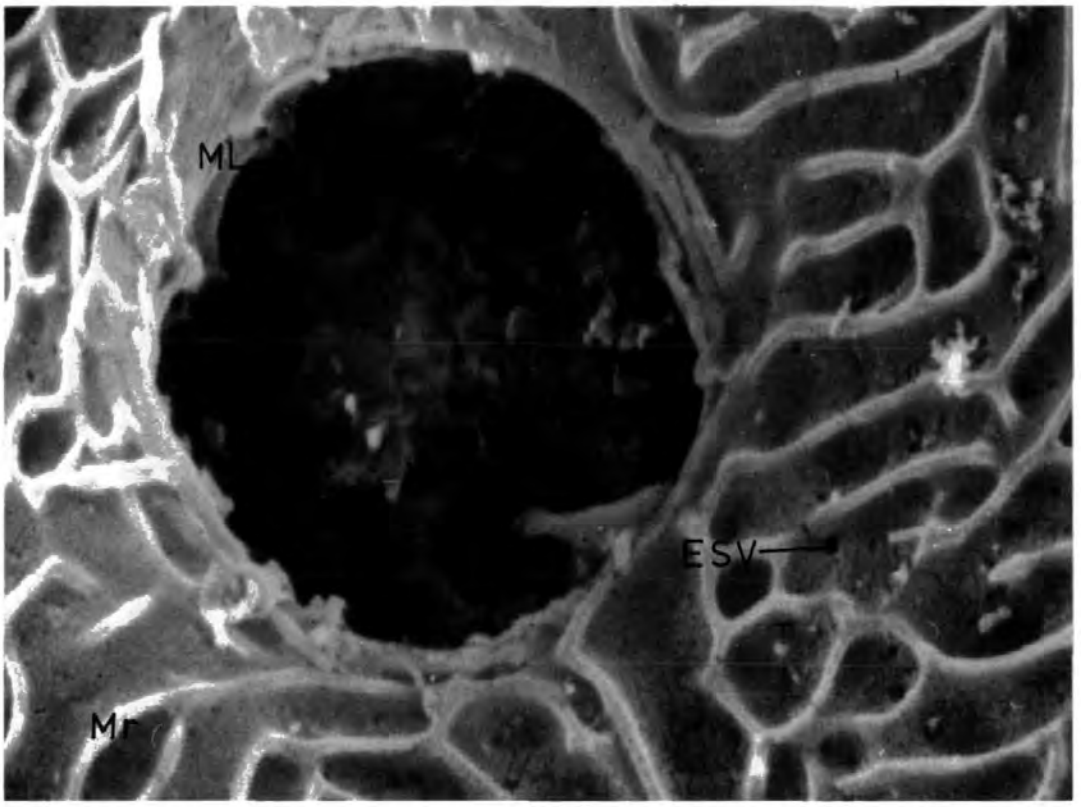

ML
ESV
Mr

PLATE 5

A. Transmission electronmicrograph of surface epidermal cells. The flattened cells at the surface with surface microridges and mucous covering can be seen with tight cell junctions (arrowed) and interdigitation between epidermal cells (x 5,850).

E Epidermal cell

ESV Epidermal cell secretory vesicle

ML Mucous layer

Mr Microridges

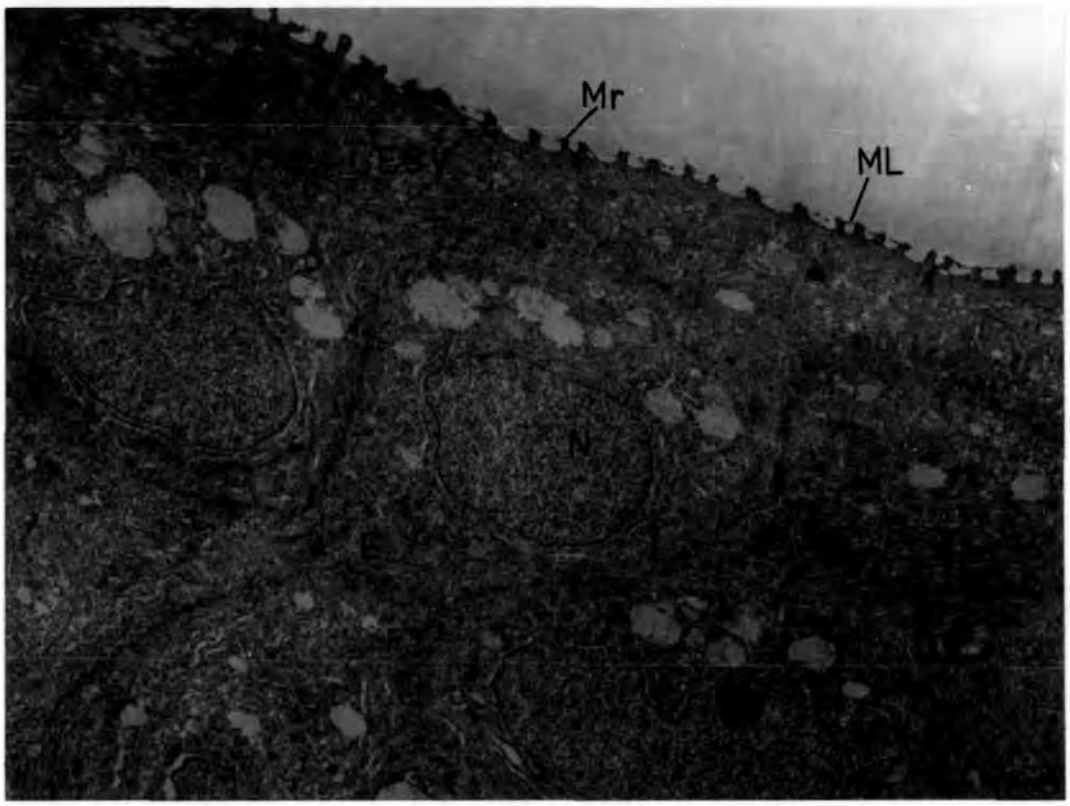
N Nuclei

B. Electronmicrograph of basal cell layer. Dark staining cuboid epidermal cells are seen to comprise the layer with a number of lymphocytes above them. Collagen fibres of the spongiosum compactum are seen immediately below the basement membrane (x 4,590).

Co Collagen

D Dermis

L Lymphocyte


Mr
ML
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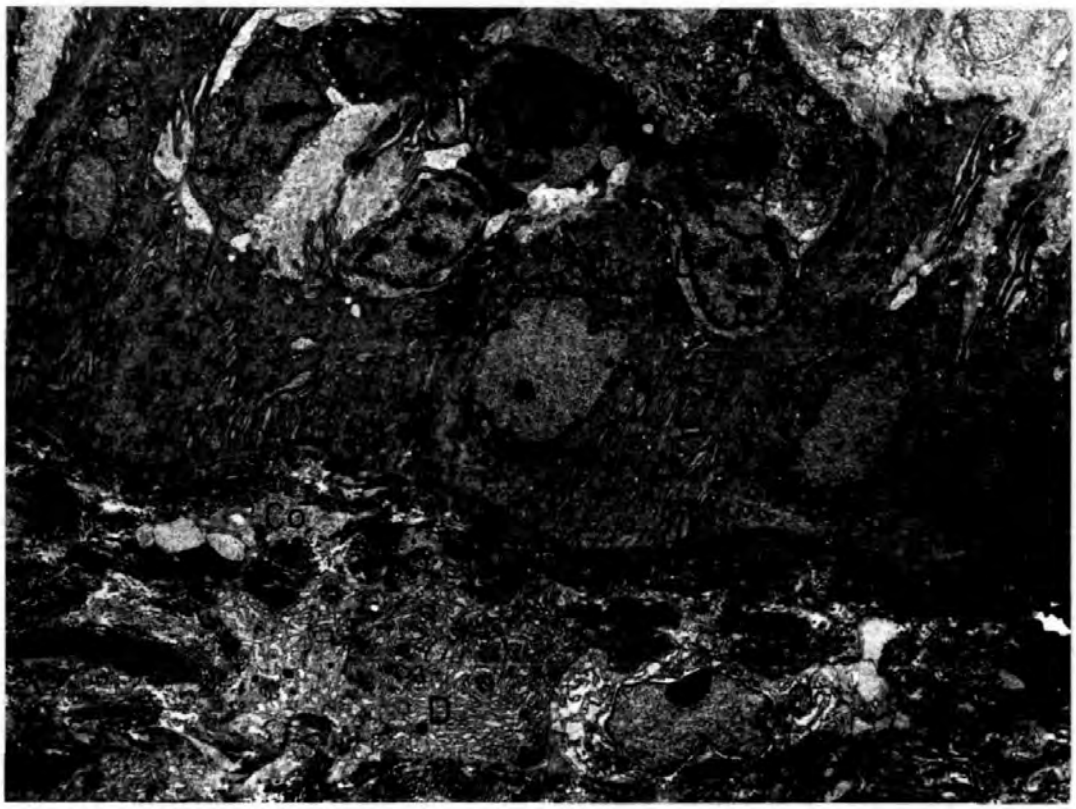

Co
D

PLATE 6

A. Electronmicrograph of basal cell layer and basement membrane. A club cell is seen between epidermal cells of the basal cell layer (x 7,600).

BM Basement membrane

C Club cell

Co Collagen

F Folds

L Lymphocyte

B. Electronmicrograph of basal cell layer and basement membrane showing folds extending from basement membrane into the basal layer cell. Hemidesmosomes are also seen between the cell and the basement membrane (x 10,000).

BM Basement membrane

F Folds

H Hemidesmosomes

N Nuclei

PLATE 7

A. Electronmicrograph of sloughing epidermal cell. Breakdown of cellular contents is apparent and numerous lysosomes are seen. Formation of underlying microridges is also seen (arrowed) (x 13,260).

EC Epidermal cell

Ly Lysosomes

B. Electronmicrograph of surface epidermal cells with mucous material covering epidermal ridges. Complex interdigitation between adjacent cells can be seen. Mucous layer measures approximately 0.5 µm thick (x 22,680).

In Interdigitation between epidermal cells

ML Mucous layer

Mr Microridges


E

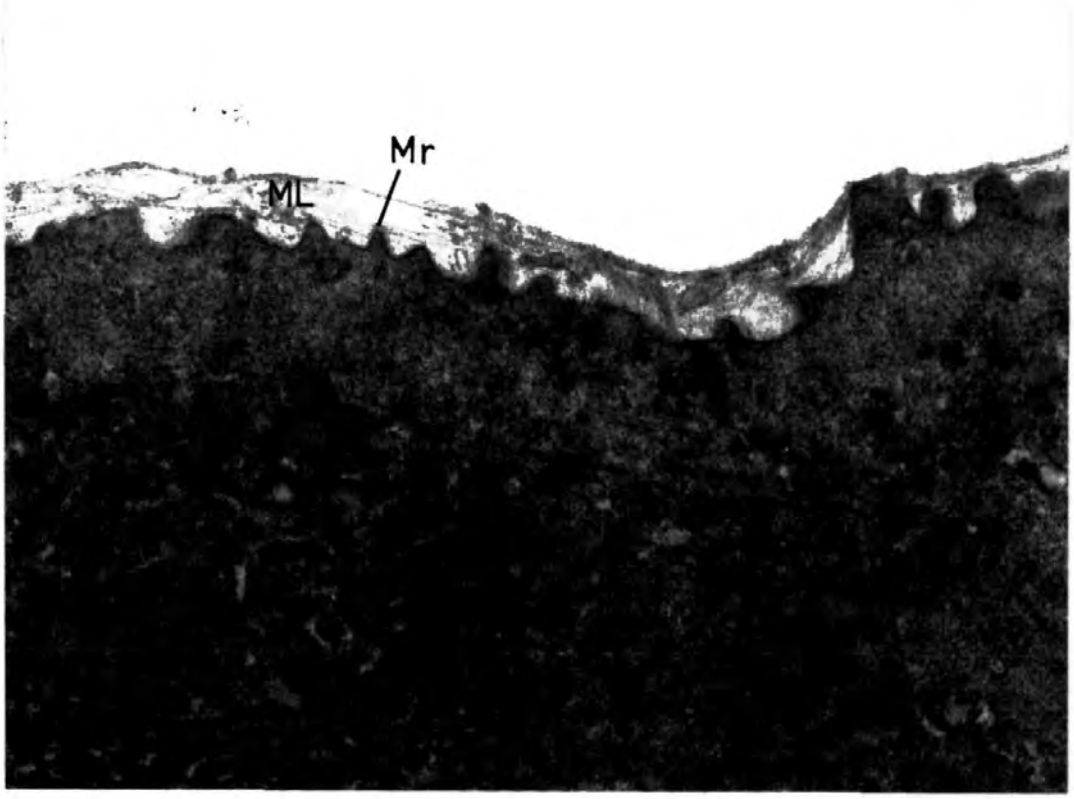

Mr
ML

PLATE 8

A. Electronmicrograph of club cells. Around the central nucleus are the cell organelles and above it the cell vacuole. Mitochondria are also seen in the peripheral regions of the cell surrounded by an electron dense layer (x 3,960).

CF Club cell filamentous material

CV Club cell vacuole

Mit Mitochondrion

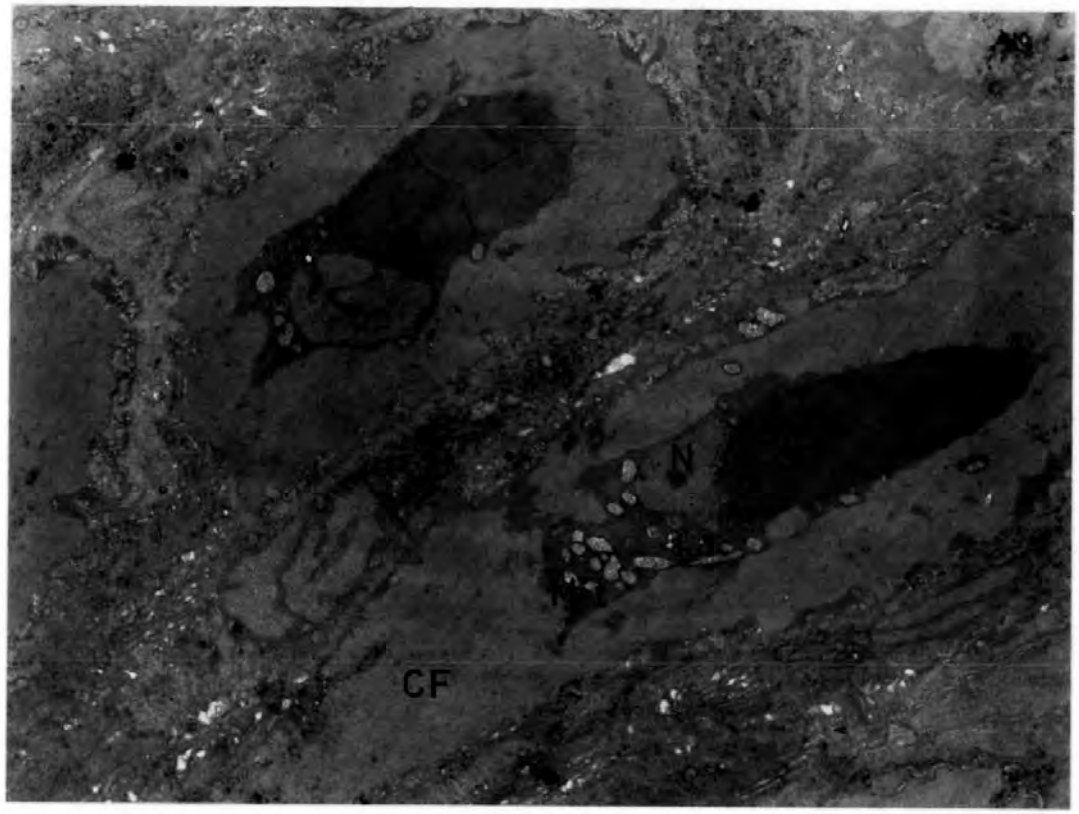
N Nucleus

B. Electronmicrograph of secreting club cell. Surface epidermal cells are seen being forced off by the club cell contents (x 4,620).

C Club cell

CF Club cell filamentous material

SEC Surface epidermal cell


N
CF

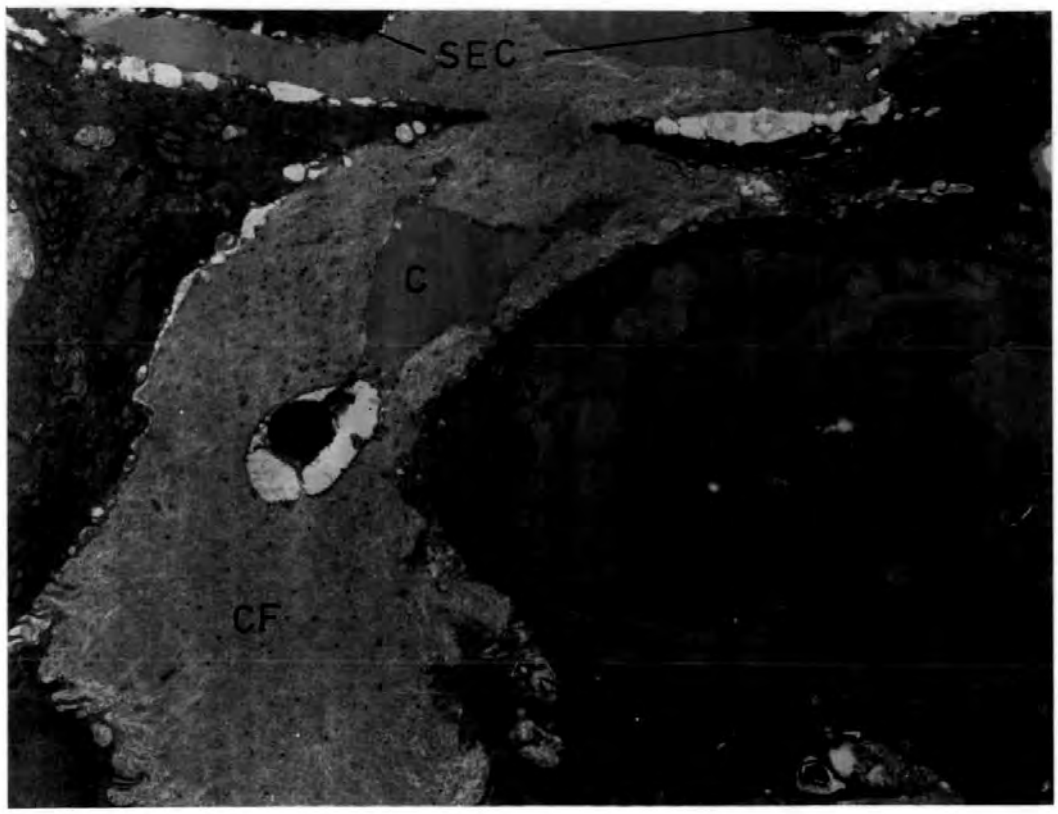

SEC
C
CF

PLATE 9

A. Electronmicrograph of club cell and epidermal cell boundary showing the complex interdigitation between cells (arrowed). Spherical bodies are seen in the club cell and filamentous bundles can be observed in the epidermal cell (x 17,680).

C Club cell

ECF Epidermal cell filaments

SB Spherical bodies (filamentous material)

B. Electronmicrograph of club cell showing the coiled structure of filaments. Filament diameter (arrowed) is 25 nm - 28 nm and distance between coils (barred arrows) is approximately 15 nm (x 64,000).

CF Club cell filamentous material

C. Electronmicrograph of perinuclear area of club cell containing mitochondria and rough-surfaced endoplasmic reticulum. Free ribosomes occur in the perinuclear and peripheral cell regions with the spherical bodies in the lighter staining region (x 13,860).

CV Club cell vacuole

Mit Mitochondrion

N Nucleus

RER Rough endoplasmic reticulum

SB Spherical bodies


SB

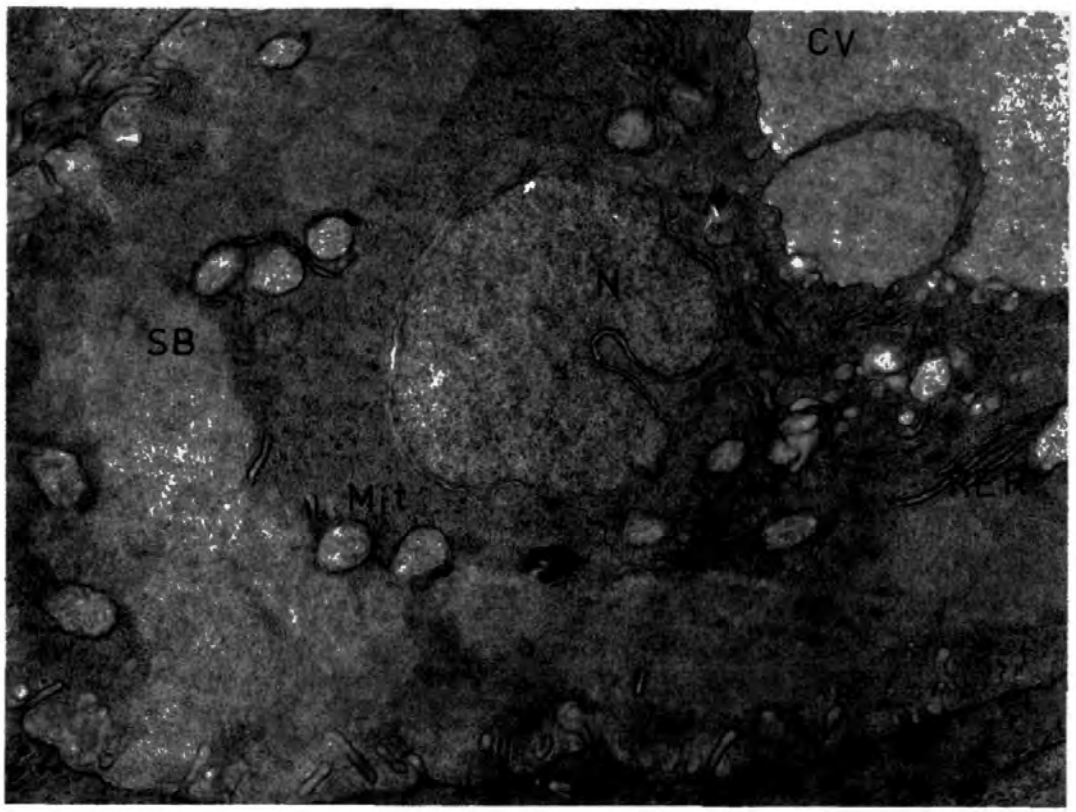

CV
N
SB
Mit

PLATE 10

A. Electronmicrograph of granular club cell with central vacuole packed with granular material. Spherical bodies are still discernible in the lighter staining region between the peripheral and perivascular regions (x 5,610).

Gr Granules

SB Spherical bodies

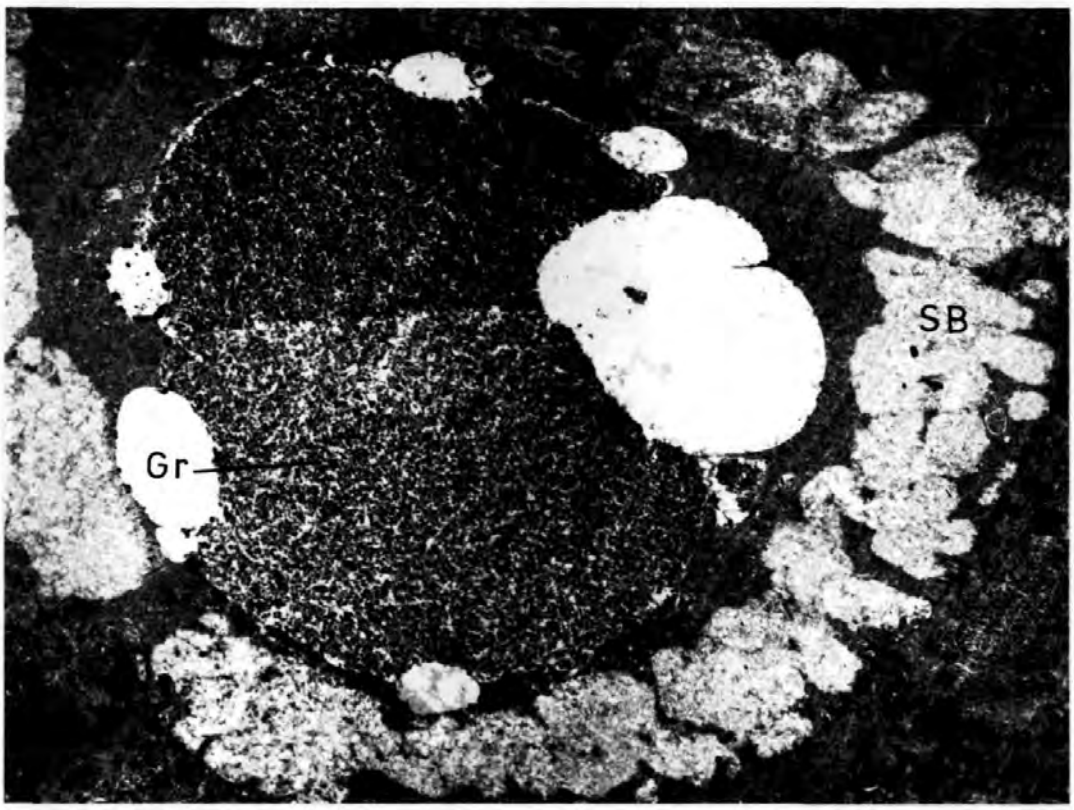
B. Electronmicrograph from mid-epidermal region. Club cell components released from the cell have spread horizontally through the epidermis. Bundles of filaments and some spherical bodies are apparent (x 5,940).

CC Club cell contents

EC Epidermal cell

FB Filamentous bundles

SB Spherical bodies


SB
Gr


FB
SB

PLATE 11

A. Light micrograph of epoxy section of epidermis. Club cells have released their contents beneath the epidermal surface (x 1,280)

C Club cell

CC Club cell contents

M Mucous cell

B. Light micrograph of epoxy section of epidermis. Club cell contents have spread horizontally through epidermis. Filamentous bundles are apparent (x 2,500).

C Club cell

CC Club cell contents

FB Filamentous bundles

M Mucous cell


M
CC

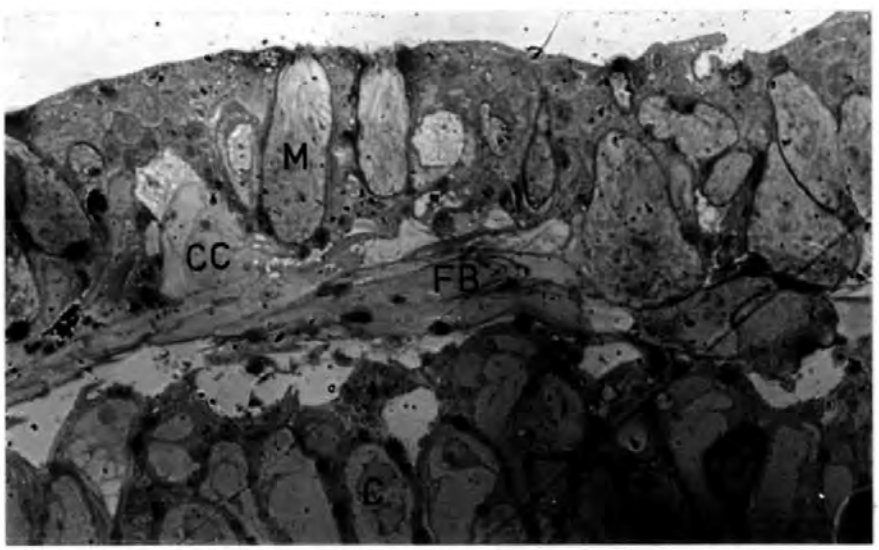

M
CC
FB
C

PLATE 12

A. Electronmicrograph of club cell filamentous material and remains of perinuclear region after rupturing of cell beneath the epidermal surface (x 8,220).

FB Filamentous bundles

PR Perinuclear region

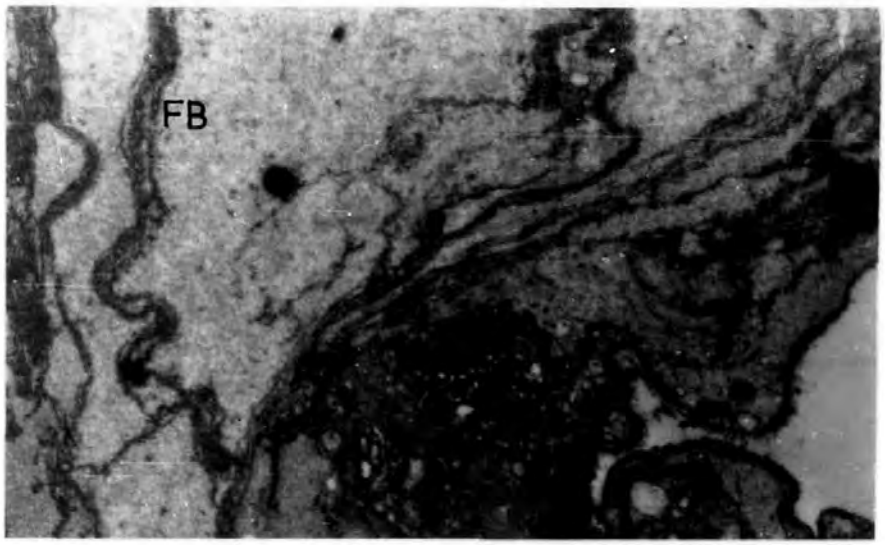
B. Electronmicrograph of club cell ruptured beneath the epidermal surface. Spherical bodies of the light staining area are still apparent (x 8,220).

SB Spherical bodies

C. Electronmicrograph of club cell ruptured beneath the epidermal surface. Filamentous threads are arranged in bundles (x 8,220).

FB Filamentous bundles

SB Spherical bodies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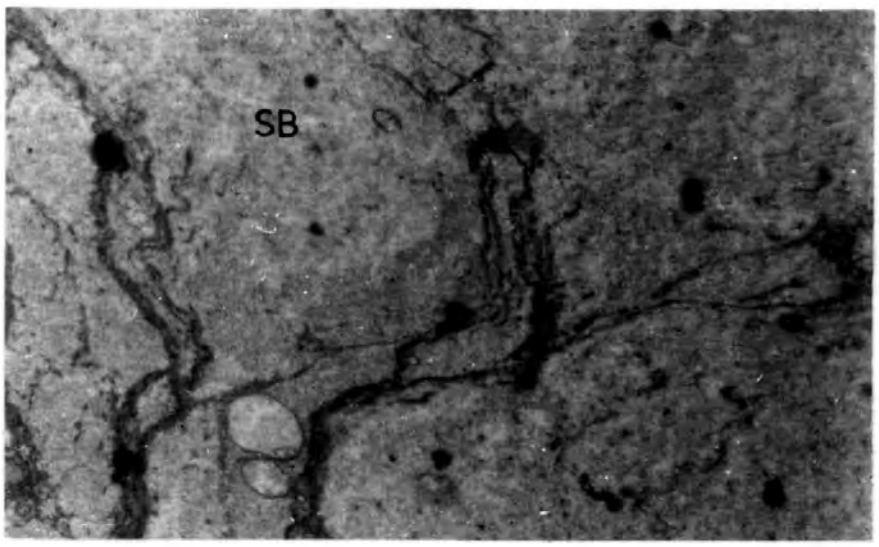
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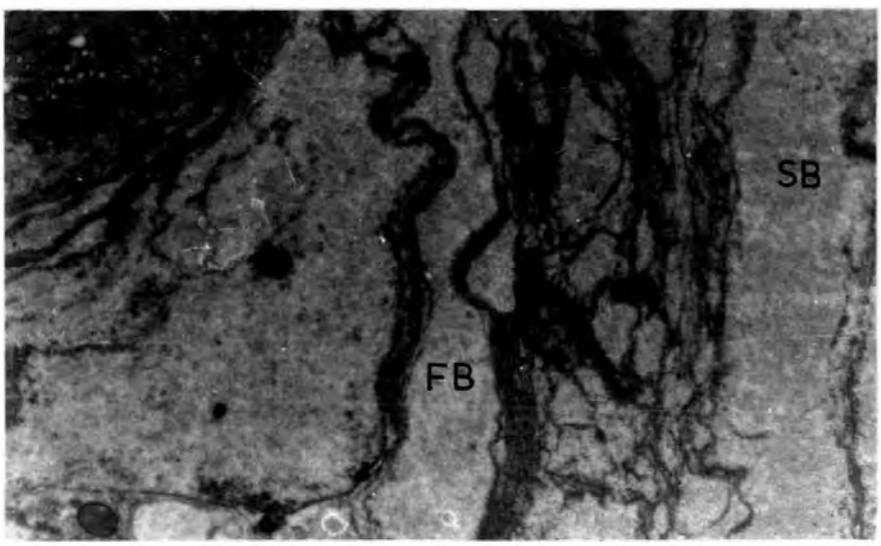

SB
FB

PLATE 13

A. Electronmicrograph of surface epidermal cell. Free melanin granules are seen and also a number of vesicles which appear to be releasing their contents to the surface (x 26,000).

E Epidermal cell

ESV Epidermal cell secretory vesicle

MGr Melanin granule

Mr Microridges

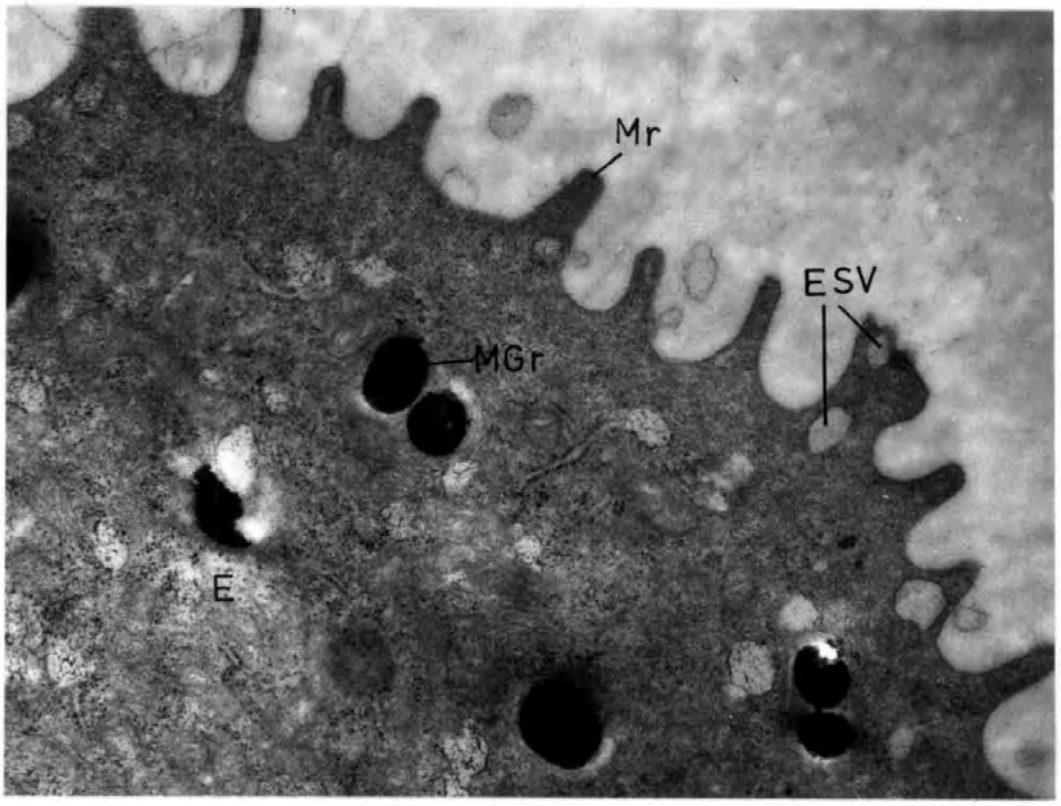
B. Electronmicrograph of surface epidermal cell. Staining with phosphotungstic acid (PTA) shows vesicles to contain protein-carbohydrate material. Some vesicles do not appear to take up stain (x 20,800).

E Epidermal cell

ESV Epidermal cell secretory vesicle

Mr Microridges

V Vesicle


Mr
ESV
MGr
E

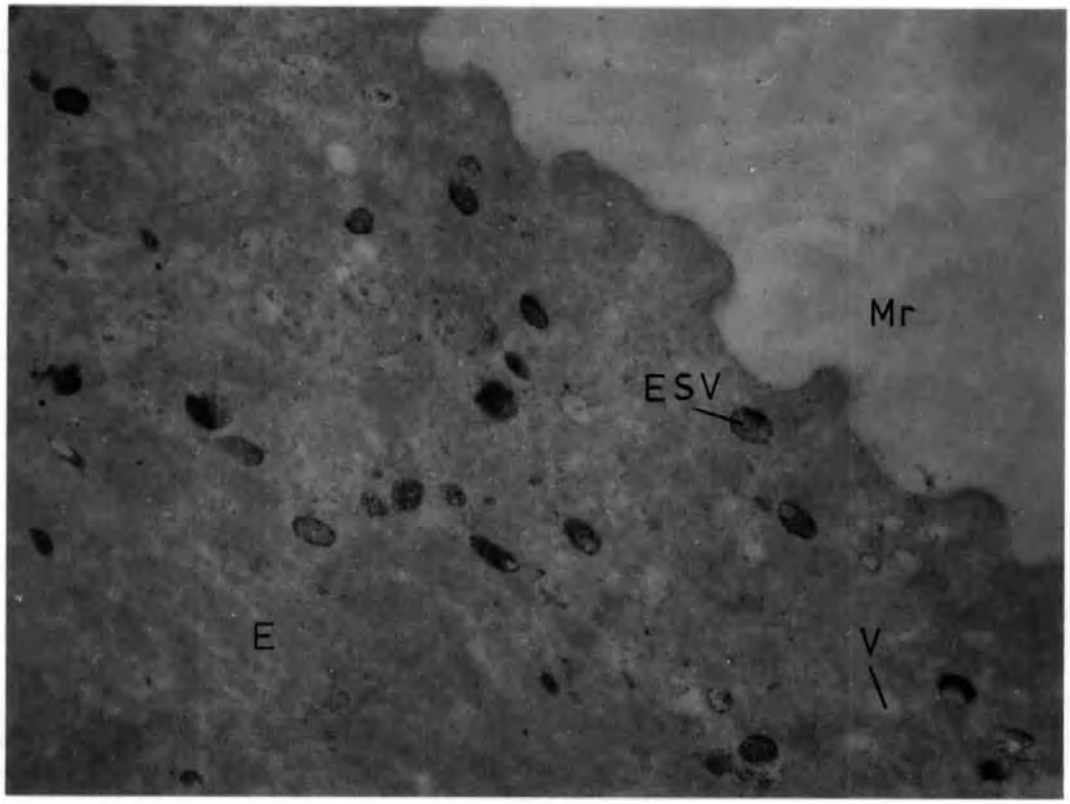

Mr
ESV
E
V

PLATE 14

A. Electronmicrograph of epidermal cells in mid-epidermal region after removal of surface mucus. Characteristic bundles of filaments are seen around the periphery of the cell. Prominent mitochondria and Golgi apparatus producing secretory droplets are also apparent (x 14,040).

Des Desmosomes

ECF Epidermal cell filaments

G Golgi apparatus

Mit Mitochondrion

N Nucleus

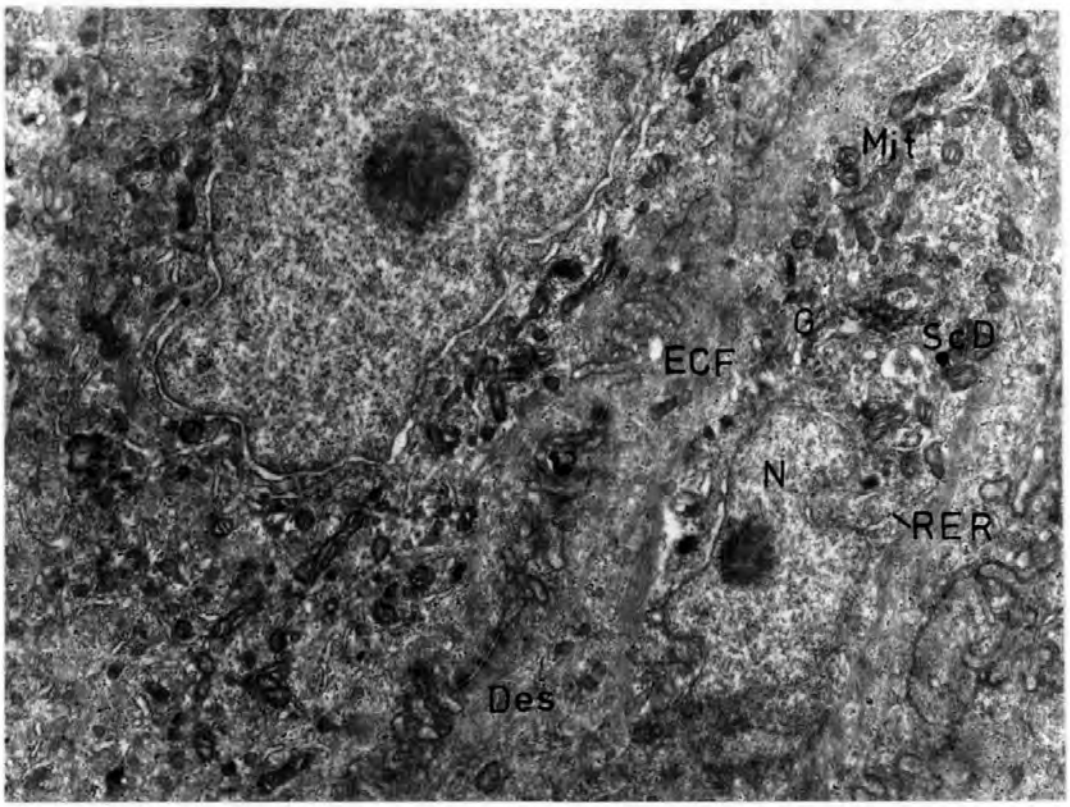
RER Rough endoplasmic reticulum

ScD Secretory droplets

B. Electronmicrograph of epidermal cells at mid-epidermis after removal of surface mucus. Coalescence of smaller secretory droplets (arrowed) is occurring forming larger secretory vesicles (x 12,600).

ECF Epidermal cell filaments

N Nucleus


Mit
G
ScD
ECF
N
RER
Des

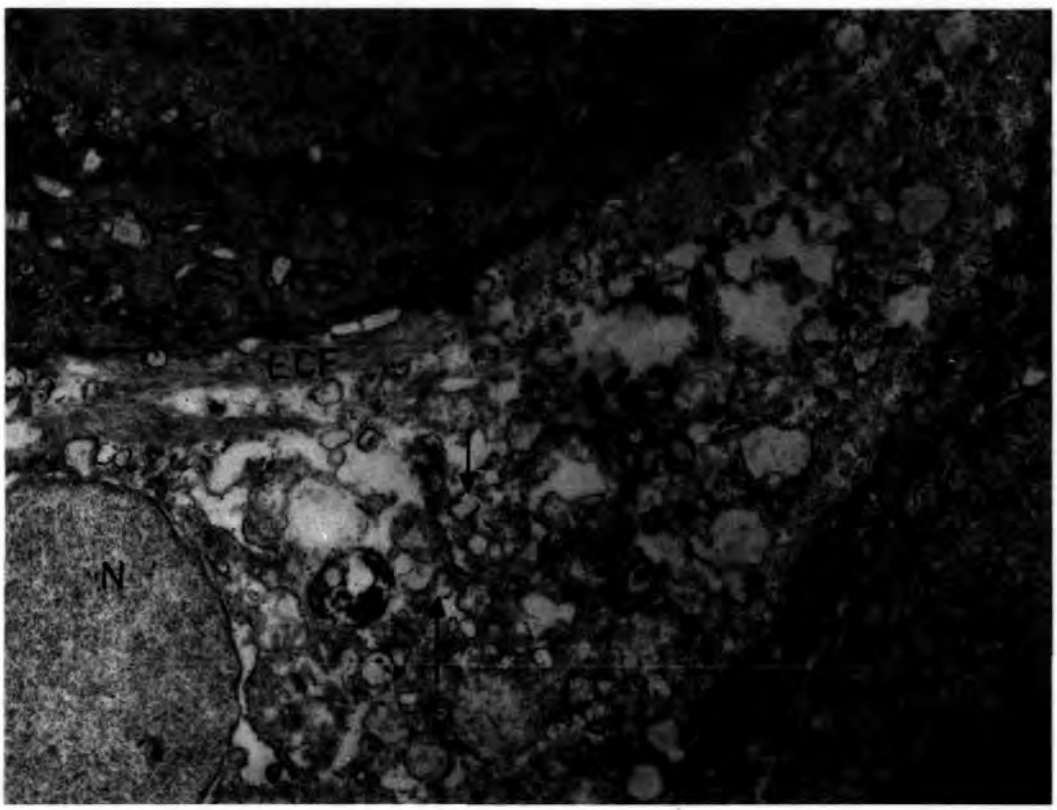

ECF
N

PLATE 15

A. Electronmicrograph of epidermal cell in middle stages of its secretory role. Characteristic epidermal cell filaments are still apparent as well as complex interdigitation with an adjacent club cell. There is an increase in numbers of secretory vesicles (x 17,600).

C Club cell

Des Desmosomes

ECF Epidermal cell filaments

In Interdigitation

Mit Mitochondrion

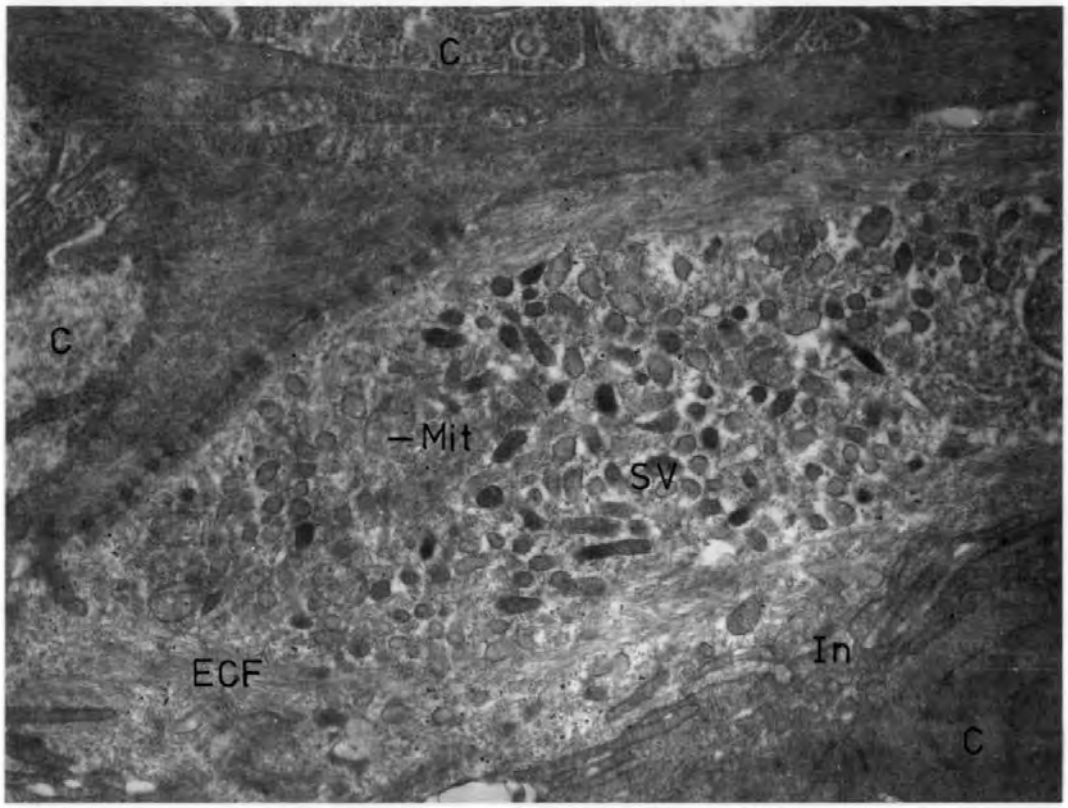
SV Secretory vesicles

B. Electronmicrograph of epidermal cell in secretory role at later stage than above. Secretory vesicle numbers increase and are now tightly packed within the cell (x 10,710).

C Club cell

N Nucleus

SV Secretory vesicle


C
C
Mit
SV
ECF
In
C

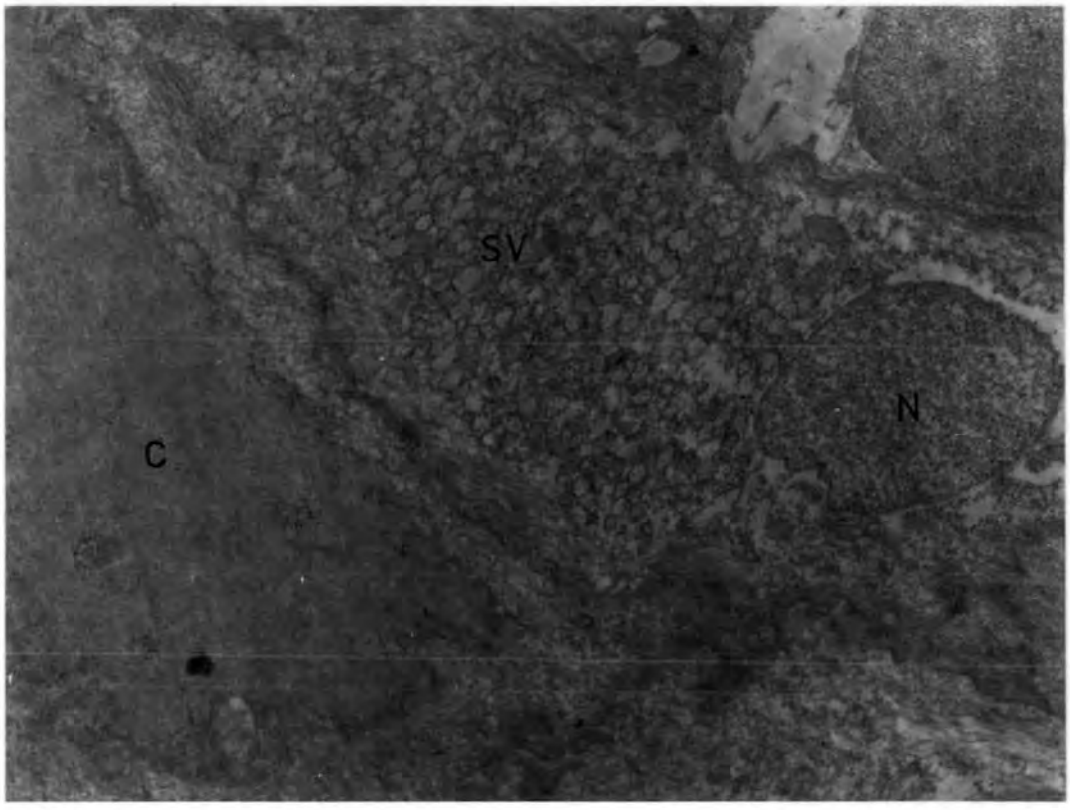

SV
N
C

PLATE 16

A. Electronmicrograph of epidermal cell at late stage in secretory role. Coalescence of secretory vesicles gives it a similar appearence to a mucous cell, although membranes are less complete. Presence of melanin granules further suggests the cell to be of the epidermal cell type (x 6,800).

ECS Epidermal cell secretory material

MGr Melanin granules

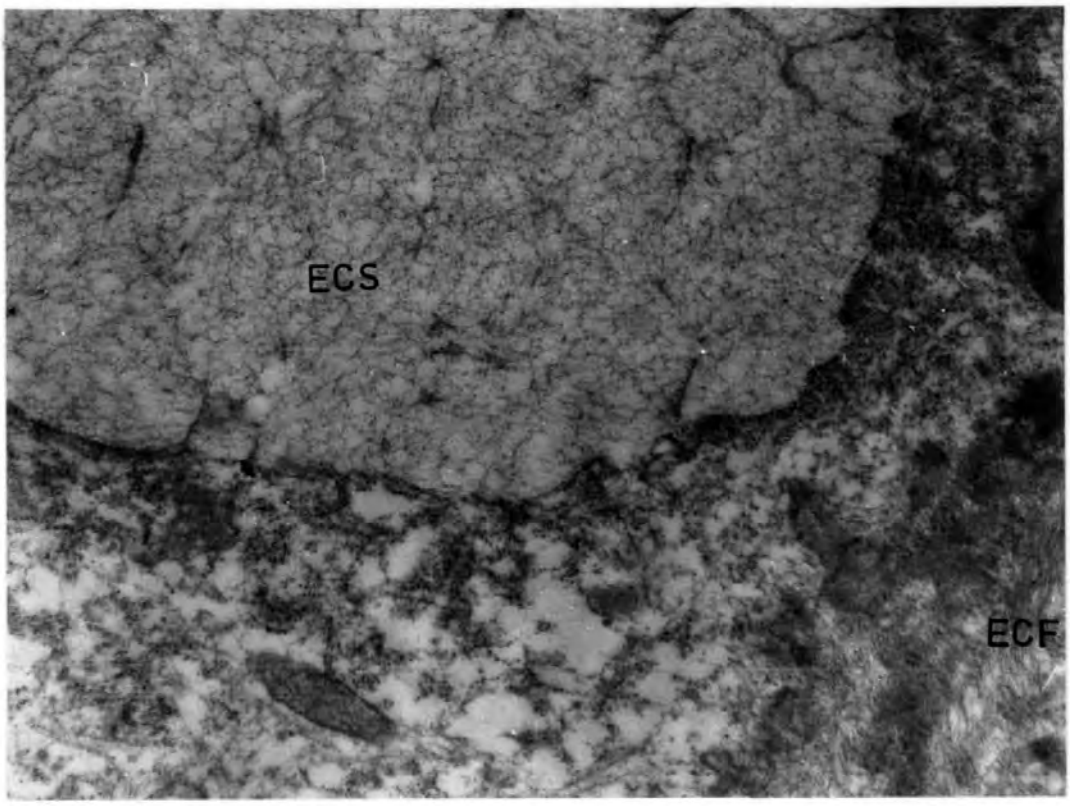
SV Secretory vesicles

B. Electronmicrograph of epidermal cell in late stage of secretory role. Coalescence of secretory vesicles gives rise to large vesicle. Note the lack of definite membrane bound 'mucin packets' seen in mucous cells. Characteristic epidermal cell filaments are also present (x 21,840).

ECF Epidermal cell filaments

ECS Epidermal cell secretory material


ECS
MGr
SV


ECS
ECF

A. Electronmicrograph of epidermal cell in early stage of its secretory role. Numerous mitochondria appear closely associated with rough endoplasmic reticulum and free ribosomes. Filaments, characteristic of epidermal cells are seen in bundles and appear to connect with desmosomal junctions (x 39,600).

Des Desmosome

ECF Epidermal cell filament

Mit Mitochondrion

N Nucleus

R Ribosomes

RER Rough endoplasmic reticulum

SV Secretory vesicle

B. Electronmicrograph of epidermal cell contents in early stages of its secretory role. Closely associated with the Golgi apparatus are secretory droplets which are seen to coalesce to form characteristic 0.25 µm secretory vesicles. Note that the organisation of the cell is markedly different to that of a developing mucous cell (Pl. 20A,B) (x 39,600).

ECF Epidermal cell filament

G Golgi apparatus

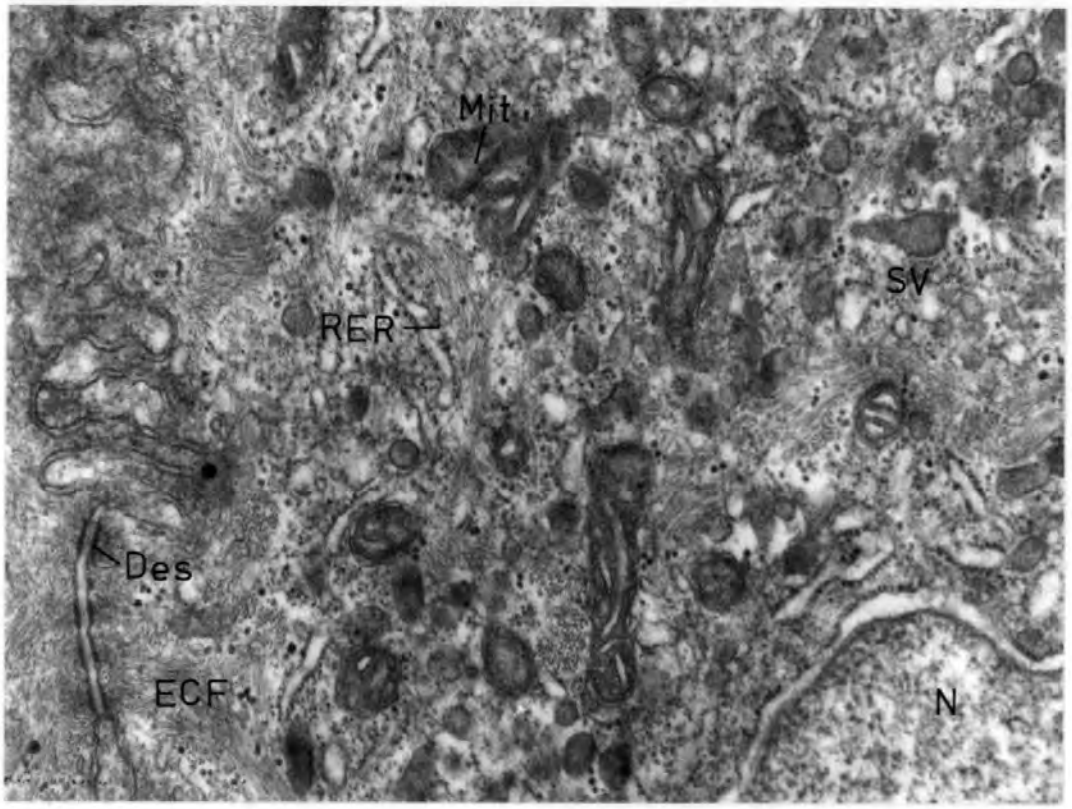
Mit Mitochondrion

R Ribosomes

RER Rough endoplasmic reticulum

SD Secretory droplet

SV Secretory vesicle


Mit
SV
RER
Des
ECF
N

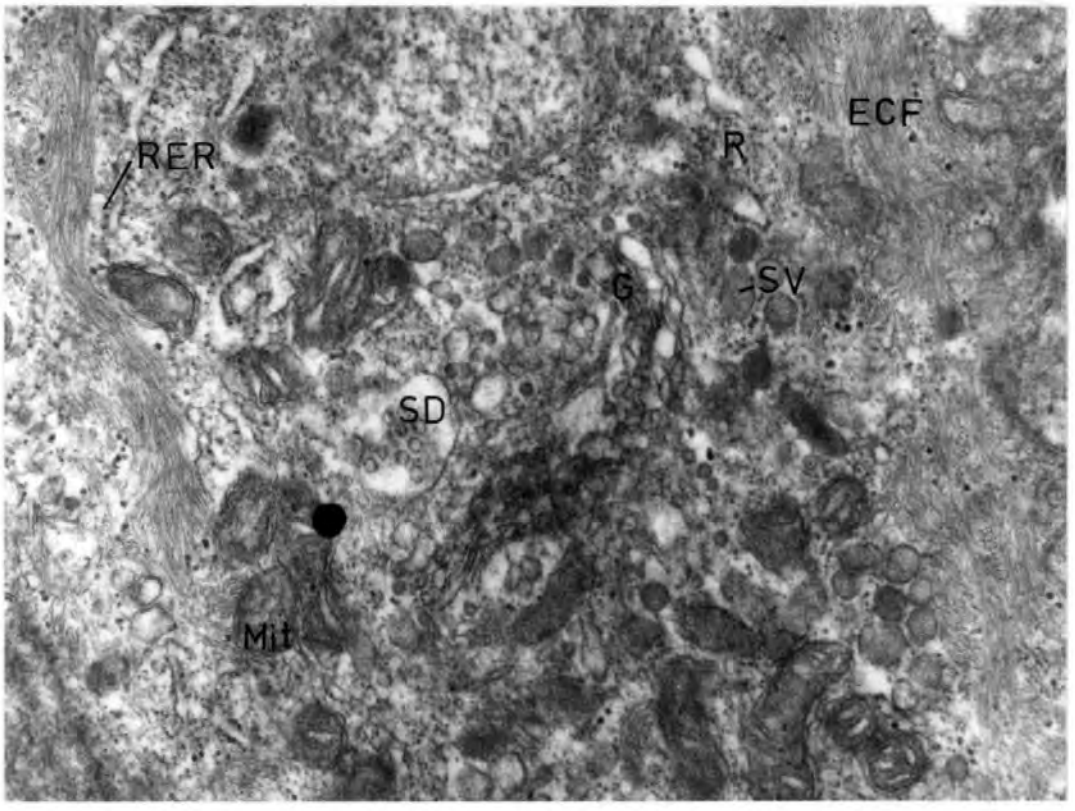

ECF
RER
R
G
SV
SD
Mit

PLATE 18

A. Electronmicrograph of immature mucous cell in lower region of epidermis. Several membrane-bound mucin 'packets' have already formed (x 8,000).

IM Immature mucous cell

MP Membrane bound mucin 'packets'

Mit Mitochondrion

N Nucleus

B. Electronmicrograph of mucous cell at the epidermal surface. Mucus is held in membrane bound 'packets' that sometimes appear incomplete (arrowed). Some mucin 'packets' are seen to stain more densely than others. Cell nucleus and other organelles are displaced and compressed basally (x 3,960).

AP Apical point

C Club cell

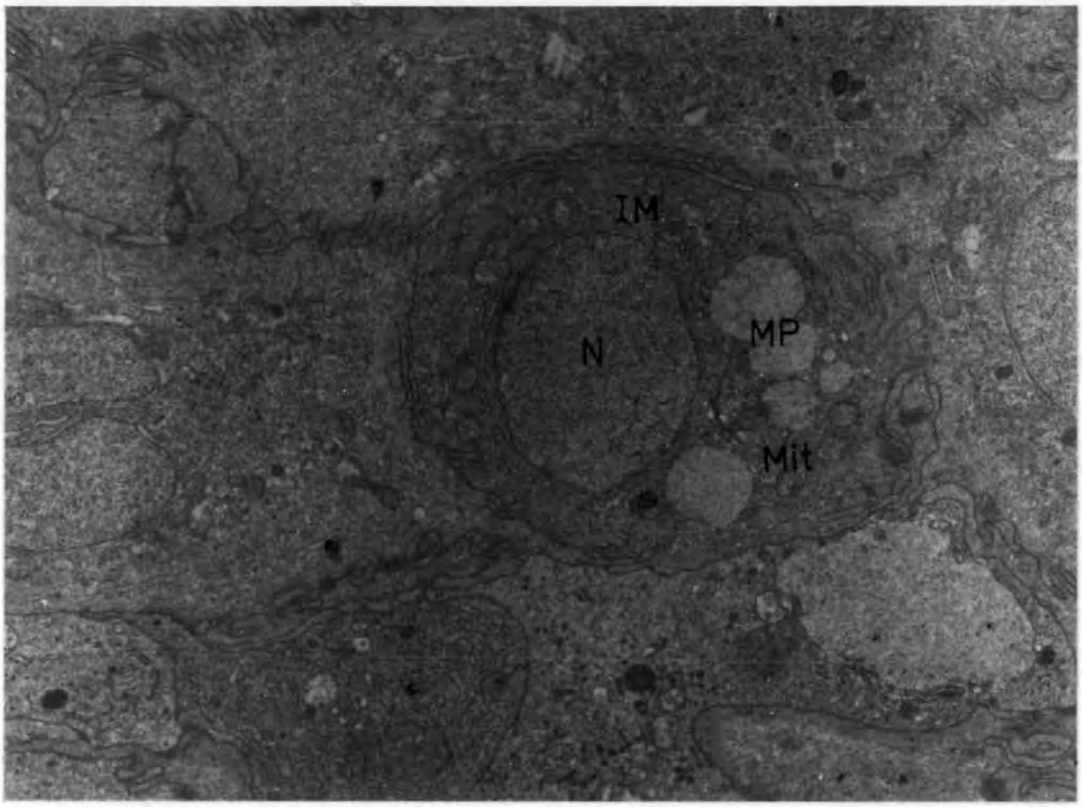
EC Epidermal cell

M Mucous cell

MP Membrane bound mucin 'packets'

Mr Microridges

N Nucleus


IM
N
MP
Mit

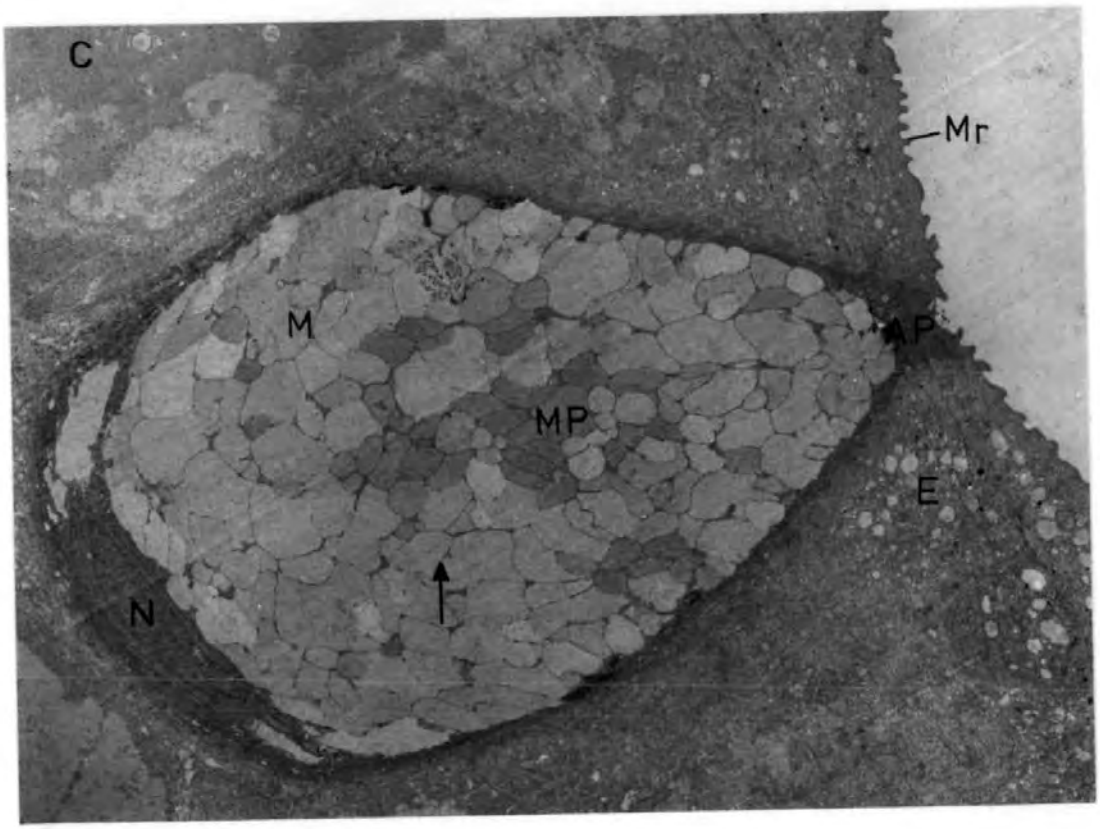

C
Mr
M
MP
E
N

PLATE 19

A. Electronmicrograph of mucous cell open at surface. Immature mucin 'packets can be seen (arrowed) in lower region of the cell (x 9,000).

C Club cell

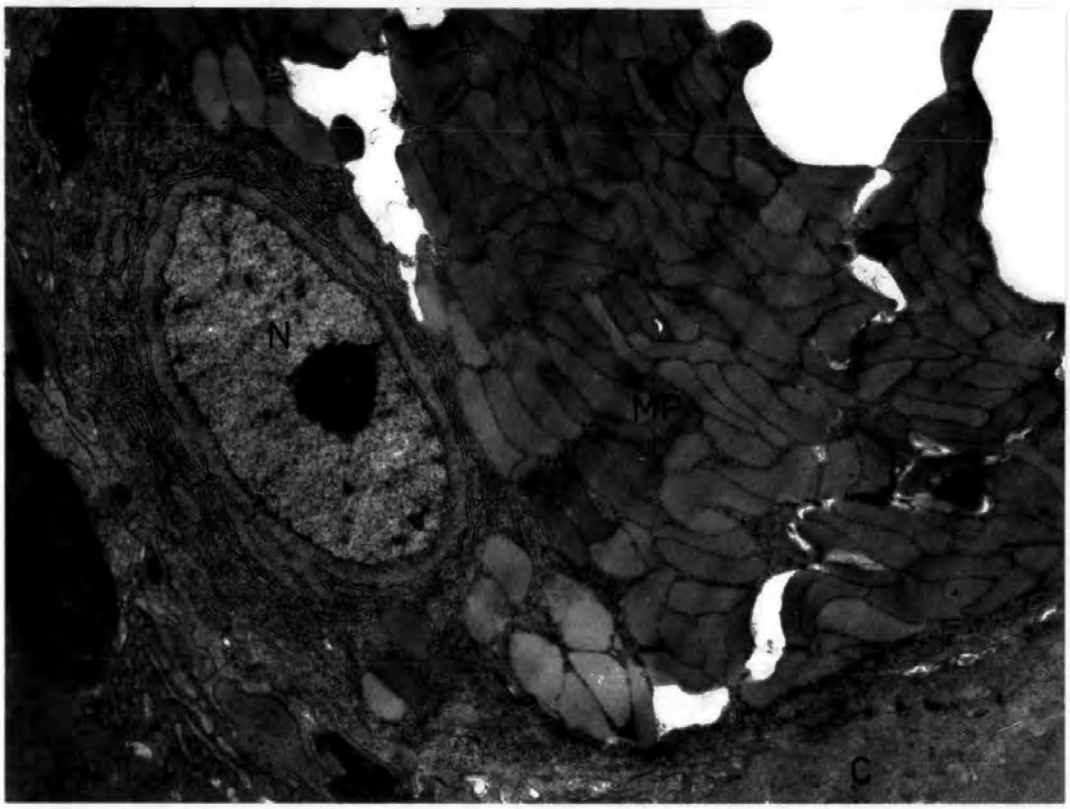
MP Membrane bound mucin 'packets'

N Nucleus

B. Electronmicrograph of mucous cell. Dense staining for protein-carbohydrate material with PTA. Note differential staining uptake of mucin 'packets' (x 4,539).

MP Membrane bound mucin 'packets'

N Nucleus


N
MP
C

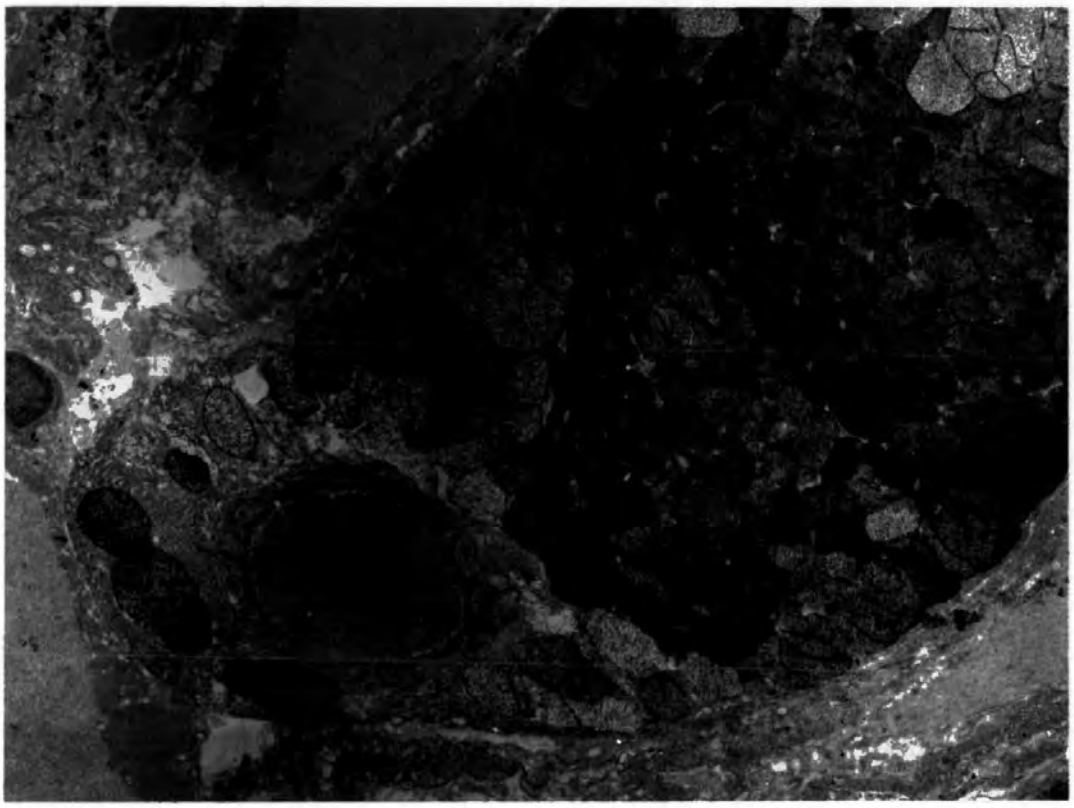


PLATE 20

A. Electronmicrograph of mucous cell in active mucin productive stage (x 8,000).

G Golgi apparatus

MP Membrane bound mucin 'packets'

RER Rough endoplasmic reticulum

SD Secretory droplets

B. Electronmicrograph of mucous cell in active producing stage. Mitochondria are surrounded by rough endoplasmic reticulum. Secretory droplets are closely associated with the Golgi apparatus and coalesce to form membrane bound mucin 'packets' (x 20,800).

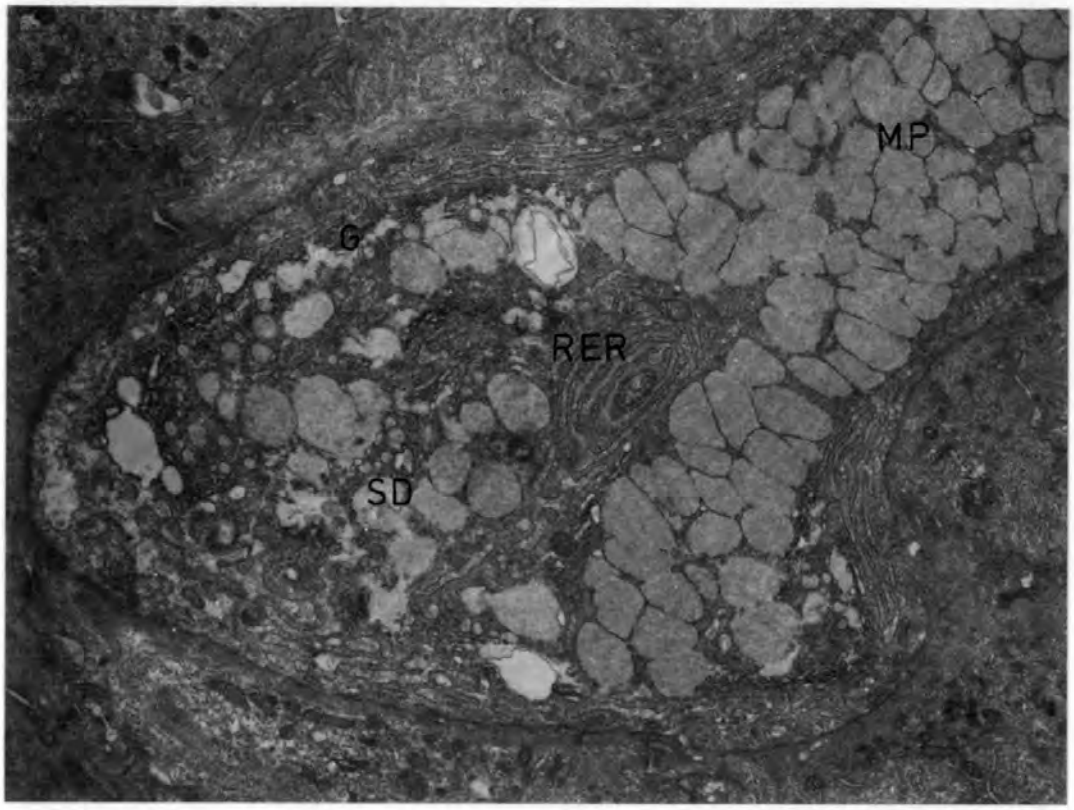
G Golgi apparatus

Mit Mitochondrion

MP Membrane bound mucin 'packets'

RER Rough endoplasmic reticulum

SD Secretory droplets


MP
G
RER
SD

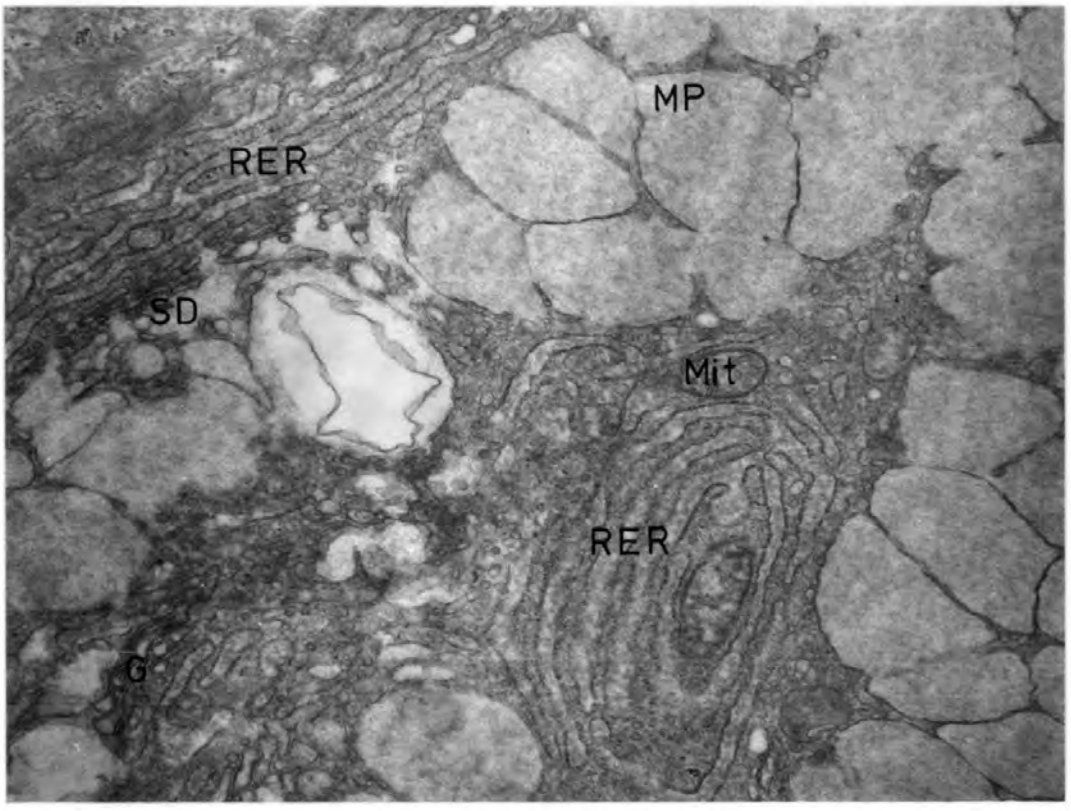

MP
RER
SD
Mit
RER
G

PLATE 21

A. Electronmicrograph of neutrophil-like cell in lower region of epidermis. Nucleus is polymorphous and dark granules are present (x 6,400).

Gr Granules

IS Intercellular space

Mit Mitochondrion

N Nucleus

B. Electronmicrograph of neutrophil-like cell. In addition to granules large numbers of tubular-like structures and also free ribosomes are apparent. Note the large intercellular spaces (x 30,000).

Gr Granules

IS Intercellular space

RER Rough endoplasmic reticulum

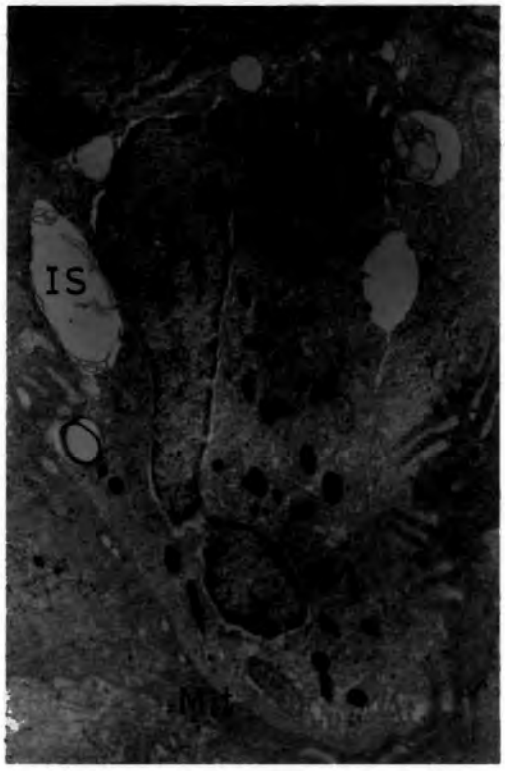
TSER Tubular smooth endoplasmic reticulum

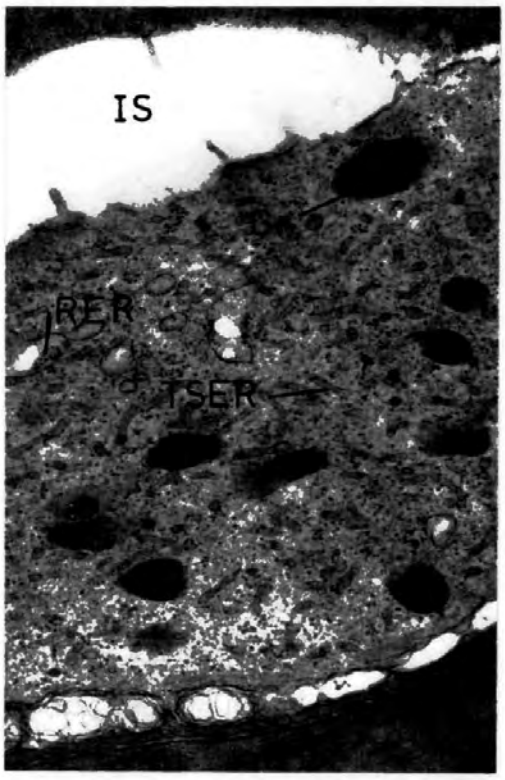
C. Electronmicrograph of neutrophil-like cell in lower region of the epidermis (x 24,700).

Gr Granules

RER Rough endoplasmic reticulum

TSER Tubular smooth endoplasmic reticulum


IS


IS
RER
TSER


RER

PLATE 22

A. Electronmicrograph of lymphocyte in upper regions of the epidermis. The intercellular space is comparatively large and the cell is irregularly shaped (x 12,480).

C Club cell

E Epidermal cell

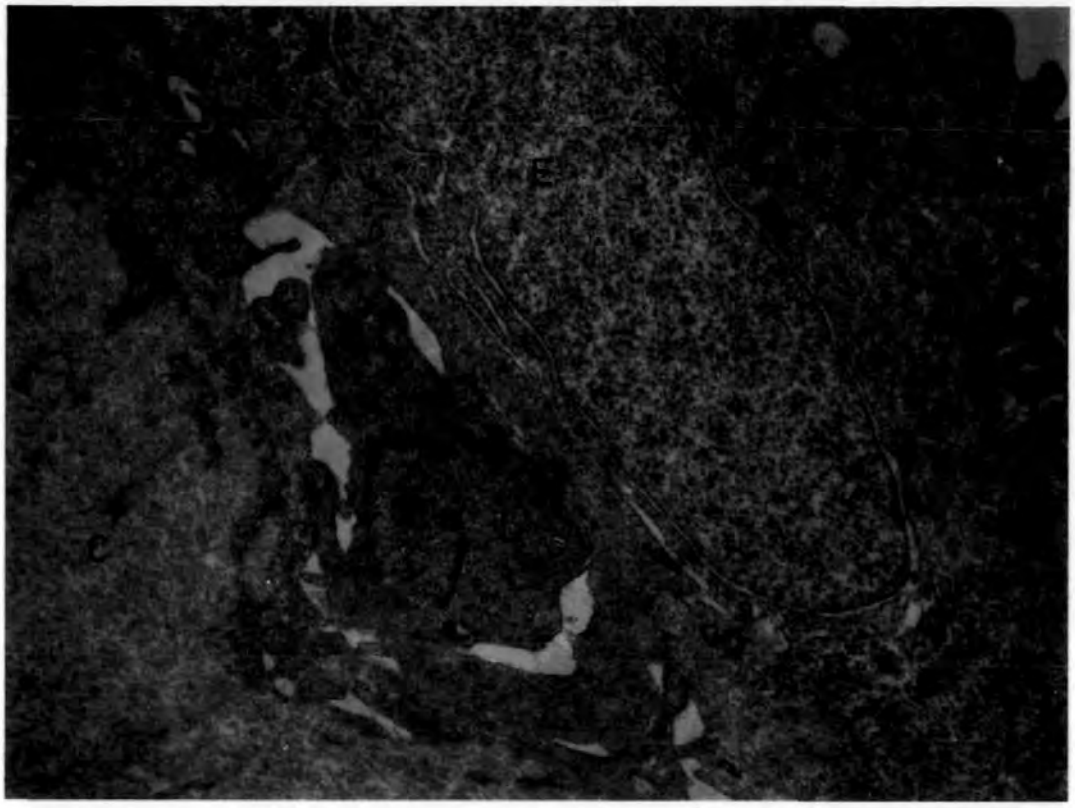
L Lymphocyte

B. Electronmicrograph of macrophage-like cell. Note the vesicular inclusions, membranous whorls and the large intercellualar space (x 12,480).

IS Intercellular space

MW Membranous whorl

VI Vesicular inclusions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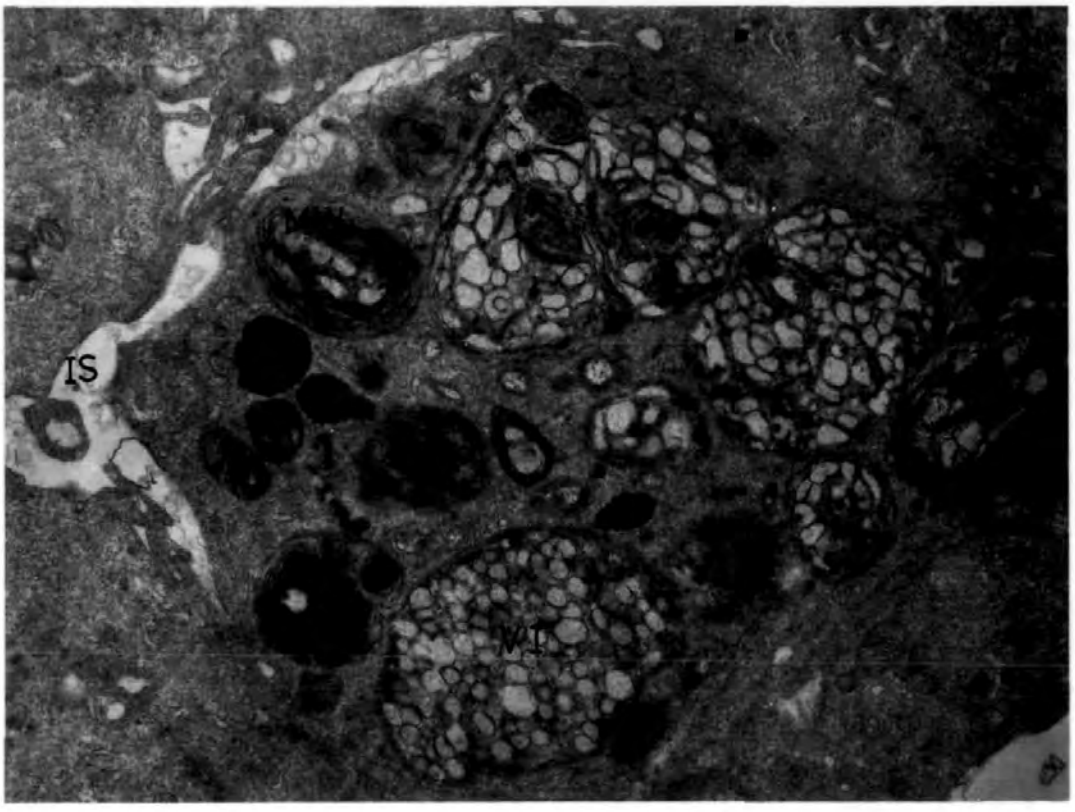

IS
M.I.

PLATE 23

A. Electronmicrograph of myelinated and unmyelinated nerve fibres and a blood capillary within the epidermis. Pinocytotic vesicles are seen in the endothelium of the capillary (x 20,800).

EnC Endothelial cell of blood capillary

MS Myelin sheath

NF Nerve fibre

N Nucleus

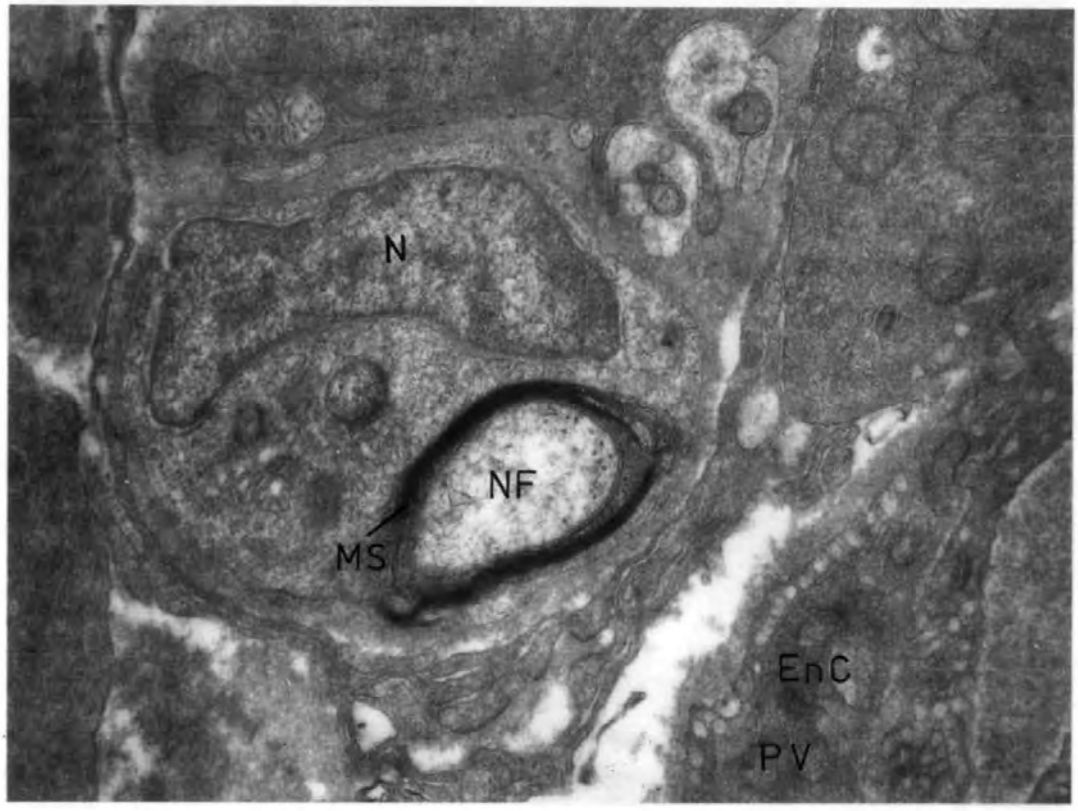
PV Pinocytotic vesicle

B. Electronmicrograph of melanin containing macrophage cell (melano-macrophage) in the mid-epidermal region (x 14,040).

C Club cell

MGr Melanin granules

MM Melanin containing macrophage


N
NF
MS
EnC
PV

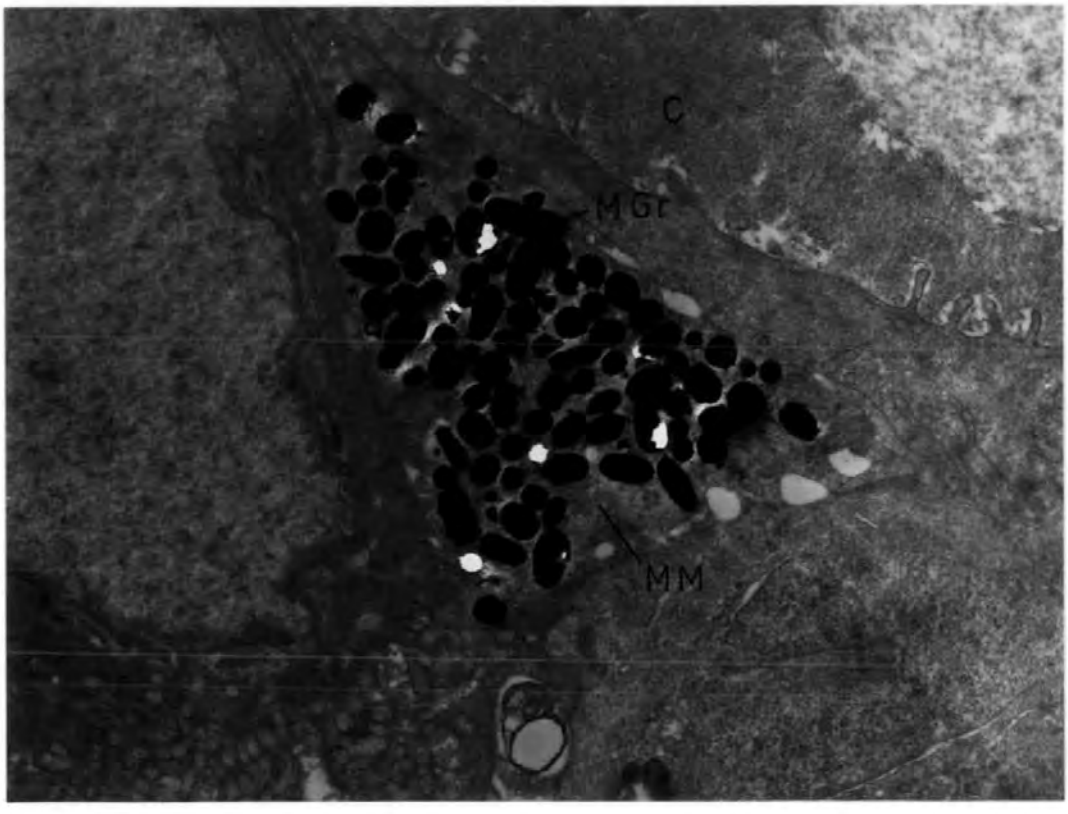

C
MGr
MM

PLATE 24

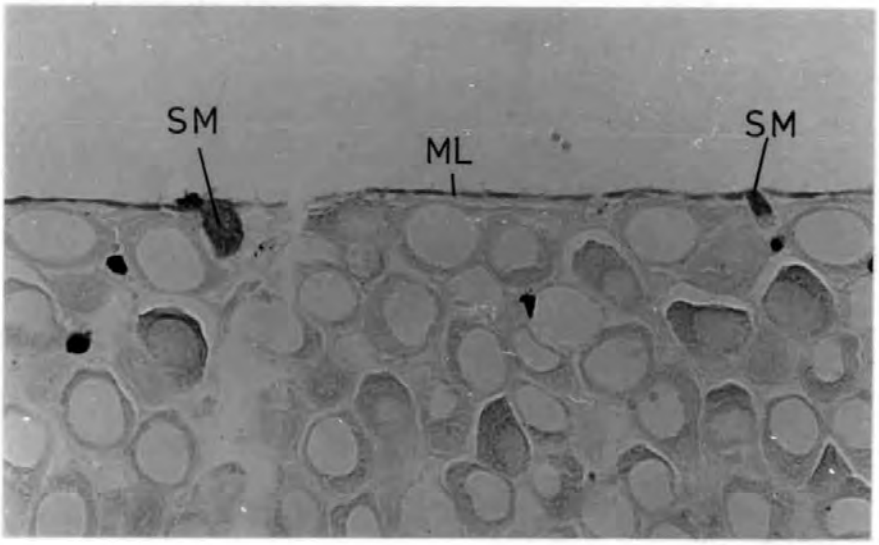
A. Light micrograph of wax section of epidermis stained from the surface with Alcian blue. Note that the stain penetrates only the mucous layer and open mucous cell (x 2,500).

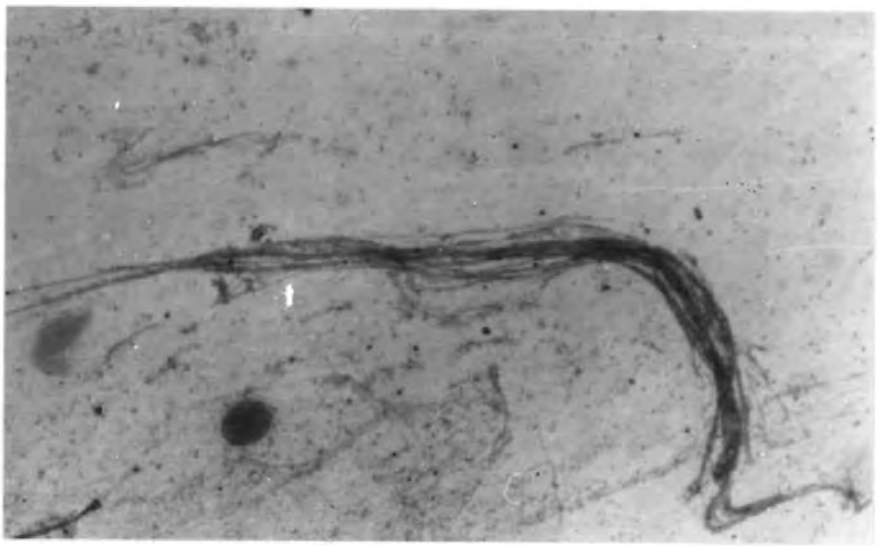
ML Mucous layer

SM Surface mucous cell

B. Light micrograph of thread bundles stained with Alcian blue as seen in a mucous smear. The threads appear to consist of small filaments (x 2,500).

C. Light micrograph of larger thread material from mucous smear. The threads appear as intact club cells probably released by the disruptive effect of compressed air (see Pl. 26A). Mercuric bromophenol blue staining (x 700).


SM
ML
SM



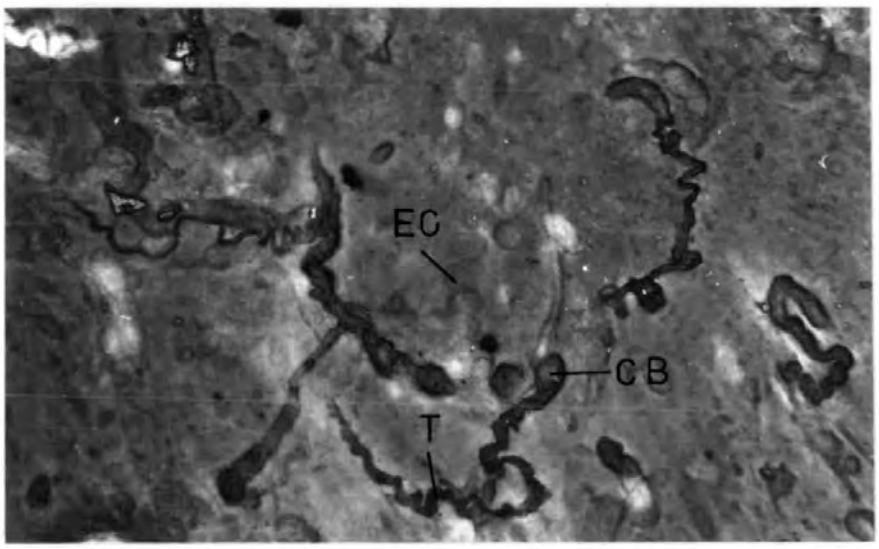

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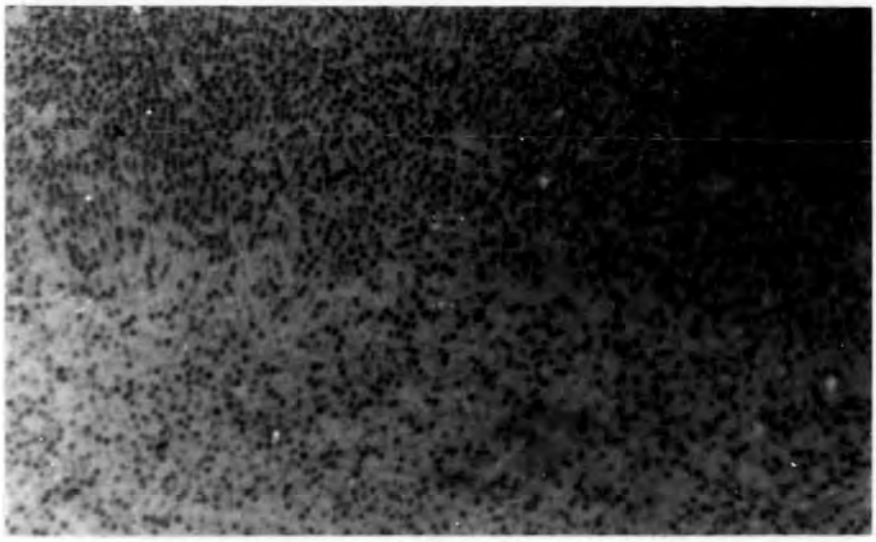
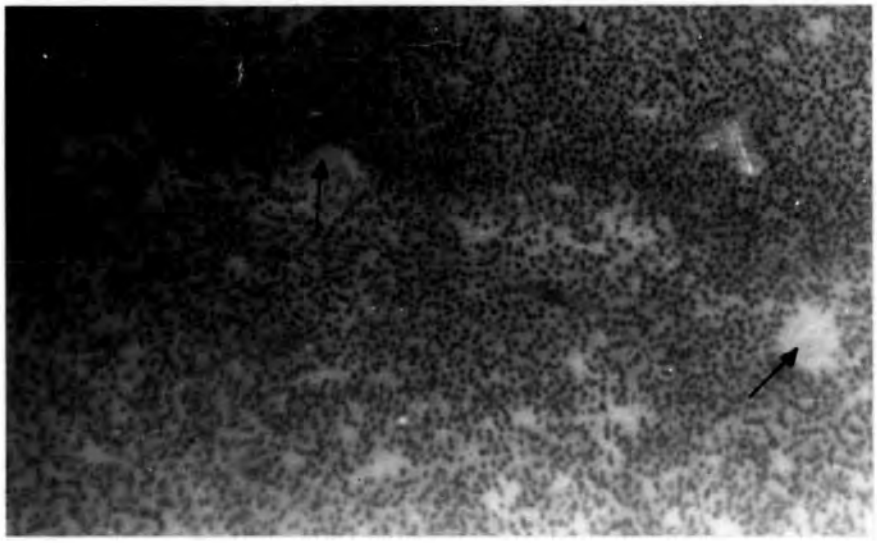
PLATE 25

A. Light micrograph of epidermis from dorsal area of region 3 (Fig. 2A). Surface stained with alcian blue to show mucous cells open at the surface (x 1,000).

C. Light micrograph of epidermis from lateral area of region 3 (Fig. 2A). Surface stained with alcian blue to show mucous cells open at the surface. Clear areas (arrowed) are openings of the lateral line canal system (x 1,000).

B. Light micrograph of epidermis from ventral area of region 3 (Fig. 2A). Surface stained with alcian blue to show mucous cells open at the surface (x 1,000).





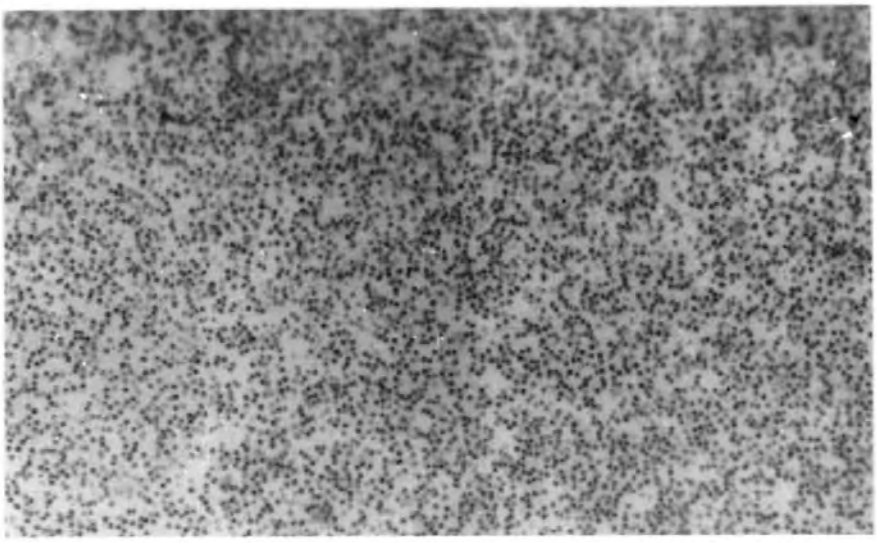


PLATE 26

A. Light micrograph of wax section from dorsal region of epidermis from which mucus has been removed by strong treatment with a compressed air jet. Serious disruption of the surface layers has taken place. Alcian blue - bromophenol blue staining (x 700).

C Club cell

D Dermis

B. Light micrograph of wax section of dorsal region of eel after 24 h treatment with atropine solution. Mucous cells have migrated to the surface but have not released their contents. Alcian blue staining (x 700).

D Dermis

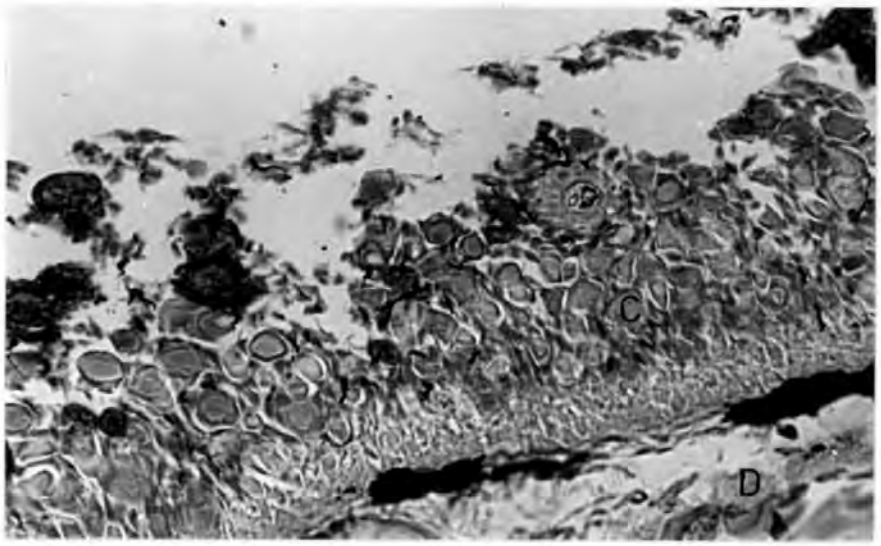
M Mucous cell

MM Melanin containing macrophage

C. Light micrograph of wax section from the dorsal region of an eel subjected to electrical stimulation. Club cells have an extended basal 'foot'. Note the mucous cells appear to open at the surface but their contents have not been secreted. Mercury bromophenol blue staining (x 700).

C Club cell

M Mucous cell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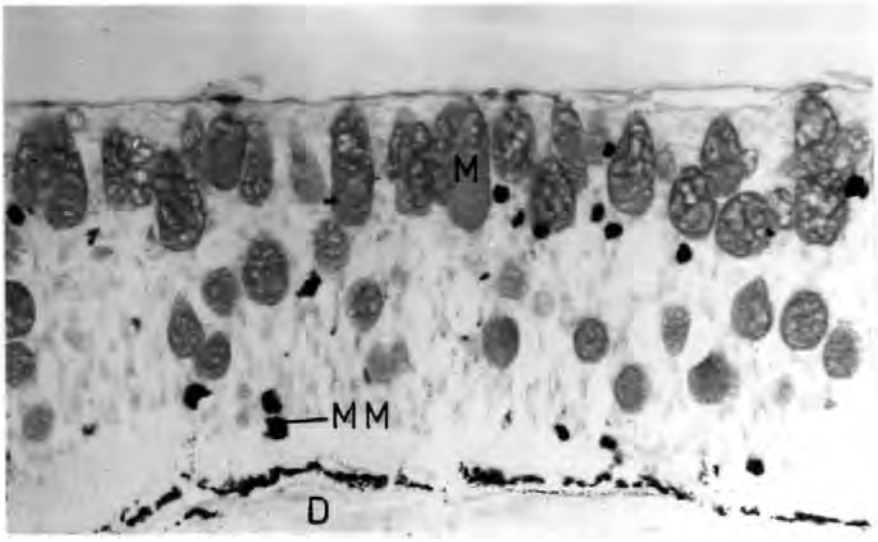
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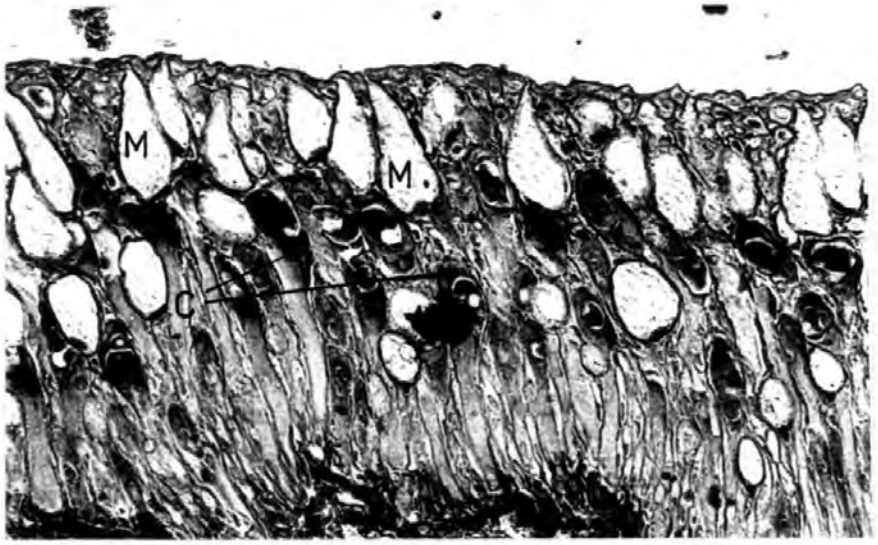
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M
C

PLATE 27

A. Light micrograph of wax section from the dorsal epidermis showing effect of chloroform vapour in stimulating release of mucus, causing sloughing of surface cells. Alcian blue staining (x 700).

D Dermis

M Mucous cell

ML Mucous layer

B. Light micrograph of wax section of the dorsal epidermis from fish exposed to pH 5.0 for 30 mins. Some stimulation of the mucous cells is apparent. Alcian blue staining (x 700).

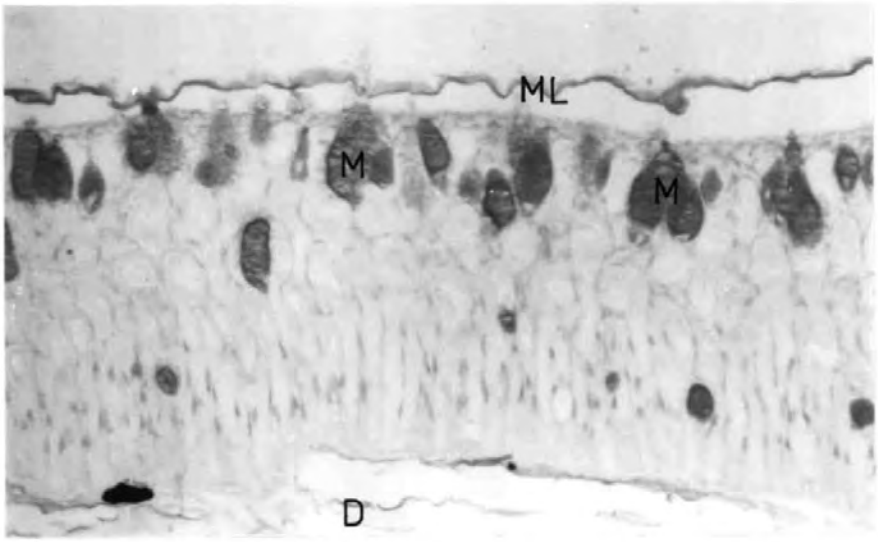
D Dermis

C. Light micrograph of wax section of the dorsal epidermis from fish exposed to pH 9.0 for 30 mins. Serious disruption of the tissue occurs with 'lift off' of surface layer. Alcian blue staining (x 700).

D Dermis

E Epidermis

Mel Melanocyte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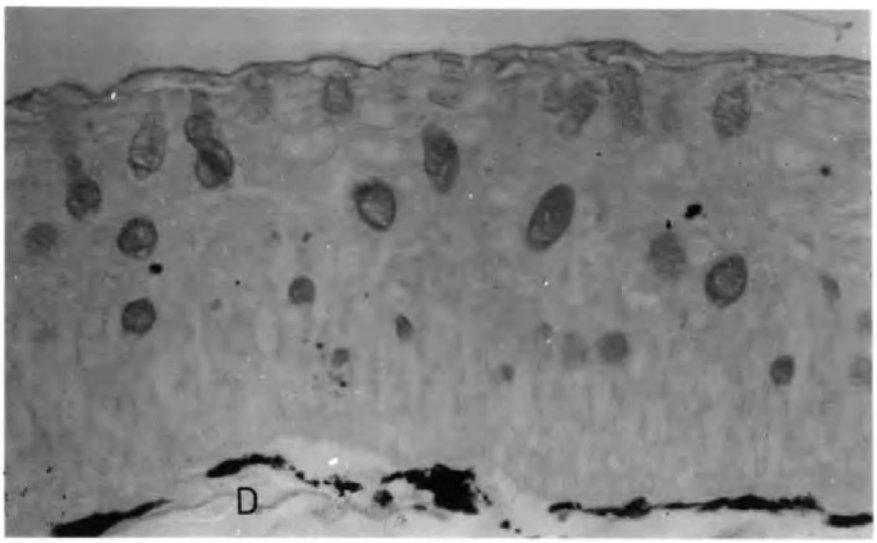
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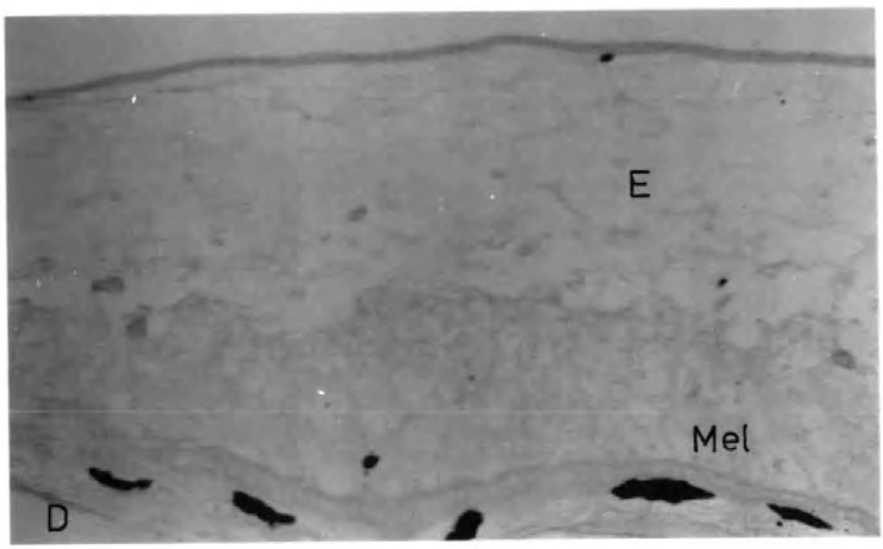

E
Mel
D

Distribution of Mucous and Club Cells

The distribution of mucous and club cells within the epidermis as seen by the surface-staining method and confirmed by analysis of sectioned material are presented in Tables 4 and 5.

Surface Staining of the Epidermis. Surface-stained epidermis, seen in section (Pl. 24A), shows positive staining of only the surface mucus and open mucous cells. Counts of stained surface mucous cells were obtained for each region (Fig. 2A) after the skin preparations had been photographed. Examples (Pls 25A,B,C) show surface-stained epidermis from the dorsal, lateral and ventral areas respectively, taken from a region just anterior to the cloacal opening (Fig. 2A, region 3). Small non-staining areas (Pl. 25B) are the openings of the lateral line canal system.

Sectioned Epidermis. a) Mucous cells. Counts of the numbers of mucous cells, within a 1 mm length of epidermis performed on 7 µm sections, (Table 5) show increased numbers of mucous cells in both head and pectoral positions (Fig. 2B, regions 1,2) when compared to the posterior regions of the body (Fig. 2B, region 5) ($P < 0.001$). Furthermore, both the dorsal and the ventral areas in all regions contain more mucous cells than the lateral areas of the same regions ($P < 0.001$).

b) Club cells. In both dorsal and lateral areas of the head region (Fig. 2B, region 1) club cells are relatively sparse but increase in numbers towards the tail. Between the mid-point of the body and the tail (Fig. 2B, regions 3,4 and5) significantly more club cells are found in the lateral areas of the fish than in the dorsal area and both contain more than the comparable ventral area ($P < 0.02$).

TABLE 4. DISTRIBUTION OF MUCOUS CELLS

a) Surface Staining Method

Area of Sample	Region of body (Fig. 2A)				
	1 Head	2 Behind Pectoral Fins	3 Anterior of Cloaca	4 Mid Cloaca - Tail	5 Tail
Dorsal	491 ± 87	406 ± 63	402 ± 49	383 ± 63	336 ± 32
Ventral	469 ± 16	401 ± 71	335 ± 36	299 ± 63	

All figures represent open surface mucous cells per square mm. Values are the mean of 6 fish followei by the standard deviation.

TABLE 5. DISTRIBUTION OF MUCOUS AND CLUB CELLS

Sectioning Method

Area of Sample	Region of Body (Fig. 2B)					
	1	2	3	4	5	6
	Head	Behind Pectoral Fins	Mid Region 2 - Region 4	Mid Head - Tail	Mid Region 4 - Tail	Tail
Dorsal						
Mucous	37.1 ± 3.5	42.7 ± 6.2	27.2 ± 5.5	34.2 ± 4.1	18.4 ± 3.5	
Club	13.8 ± 3.5	31.5 ± 5.5	23.5 ± 4.8	33 6 ± 4.1	32.0 ± 3.5	
Lateral						
Mucous	23.3 ± 3.5	18.1 ± 3.5	21.0 ± 3.5	23.7 ± 3.5	15.9 ± 4.1	26.0 ± 4.8
Club	16.3 ± 4.1	26.4 ± 4.8	36.9 ± 6.9	40.2 ± 4.1	36.6 ± 5.5	9.8 ± 3.5
Ventral						
Mucous	37.8 ± 7.5	34.7 ± 7.5	34.6 ± 8.2	30.0 ± 4.1	21.8 ± 4.4	
Club	32.5 ± 4.1	32.5 ± 4.1	27.8 ± 4.8	22.2 ± 4.8	24.2 ± 8.2	

Figures represent counts of mucous cells/club cells per 1 cm of epidermis from 7 µm sections followed by the standard deviation.

Epidermal Thickness. The thickness of the epidermis does not vary greatly over the body surface (Table 6). The epidermis of the lateral area just behind the pectoral fins (Fig. 2B, region 2) is however, significantly thinner ($P < 0.002$) than that of the rest of the body.

Observations of Mucous Smears

Visual examination of a suspension of the mucous secretion removed by the compressed air method reveals the presence of numerous threads. When examined, as a freshly prepared smear, at the light microscope level, the threads appear as one of two forms. The first (Pl. 24B) are seen as bundles of filaments (approx. 2 μm in diameter) that appear very similar to the threads found in ruptured club cells within the epidermis (Pls 11A,B) and also observed at the electron microscope level (Pls 12A,B,C). The second type (Pl. 24C) of thread appears with a cellular body at one end. The thread becomes progressively thinner away from the cell body and sometimes appears to be coiled. These threads appear to be whole club cells, possibly released from the epidermis by the action of the compressed air jet used in the collection of the mucous sample. Further evidence to support this view is the presence of epidermal cells within the same smear preparation (Pl. 24C). In addition, the cell bodies of these threads have staining reactions similar to those of club cells.

Experimental Procedures

Effect of Atropine. The effect of atropine sulphate, a compound which blocks the sympathetic nervous system, also effectively prevents the release of the contents of mature mucous cells at the surface of the epidermis (pl. 26B). The mucous cells are, however, still active and increase in size, moving up through the epidermis to accumulate

TABLE 6. VARIATION OF EPIDERMAL THICKNESS

Area of Sample	Region of Body (Fig. 2B)					
	1 Head	2 Behind Pectoral Fins	3 Mid Region 2 – Region 4	4 Mid Head – Tail	5 Mid Region 4 – Tail	6 Tail
Dorsal	0.68 ± .06	1.00 ± .13	0.64 ± .11	0.59 ± .05	0.63 ± .05	
Lateral	0.54 ± .07	0.53 ± .05	0.71 ± .09	0.58 ± .05	0.60 ± .07	0.51 ± .08
Ventral	0.87 ± .06	0.86 ± .12	0.76 ± .13	0.56 ± .05	0.74 ± .08	

All values are the mean of 20 measurements and show the standard deviations (in mm).

just beneath the epidermal surface. The enlargement of the cells thus indicates continued synthesis of the mucus although the release of the contents of the surface mucous cells is inhibited.

Effect of Electrical Stimulation. Electrical stimulation of the epidermis produces an elongation of the basal 'foot' of club cells of the lower and mid-epidermis (Pl. 26C). The mucous cells just beneath the epidermal surface also appear elongated to reach the surface and although some are open at the surface (Pl. 26C) they have not released their contents. The surface layers of epidermal cells appear to be less flattened than those of the control sections giving the epidermis an irregular surface.

Effect of Chloroform. When chloroform vapour is used to stimulate the epidermis (Pl. 27A), the superficial mucous cells are immediately stimulated and release their contents. This secretion from the mucous cells appears to 'blow off' the non-cellular mucous layer from the surface of the epidermis. This layer of mucus may appear to be thicker than normal (Pl. 27A) due to the precipitating action of chlorform.

Effect of Acidic Conditions. The epidermis of eels kept in acidic (pH 5.0) conditions (Pl. 27B) shows the release of mucous cell secretion at the surface of the epidermis. There is also a noticeable reduction of Alcian blue staining of the superficial mucous cells.

Effect of Alkaline Conditions. The epidermis of eels held in alkaline (pH 9.0) conditions show a serious disruption in the structure (Pl. 27C). The mucous cells are not recognisable and both mucous and club cells appear to have ruptured beneath the epidermal surface. The surface layer stains strongly with Alcian blue and appears to have lifted from the remains of the epidermis.

HISTOCHEMISTRY

The histochemical staining reactions of the epidermis are presented in Tables 7 - 10.

Mucous Cells. The mucous cells of the epidermis show many positive staining reactions for carbohydrates (Table 8). A positive reaction to PAS, combined PAS - Alcian blue at pH 1.0 and pH 2.5 and also aldehyde fuchsin - AB indicate the presence of both sulphated and non-sulphated mucopolysaccharides (the histological use of the term mucopolysaccharide is explained in Appendix I). Positive reactions are also found with low iron diamine and high iron diamine techniques and indicate the presence of both a sulphate containing and a carboxylated mucosubstance. The intensity of Alcian blue staining is reduced after incubation with neuraminidase, when compared to a control section (neuraminidase cleaves terminal sialic groups from a polysaccharide chain) and thus part of the positive reaction to Alcian blue is due to neuraminic (sialic) acid groups. The presence of sulphate is confirmed by the tetrazonium method (Table 9).

Incubation with testicular hyaluronidase preceeding Alcian blue staining does not reduce the staining intensity when compared to the control, indicating the absence of uronic acids. Similarly, incubation with amylase followed by staining with PAS technique indicates that the positive response of the mucous cells is not due to the presence of glycogen. Mild methylation (37 °C, 4 h) is found to slightly reduce the staining uptake of Alcian blue, whereas active methylation (60 °C, 4 h) effectively inhibites all Alcian blue staining, which is in part restored by saponification. This is a further indication of the presence of sulphate. The β - metachromasia exhibited with toluidine blue (Table 8) indicates the presence of free electronegative surface charges.

Mucous cells stain only weakly for proteins by the mercuric - bromophenol blue method with only the fine network of the membranes of the mucin 'packets' showing a positive staining reaction. Alkaline fast green (for basic and nuclear proteins) shows positive staining for only the cell nuclei and the failure to stain with Millon's reagent indicates the absence of tyrosine containing proteins.

Positive uptake of Sudan black B and Nile blue sulphate (both stain for lipids) by the mucous cells does occur (Table 10) but no further confirmation of this response can be ascertained by any of the other lipid staining procedures.

Club Cells. In contrast to the mucous cells, the club cells do not show strong positive staining reactions for carbohydrates, although they do show a very weak reaction to both PAS and Aldehyde fuchsin indicating the possible presence of some carbohydrate material. Strong staining reactions do occur, however, with methods for the detection of proteins (Table 9). Thus positive reactions for mercuric bromophenol blue, Millon reaction and alkaline fast green indicate the presence of tyrosine and alkaline amino acids (arginine and lysine). Negative reactions are also seen for both amyloid and elastic fibres and all classes of lipid, although a weak reaction to Nile blue sulphate was observed. This stain, however, is not specific for lipid material (Pearse, 1968). Oxidation with performic acid followed by Schiff's reagent shows a strong positive reaction which is inhibited by bromination. Some phospholipid, keratin and proteins containing methionine, cysteine and tryptophan are all stained by this method. Methylene blue extinction below pH 4.93 indicates the presence of carboxyl groups (probably of acidic amino acids e.g. aspartic and glutamic acid) within the club cells.

Epidermal Cells. The epidermal cells show a number of different staining reactions dependent on their position in the epidermis. In the surface layers, they frequently, although not always show positive reactions to carbohydrate stains, similar to those seen in the mucous cells. There is also a strong positive reaction the the PFAS technique indicative of keratin or proteins containing methionine, cystine and tryptophan or phospholipid. Positive reactions to Sudan black B and Nile blue sulphate are also apparent and in common with mucous cells other methods for lipids give negative reactions. There was, however, a strong positive reaction for cholesterol and its esters in the surface epidermal cells as shown by Schultz's reaction, although this could not be confirmed by the Okamoto method. Positive reactions for nuclear material and proteins are observed in all the epidermal cells, although the epidermal cells do not contain tyrosine as shown by a negative reaction to Millon's reagent.

TABLE 7. HISTOCHEMICAL STAINING REACTIONS OF EEL EPIDERMIS

1) General Procedures

Staining Procedure	Epidermal Cells	Club Cells	Mucous Cells
Mallory's Triple	+++ Dk B	+++ Dk B	+++ Lt B
Masson's Triple	++++ Dk B	+++ P	++++ Lt B
Haematoxylin and Eosin	++++ Pk, nuclei P	++++ Pk	++ Pk
Methylene Blue Extinction	N.D.	No staining below pH 4.93	Staining to pH 2.12

Key:-

N.D.	Not determined
-	No reaction
+	Very weak staining
++	Weak staining
+++	Moderate staining
++++	Strong staining
Dk	Dark
Lt	Light
B	Blue
P	Purple
Pk	Pink

TABLE 8. HISTOCHEMICAL STAINING REACTIONS OF EEL EPIDERMIS

2) Carbohydrate Procedures

Staining Procedure	Epidermal Cells		Club Cells	Mucous Cells	Inference
	Throughout Epidermis	At Surface			
Periodic-acid Schiff (PAS)	++ M	++++ M	+ Pk	++++ M	Vicinal hydroxyl groups present
Amylase - PAS	++ M	++++ M	+ Pk	++++ M	PAS activity not due to glycogen
Alcian Blue (AB) pH 2.5	++ B	+++ B	-	++++ B	Acidic mucosubstances present (except in club cells)
AB pH 1.0	-	-	-	++ B	Sulphated mucosubstances present in mucous cells only
PAS - AB pH 2.5	++ M	+++ M	+ Pk	++++ B - P	Strongly acidic sulphated mucosubstances absent
PAS - AB pH 1.0	++ M	+++ M	+ Pk	++++ M - P	
Neuraminidase - AB pH 2.5	N.D.	N.D.	-	++ B	Neuraminic acid present
Testicular hyaluronidase - AB pH 2.5	++ B	+++ B	-	++++ B	Hyaluronic acid absent

TABLE 8. Continued

Staining Procedure	Epidermal Cells: Throughout Epidermis	Epidermal Cells: At Surface	Club Cells	Mucous Cells	Inference
Mild Methylation (37 °C, 4 h) - AB pH 2.5	++ B	+++ B	-	++++ B	Both carboxylated and sulphated mucosubstances present
Active Methylation (60 °C, 4 h) - AB pH 2.5	-	-	-	-	
+ Saponification	N.D.	N.D.	-	++ B	
Aldehyde Fuchsin (AF)	++ P	+++ P	-	++++ P	Sulphated and carboxylated groups present
AF - AB pH 2.5	++ P	+++ P	+ P	++++ B	
AF - AB pH 1.0	++ P	+++ P	+ P	+++ B	
Low Iron Diamine (LID)	+ Bl	+++ Bl	+ Bl	+++ Bl	Sulphated mucosubstances present
High Iron Diamine (HID)	+ Bl	+++ Bl	+ Bl	+++ Bl	
Periodic Acid Para-diamine (PAD)	+ Br	++ Br	+ Br	+++ Br	Periodic acid reactive mucosubstances present

TABLE 8. Continued

Staining Procedure	Epidermal Cells		Club Cells	Mucous Cells	Inference
	Throughout Epidermis	At Surface			
Toluidine Blue	+++ B	+++ B	-	+++ P	Free electronegative charge
for β-metachromasia	-	-	-	R - Pk	

Key:-

N.D.	Not determined
-	No reaction
+	Very weak staining
++	Weak staining
+++	Moderate staining
++++	Strong staining
B	Blue
Bl	Black
Br	Brown
M	Magenta
P	Purple
Pk	Pink
R	Red

TABLE 9. HISTOCHEMICAL STAINING REACTIONS OF EEL EPIDERMIS

3) Protein Procedures

Staining Procedure	Epidermal Cells: Throughout Epidermis	Epidermal Cells: At Surface	Club Cells	Mucous Cells	Inference
Methyl Green - Pyronin Y	nuclei +++ G	++ G	nuclei ++ G	nuclei +++ G	Nuclear DNA stained
Alkaline Fast Green	nuclei ++ G	nuclei ++ G	++ G	nuclei ++ G	Club cell contents contain arginine and lysine
Mercuric Bromophenol Blue	++ B	++ B	++++ B	+ B mmp	Protein present in epidermal and club cells
Millon's Reagent	-	-	+++ R	-	Club cells contain tyrosine
Methyl Violet	-	-	-	-	Amyloid absent
Tetrazonium Method	+ RV	+ RV	+ RV	+++ RV	Sulphate present in mucous cells
Orcinol Method	-	-	-	-	No elastic fibres

Key:-

-	No reaction	mmp	membranes of mucin 'packets'
+	Very weak staining	B	Blue
++	Weak staining	G	Green
+++	Moderate staining	R	Red
++++	Strong staining	RV	Red-violet

TABLE 10. HISTOCHEMICAL STAINING REACTIONS OF EEL EPIDERMIS

4) Lipid Procedures

Staining Procedure	Epidermal Cells: Throughout Epidermis	Epidermal Cells: At Surface	Club Cells	Mucous Cells	Inference
Sudan Black B	-	+++ Bk	-	++++ Bk	Lipid present
Sudan Blue	-	-	-	-	Neutral lipid absent
Sudan III	-	-	-	-	Neutral lipid absent
Nile Blue Sulphate@	++ Pk	+++ B	++ P - B	++++ B	Acidic lipids present
Oil Red O	-	-	-	-	Neutral lipid absent
For Phospholipids					
Menschik Reaction	-	-	-	-	Phospholipid absent
Pseudoplasmal Reaction	-	-	-	-	Phospholipid absent
Luxol Blue (+ unmasking)	-	-	-	-	Phospholipid absent
Performic-acid Schiff	-	+++ M	+++ M	+ M	Aldehyde or tyrosine present
Bromine control	-	+ M	-	+ M	Blocks tyrosine and ethylene bonds

TABLE 10. Continued

Staining Procedure	Epiiermal Cells: Throughout Epidermis	Epiiermal Cells: At Surface	Club Cells	Mucous Cells	Inference
Sugar-containing Lipid					
Modified Bruckner Reaction	-	-	-	-	Absent
Modified Molische Reaction	-	-	-	-	Absent
Cholesterol and Esters					
Schultz Method	-	+++ G	-	-	Present in surface epidermal cells
Okamoto Method	-	-	-	-	Absent

@ Nile blue sulphate stained club cell contents pink and central vacuole blue.

Key:-

-	No reaction
+	Very weak staining
++	Weak staining
+++	Moderate staining
++++	Strong staining
B	Blue
Bk	Black
G	Green
M	Magenta
P	Purple
Pk	Pink

SPECTROSCOPIC ANALYSIS OF MUCUS

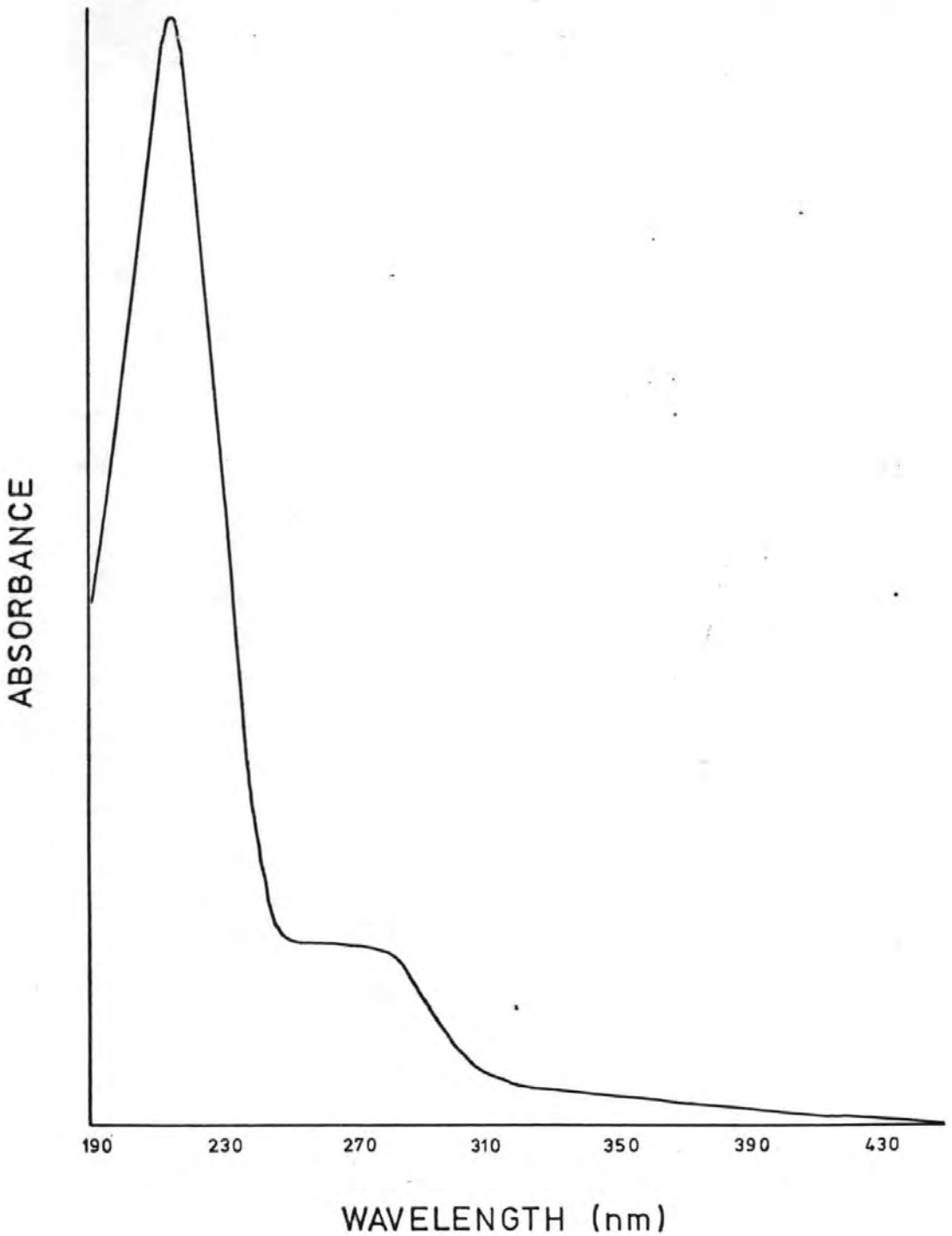
Ultra-Violet Spectrum

The ultra-violet spectrum for the soluble component of eel mucus (Fig. 3) shows maximum absorption to occur at 212 nm and this is probably due to the carbohydrate portion of the molecule (carbohydrates absorb strongly at this wavelength). The plateau region found between 245 and 280 nm is probably due to protein and although the protein content of mucus is high, the low extinction coefficient is likely to be due to the low levels of the amino acids, typtophan, tyrosine and phenylalanine. This is characteristic of mucous glycoproteins in general (Masson, 1973).

Infra-Red Spectrum

The infra-red spectrum of eel mucus (after centrifugation at 24,000 g and freeze drying) is presented in Fig. 4. Absorption of infra-red wavelengths corresponds to the functional groups present in the compound, each group possessing one or more characteristic wavelength(s) of maximum absorbance (Scheinmann, 1970). Thus bands seen (Fig. 4) at 1650 and 1540 cm^{-1} correspond to primary and secondary amide groups ($RCONH_2$, RCONH-) although that at 1650 cm^{-1} may be in part due to water. The band at 1320 cm^{-1} may also be attributed to amino acids. Characteristic banding of the carboxylate ion ($-COO^-$) occurs at 1440 cm^{-1} whilst that at 1380 cm^{-1} may be indicative of methyl (CH_3) groups. Banding at 1240 cm^{-1} and between 1130 - 1080 cm^{-1} are attributable to sulphate ($SO_4^=$) groups whilst that at 1050 cm^{-1} may be due to alcohol $R-CH_2OH$ groupings.

Fig.3 ULTRA VIOLET SPECTRUM FOR EEL MUCUS



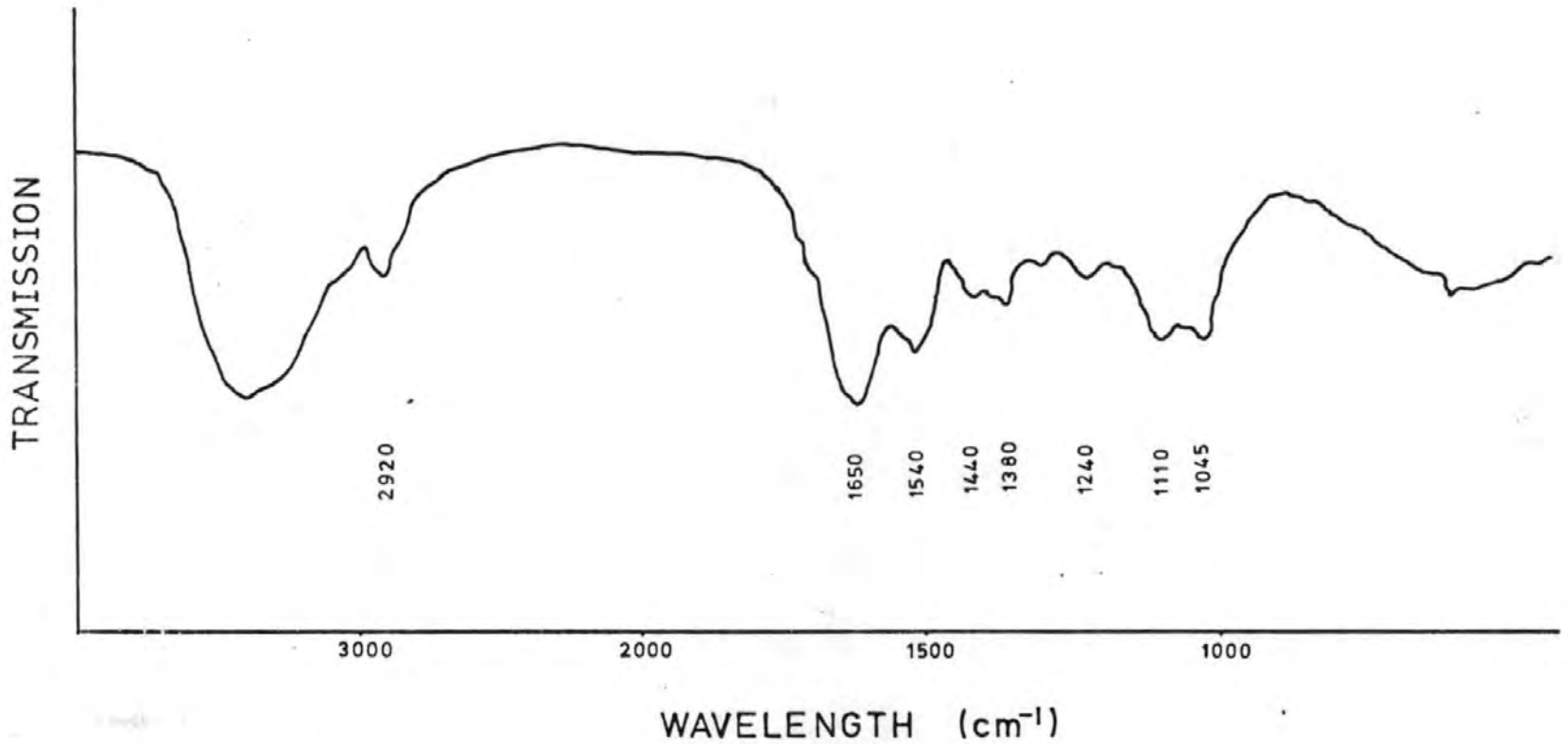


Fig. 4 INFRA-RED SPECTRUM FOR EEL MUCUS

GROSS BIOCHEMICAL ANALYSES OF MUCUS

The biochemical analyses of whole mucus (centrifuged at 24,000 g and freeze dried) as determined by spectrophotometric methods (Table 11) are expressed as a percentage of the total freeze dried mucus and are corrected for moisture and ash content. The results of the assays, except those for fucose and sialic acid are determined by comparison with a standard curve from a known compound. This compound may not necessarily be present (or the only compound of that class present) in the mucus. For example, all non-nitrogenous sugar was measured against a mannose standard curve. This may or may not have a different reactivity (for the same quantity) as the non-nitrogenous sugars present in the mucus.

The results show a discrepancy in the quantity of protein present and this is a reflection of the different biochemical reactions involved in the determination of protein. It is noted that the levels of non-nitrogenous sugar (hexoses), hexosamine and sialic acid appear in an approximate ratio of 1:1:1 and the levels of uronic acid and fucose present are lower than those of the other carbohydrates.

TABLE 11. BIOCHEMICAL ANALYSES OF EEL MUCUS

Assay	Number of Determinations	% Total
Protein (Biuret)	5	30.90 ± 1.70
Protein (Lowry)	5	22.60 ± 1.00
Carbohydrate	5	6.80 ± 0.90
Non-nitrogenous sugar	5	4.10 ± 0.20
Hexosamine	5	4.26 ± 0.04
Uronic Acid	3	1.03 ± 0.16
Fucose	3	0.32 ± 0.08
Sialic Acid	5	4.27 ± 0.24

MEASUREMENT OF LYSOZYME IN MUCUS

Lysozyme was measured as a percentage reduction in optical density (550 nm wavelength) of a suspension of killed M. lysodiekticus cells between 30 - 60 seconds after the introduction of the enzyme. The percentage reduction (between 30 - 60 sec) was then compared to a standard curve produced for egg white lysozyme. The results of the assay (Table 12) indicate the absence of lysozyme in both concentrated and freeze dried mucous samples. Lysozyme was found however, in two samples of the precipitate obtained after centrifugation (2,000 g, 30 min). This precipitate could possibly contain cells and cellular debris removed from the epidermis by the action of the compressed air method used to collect the mucous sample.

TABLE 12. ASSAY OF LYSOZYME OF MUCUS

Sample	Number of Estimations	Lysozyme ($\mu g\ cm^{-3}$)
Concentrated Mucus	3	0.0
Freeze Dried Mucus	2	0.0
Precipitate from Mucus	2	2.0 2.0

CHROMATOGRAPHIC AND ELECTROPHORETIC ANALYSES OF MUCUS

Gel Chromatography

Separation of whole eel mucus after centrifugation (24, 000 g) on both Sephadex G.200 and Sepharose 6B is unsuccessful. With Sephadex G.200, the mucus does not enter the gel layer. Mucus enters the gel matrix of Sepharose 6B but no separation of components takes place, the molecule appearing to be too large for the pore size selected.

The mucus does separate on Sepharose 4B (Fig. 5) to reveal two major peaks and a number of minor ones. Maximum values for sialic acid (Fig. 5C) (measured as n-acetyl neuraminic acid) are found on the shoulder of the first peak.

Ion-Exchange Chromatography

Separation of whole eel mucous (after removal of cellular debris) on D.E.52, and ion-exchange cellulose of the D.E.A.E. type indicates the presence of three fractions (Fig. 6A). The first is eluted by buffer (Tris-HCl, 0.01 M, pH 7.4), the second by addition of buffer containing 0.2 M sodium chloride and the third fraction is eluted from the column by buffer containing 0.5 M sodium chloride. Protein analysis of the separated fractions (Fig. 6B) indicates the presence of three protein peaks contained with the second fraction (that fraction eluted by buffer + 0.2 M NaCl).

Polyacrylamide Gel Electrophoresis

Eel mucus does not separate with electrophoresis on a 7% polyacrylamide gel matrix. This is further confirmation of the large molecular size as the mucous sample remains at the point of application and does not enter the gel. There is, however, separation of the mucus in 3% polyacrylamide gel after ultrasonification of the sample (Fig. 7). Weak staining with both Alcian blue for carbohydrate and Coomassie blue

for protein indicates the high dilution of mucus in its natural state. Five bands stain with Alcian blue (Fig. 7), one of which is also stained with Coomassie blue. It is interesting that when Coomassie blue is applied to sections of the epidermis there is no staining of the mucous cell contents except the fine membrane surrounding the 'packets' of mucin.

Fig. 5 CHROMATOGRAPHY ON SEPHAROSE 4B

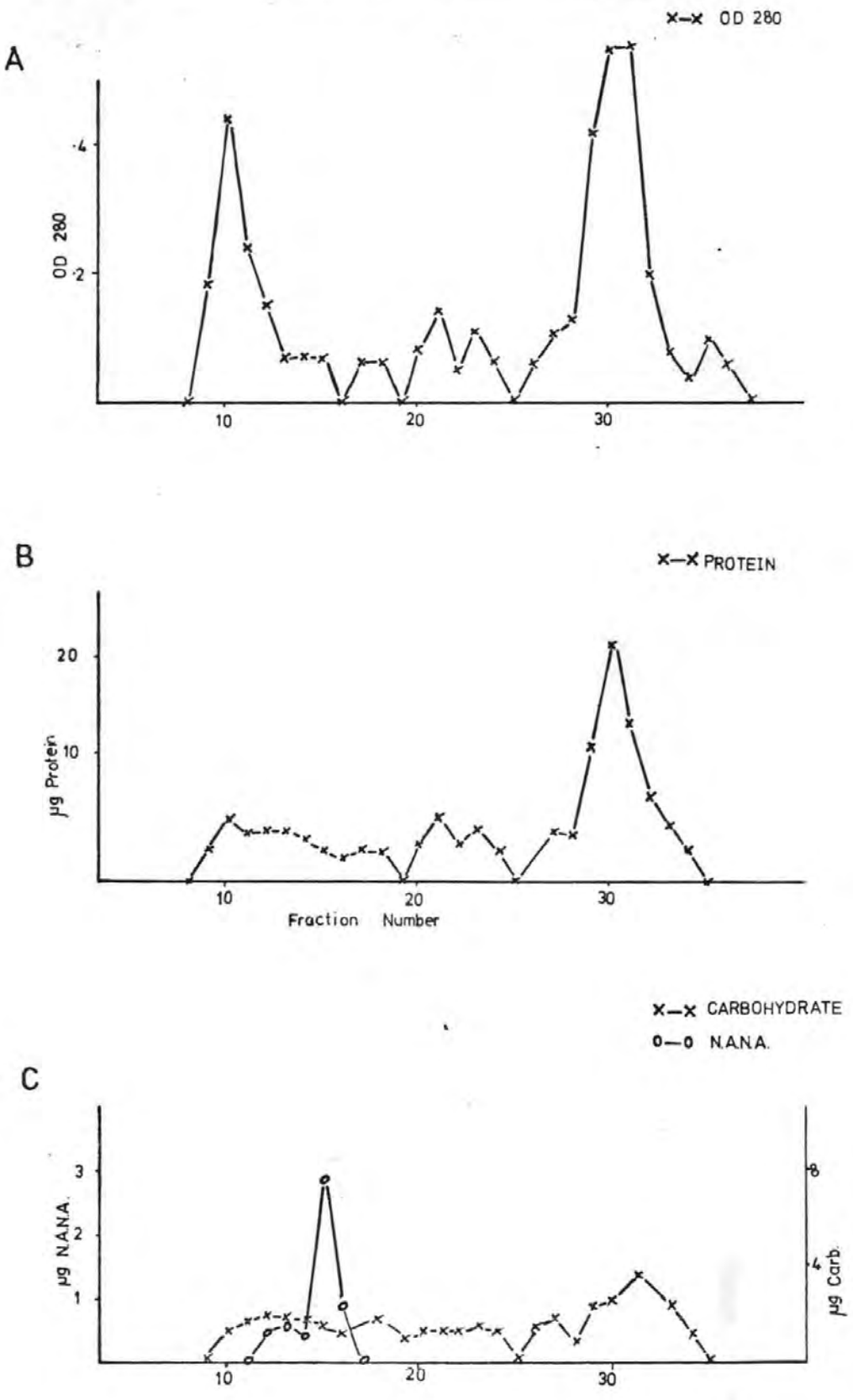


Fig 6 CHROMATOGRAPHY ON D.E. 52

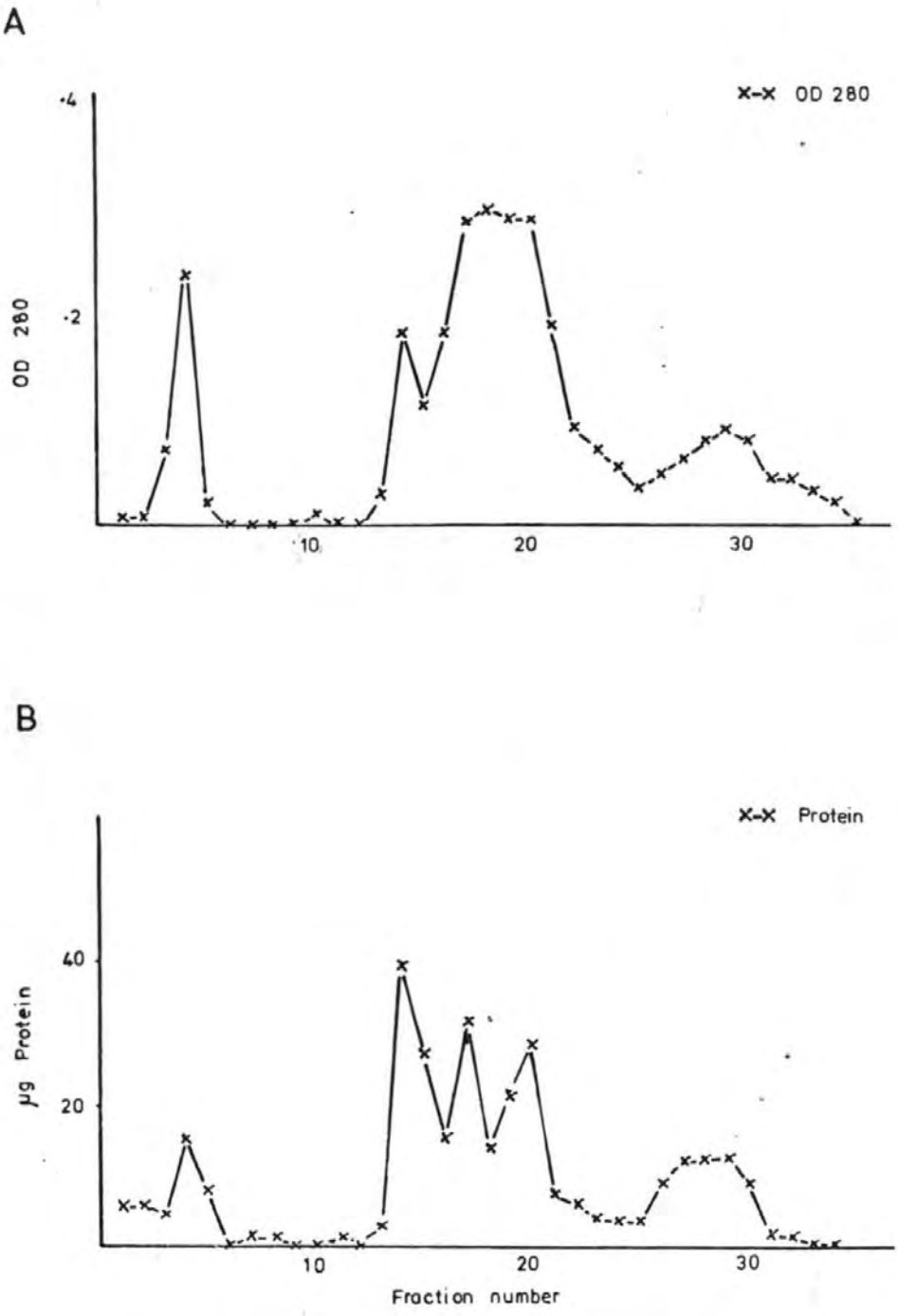
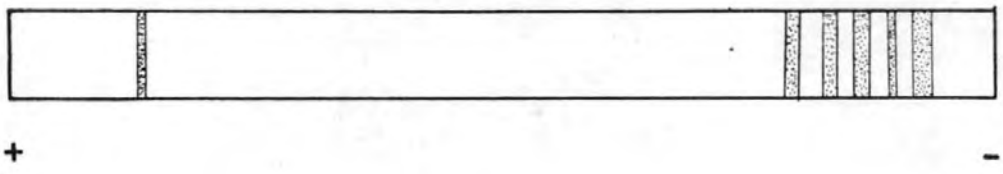


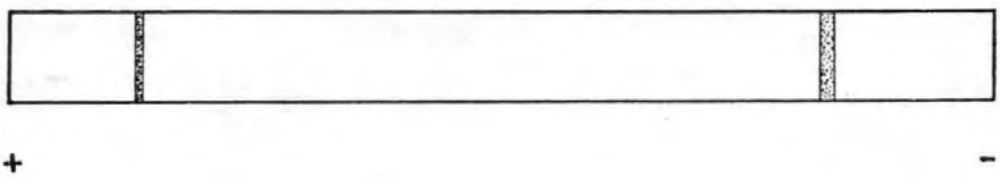
Fig. 7

POLYACRYLAMIDE GEL ELECTROPHORESIS

Alcian blue staining



Coomassie blue staining



RHEOLOGY OF MUCUS

Eel mucus may be drawn into strands which show elasticity and when stirred in a beaker the mucus or a solution of mucus will show elastic recoil when the stirring is stopped. The mucus also exhibits shear thinning properties i.e. the viscosity is reduced with an increase in the shear rate, when measured on a Weissenberg rheogonimeter (Fig. 8A) (further information on shear thinning behaviour appears in Appendix II). The response of a mucus sample centrifuged at 2,000 g, to remove cellular material only, compared to that of a sample centrifuged at 24,000 g, to remove both cellular and thread material (Fig. 8A), indicates that the presence of thread material has no significant effect on the shear thinning property of mucus. For comparative purposes the relationship between viscosity and rate of shear for a long chain molecule (7-hydroxylethylcellulose) is shown in Figure 8B.

The intrinsic viscosity (viscosity when shear rate = 0) is obtained by plotting log. viscosity against log. shear rate and extrapolation of the regression to x = 0 (an example is seen in Fig. 9A). The relationship between intrinsic viscosity and mucus concentration as determined by protein analysis, is shown in Figure 9B. At very low mucous concentrations the viscosity becomes similar to the viscosity of water (viscosity of water = 0.01 poise).

The effects of materials added to the mucous solutions are summarised in Table 13. Some variation between the viscosities of control mucous solutions is indicative of the differences in the concentration of the various mucous samples required for these experiments. Mucus is significantly 'thickened' (Student's t-test, $P < 0.05$) by the addition of solutions of divalent ions Mg^{2+} and Ca^{2+} and also tris-buffer (pH 7.2). The mucus is significantly 'thinned' by the addition of

Cd^{2+} cations ($P < 0.01$) and also DDT ($P < 0.001$). The shear thinning behaviour of mucus is destroyed and is accompanied by a decrease in viscosity with the addition of β-mercaptoethanol ($P < 0.001$), neuraminidase ($P < 0.001$) and pronase ($P < 0.002$). This is probably due to a breakdown of the molecular structure by these compounds.

INDEX OF RHEOLOGICAL RESULTS

Viscosity (poise) plotted against Rate of Shear (sec^{-1}).

Fig. 8A. Mucus centrifuged at 24,000 g and 2,000 g.
B. 7-Hydroxyethylcellulose.

Fig. 9A. Intrinsic viscosity, logarithmic plot.
B. Intrinsic viscosity plotted against mucous concentration.

Viscosity (poise) plotted against Rate of Shear (sec^{-1}) for mucus plus 'additives'.

Fig. 10A. Mucus + NaCl
B. " + KCl
C. " + $CaCl_2$

Fig. 11A. Mucus + $MgCl_2$
B. " + $PbCl_2$
C. " + $CdCl_2$

Fig. 12A. Mucus + Tris-HCl buffer
B. " + DDT
C. " + β-mercaptoethanol

Fig. 13A. Mucus + Neuraminidase
B. " + Pronase
C. " + Hyaluronidase

Fig. 14A. Mucus + Chymotrypsin
B. " + Glucuronidase
C. " + Galactosidase.

Fig. 8

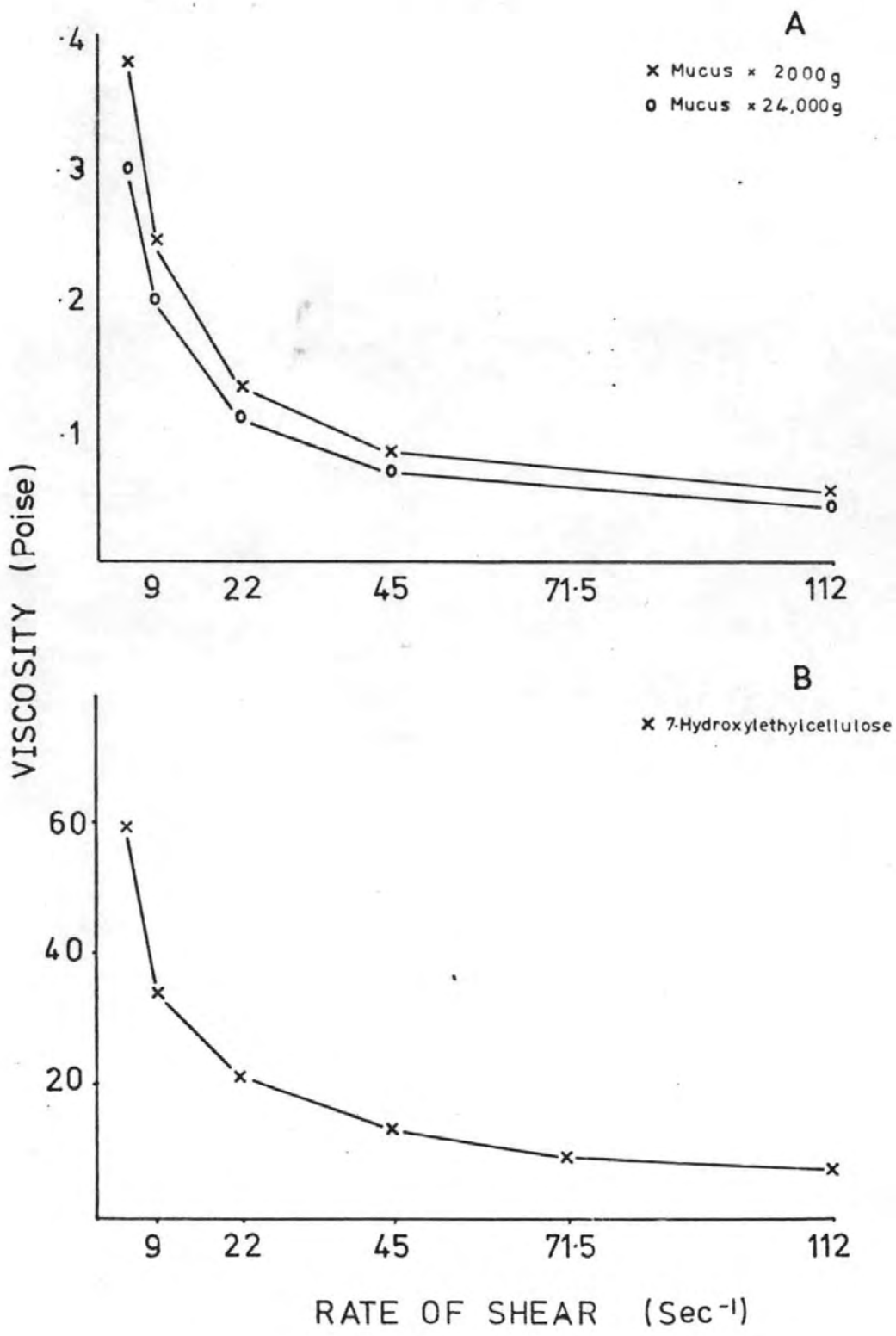

A
× Mucus × 2000g
o Mucus × 24,000g
·4
·3
·2
·1
VISCOSITY (Poise)
9
22
45
71·5
112
B
× 7-Hydroxylethylcellulose
60
40
20
9
22
45
71·5
112
RATE OF SHEAR (Sec⁻¹)

Fig. 9

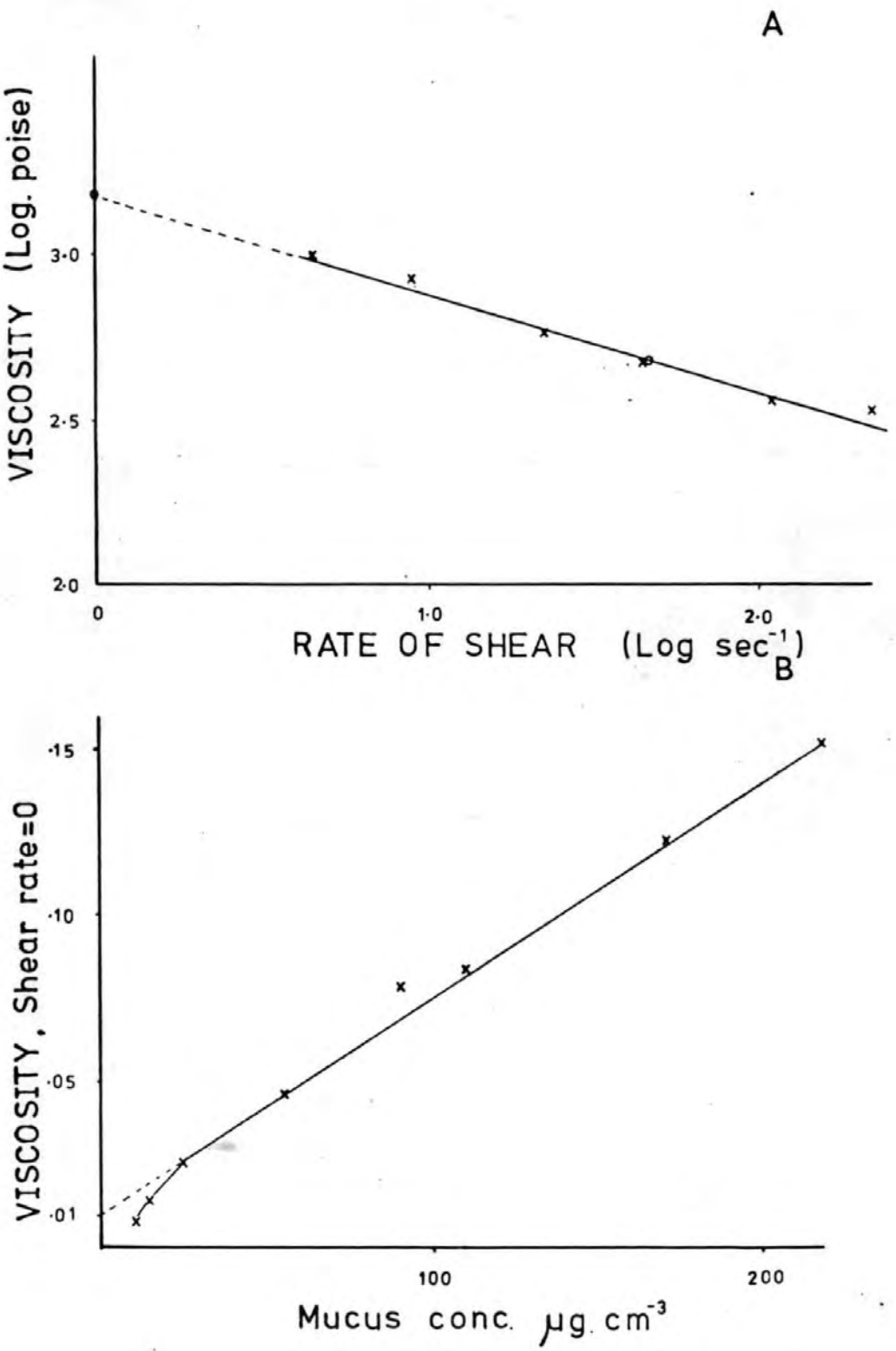

A
VISCOSITY (Log. poise)
3·0
2·5
2·0
0
1·0
2·0
RATE OF SHEAR (Log sec^{-1})
B
VISCOSITY, Shear rate=0
·15
·10
·05
·01
100
200
Mucus conc. µg. cm^{-3}

Fig. 10

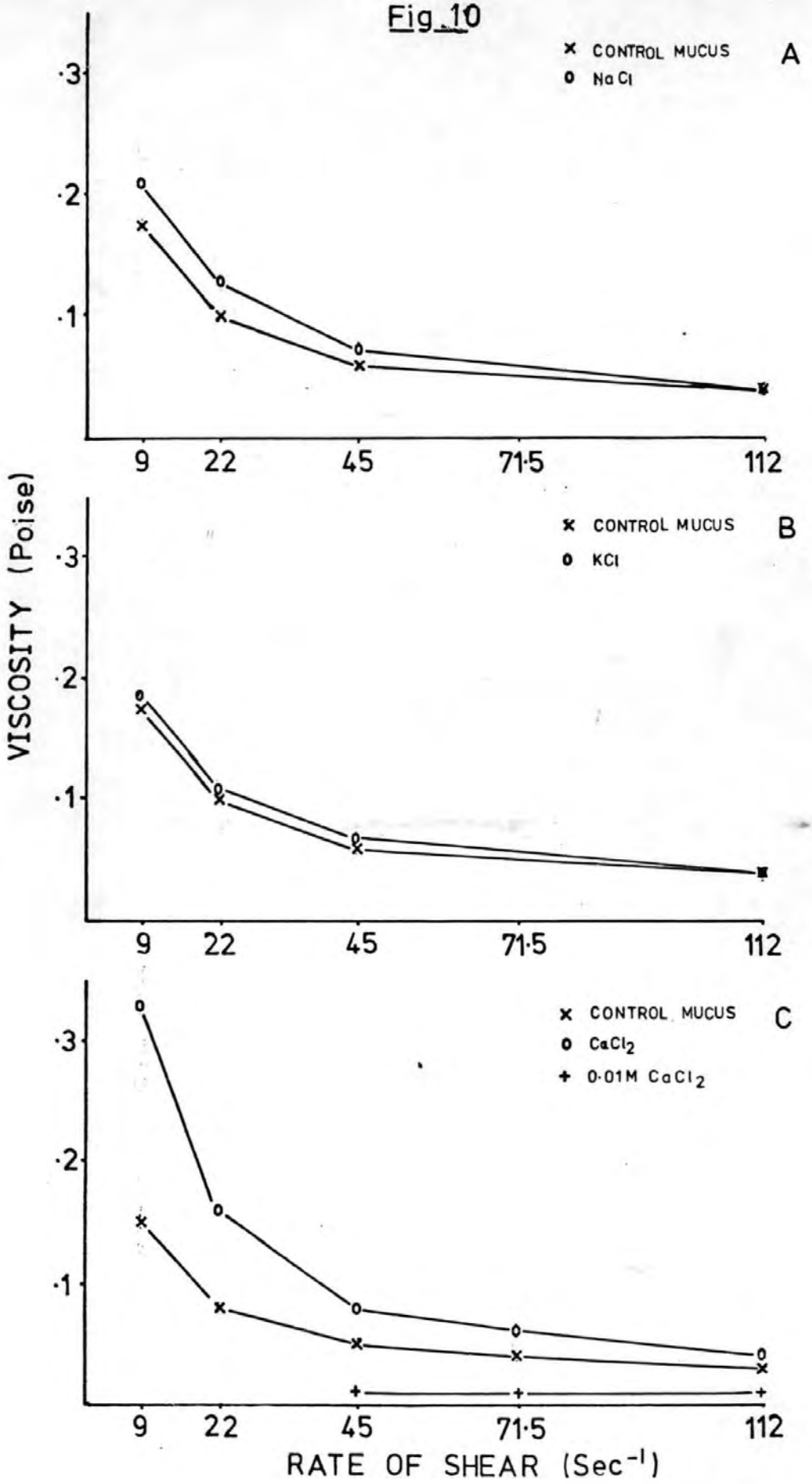

× CONTROL MUCUS
o NaCl
A
·3
·2
·1
9
22
45
71·5
112
× CONTROL MUCUS
o KCl
B
VISCOSITY (Poise)
× CONTROL MUCUS
o CaCl2
+ 0·01M CaCl2
C
RATE OF SHEAR (Sec-1)

Fig. 11

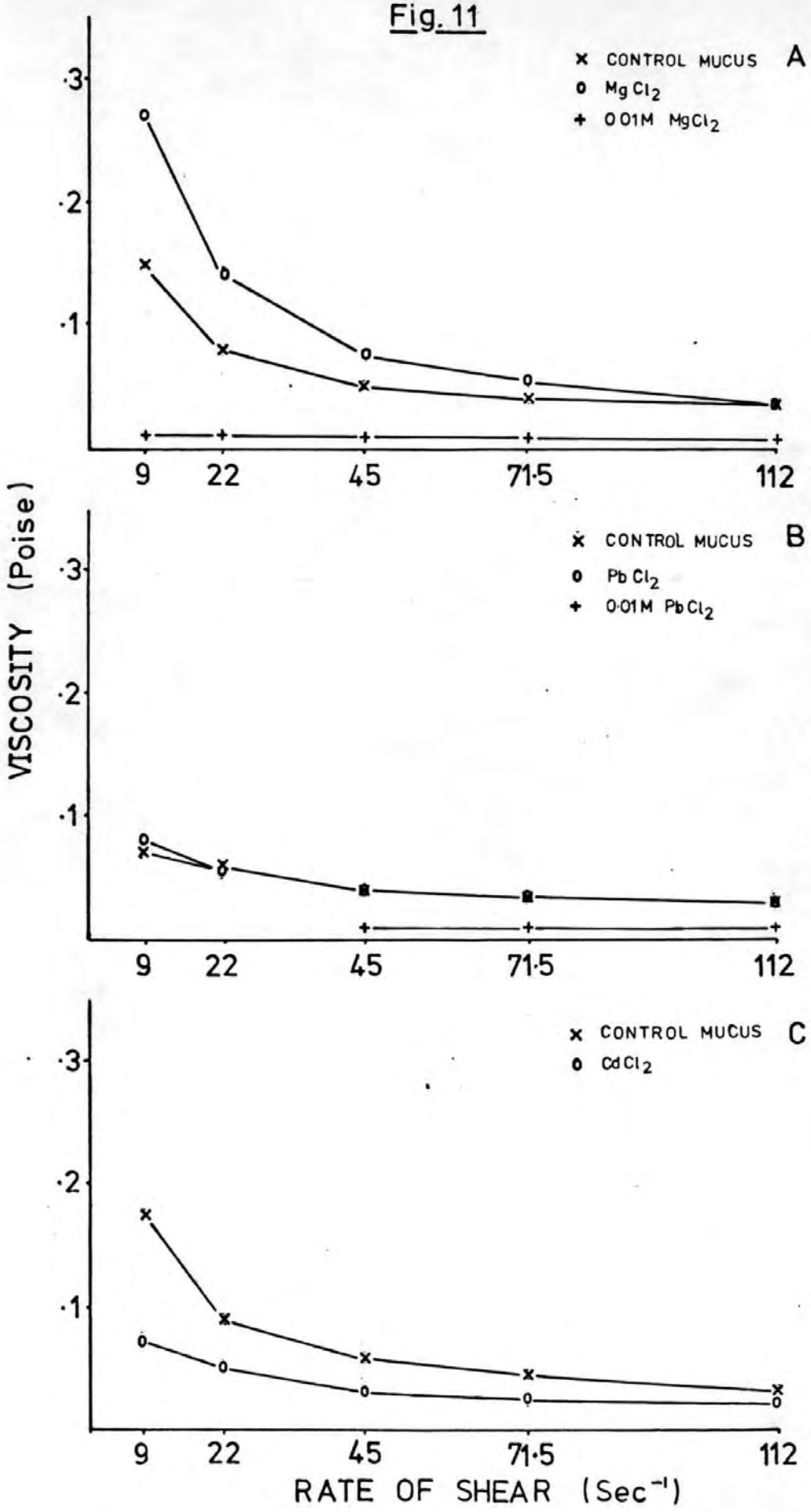

A
× CONTROL MUCUS
o $MgCl_2$
+ 0·01M $MgCl_2$
B
× CONTROL MUCUS
o $PbCl_2$
+ 0·01M $PbCl_2$
C
× CONTROL MUCUS
o $CdCl_2$
VISCOSITY (Poise)
·3
·2
·1
9
22
45
71·5
112
RATE OF SHEAR (Sec^{-1})

Fig. 12

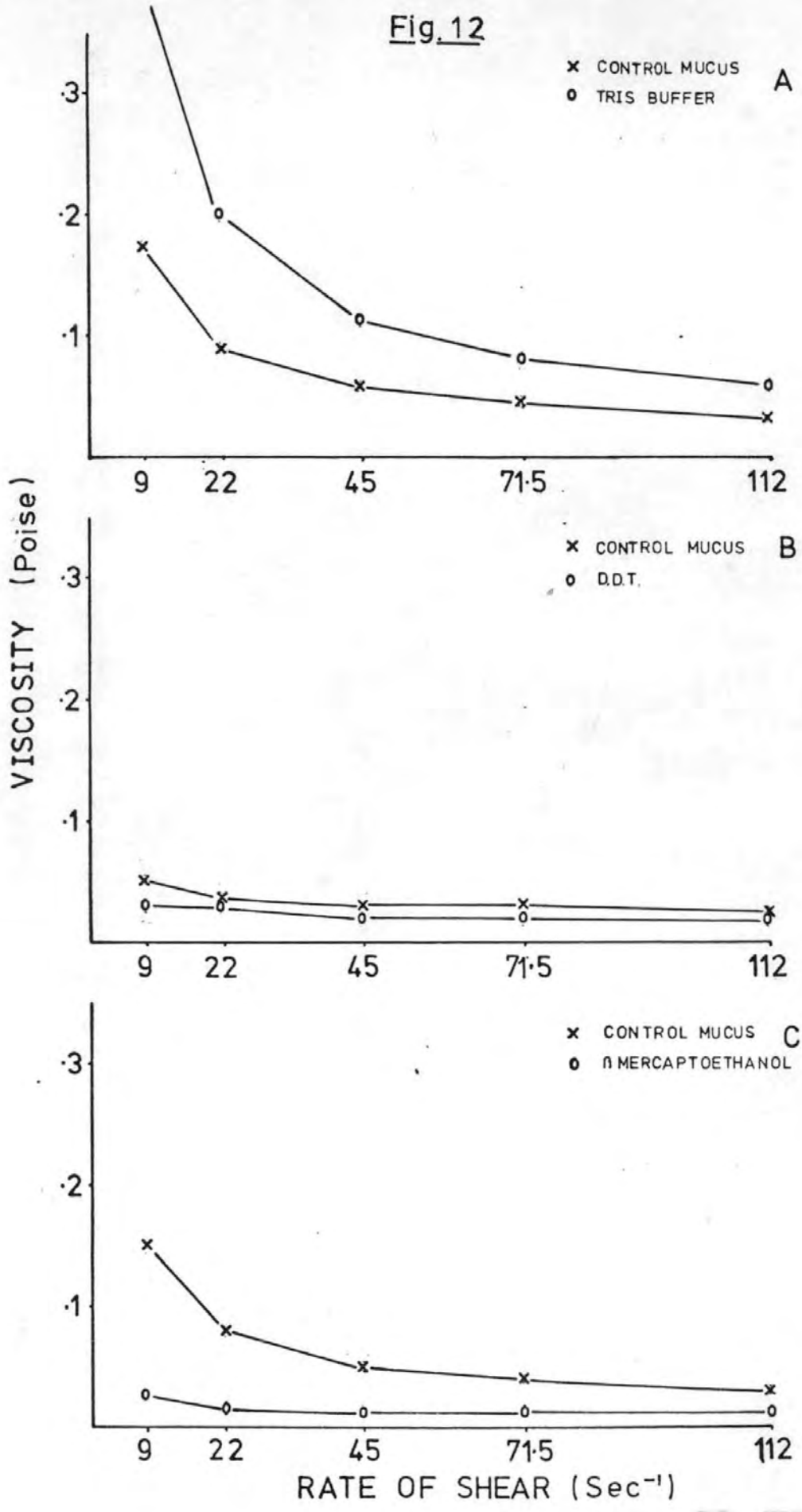

× CONTROL MUCUS
o TRIS BUFFER
A
·3
·2
·1
9
22
45
71·5
112
× CONTROL MUCUS
o D.D.T.
B
·3
·2
·1
9
22
45
71·5
112
× CONTROL MUCUS
o ß MERCAPTOETHANOL
C
·3
·2
·1
9
22
45
71·5
112
VISCOSITY (Poise)
RATE OF SHEAR (Sec⁻¹)

Fig. 13

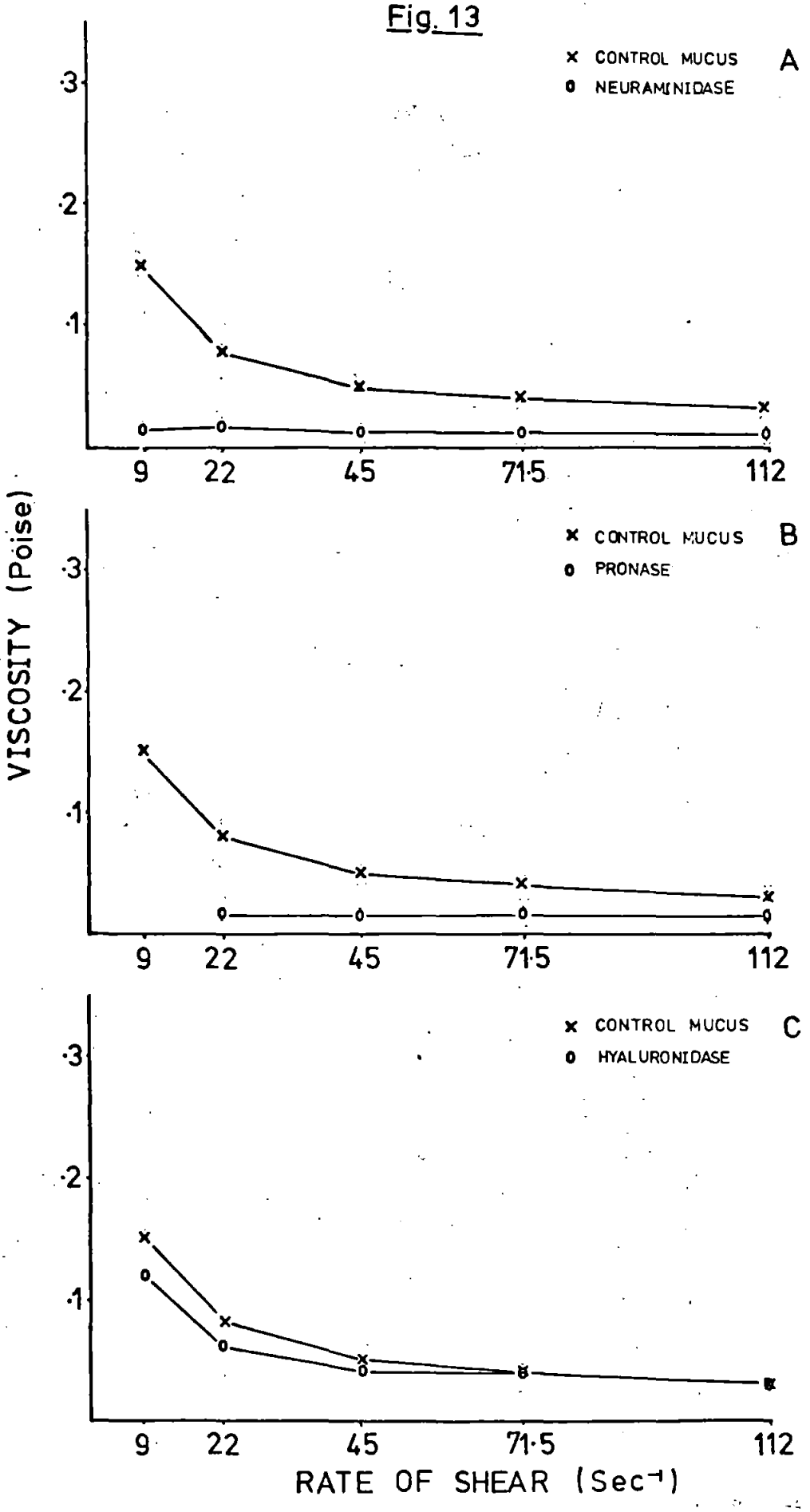

× CONTROL MUCUS
o NEURAMINIDASE
A
·3
·2
·1
9
22
45
71·5
112
× CONTROL MUCUS
o PRONASE
B
VISCOSITY (Poise)
× CONTROL MUCUS
o HYALURONIDASE
C
RATE OF SHEAR (Sec⁻¹)

Fig. 14

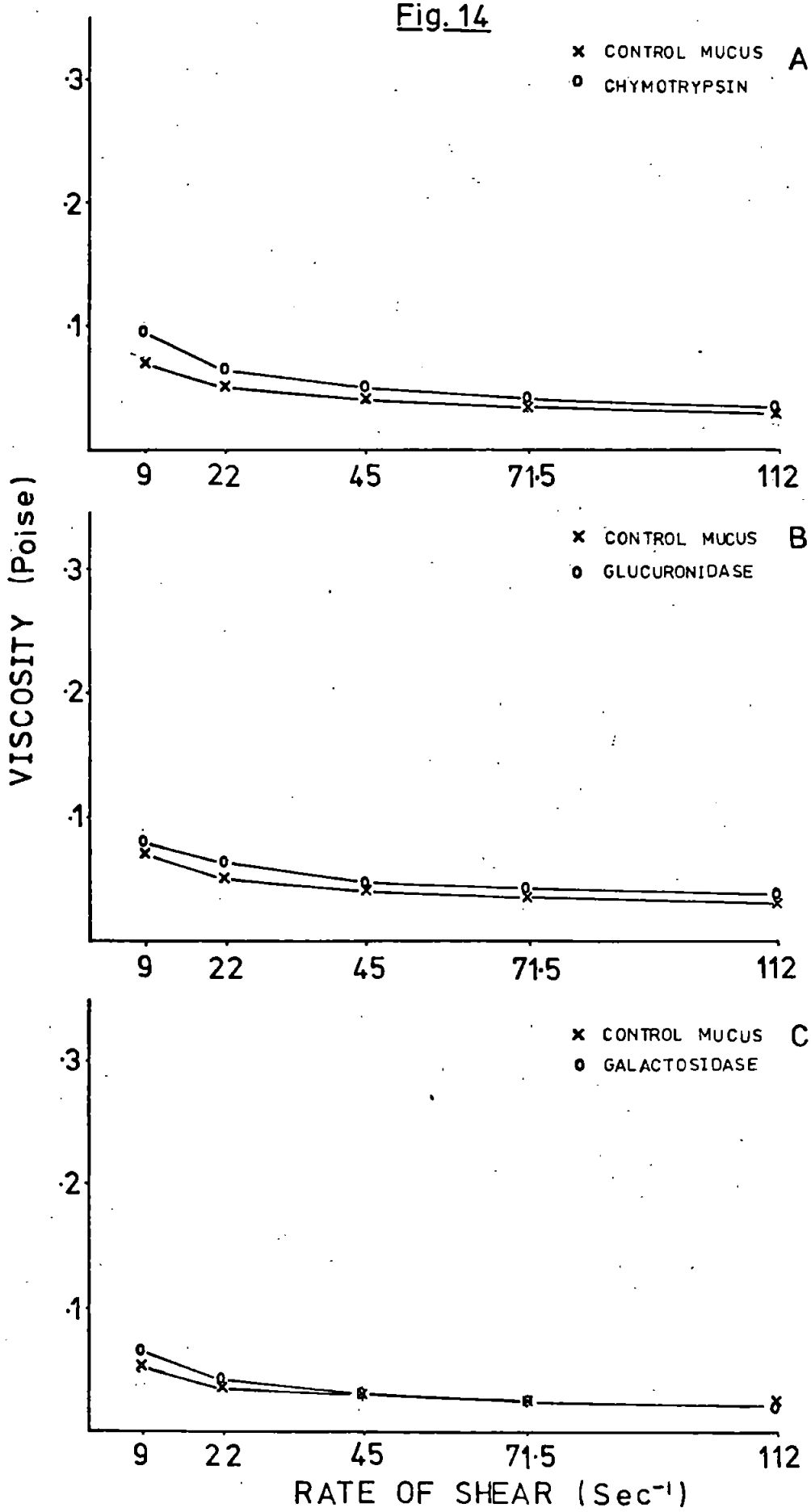

× CONTROL MUCUS
o CHYMOTRYPSIN
A
× CONTROL MUCUS
o GLUCURONIDASE
B
× CONTROL MUCUS
o GALACTOSIDASE
C
·3
·2
·1
9
22
45
71·5
112
VISCOSITY (Poise)
RATE OF SHEAR (Sec⁻¹)

TABLE 13. SUMMARY OF EFFECTS OF MUCOUS 'ADDITIVES'

A. Materials that significantly thicken mucus.

$MgCl_2$	$P < .05$
$CaCl_2$	$P < .05$
Tris-HCl Buffer	$P < .05$

B. Materials that significantly thin mucus.

$CdCl_2$	$P < .01$
DDT	$P < .001$
β-mercaptoethanol	$P < .001$
Neuraminidase	$P < .001$
Pronase	$P < .002$

C. Materials that have no significant effect.

NaCl

KCl

$PbCl_2$

Hyaluronidase

Chymotrypsin

Glucuronidase

Galactosidase

No agglutinating properties were detected in the concentrated mucus of eels, injected with Salmonella H antigen a by a direct combination technique. Furthermore when concentrated mucus from injected fish was added to wells cut in agar plates (Ouchterlony technique) no evidence of precipitin lines between the wells containing the mucus and wells containing antigen were seen.

Measurement of Viable Bacteria in the Experimental System

The results of viable bacterial cell counts for duplicate water samples taken from an untreated system (three vessels each containing 500 cm^{-3} sterile distilled water and a single eel) and a similar system treated with streptomycin (100 mg) are presented as mean and total range (Fig. 15). In untreated vessels the maximum numbers of viable bacteria were found after 84 h (6.7×10^6 cells $.cm^{-3}$). After 24 h and 48 h bacterial numbers were 8×10^3 cells $.cm^{-3}$ and 13×10^4 cells $.cm^{-3}$ respectively. The addition of steptomycin to the vessels effectively maintained the viable bacterial count at zero (as determined by the 'pour plate' method with growth on nutrient agar), during the first 48 h. The cell count subsequently rose during the following 54 h.

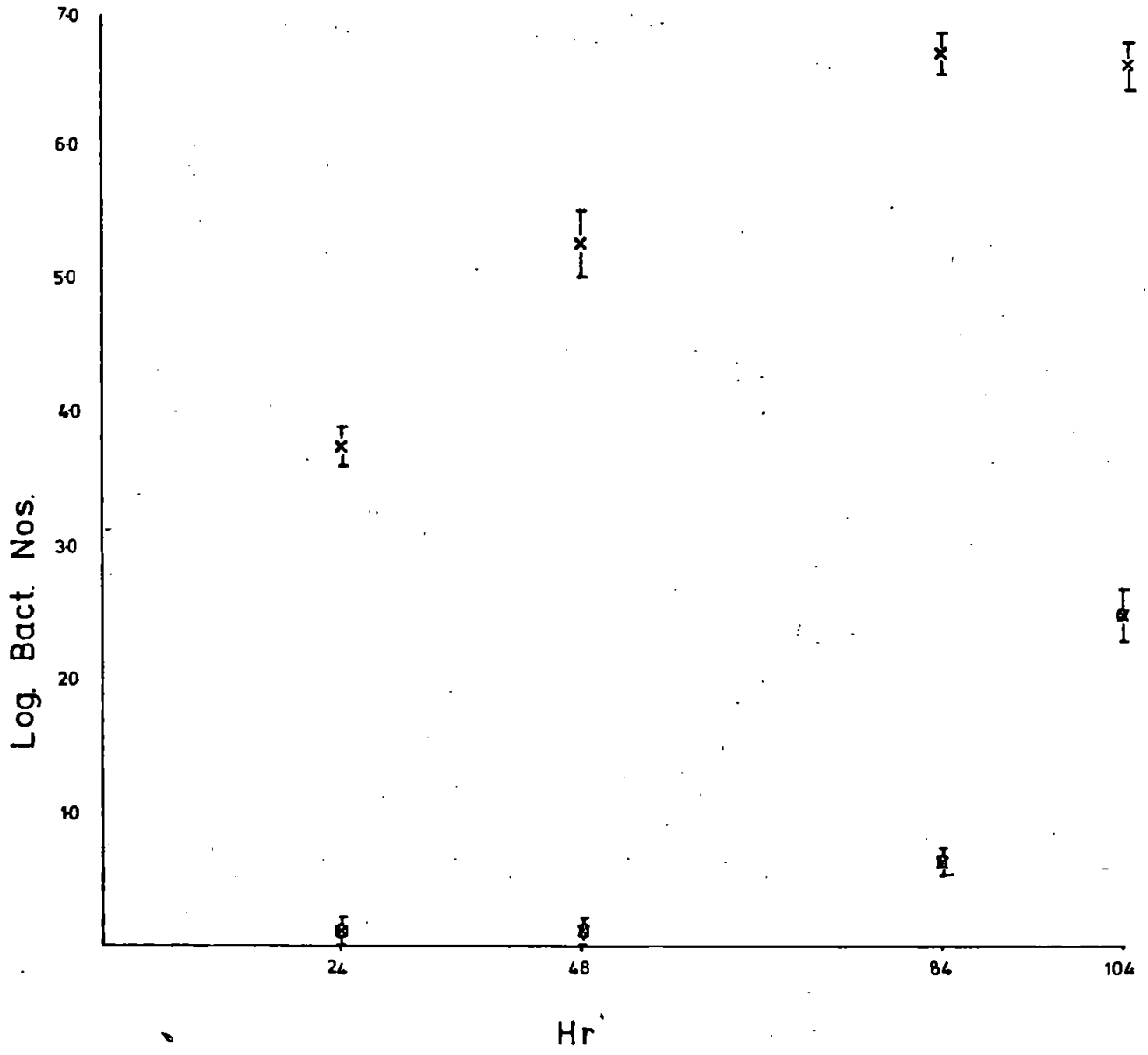
Filtration of Water Samples

Protein analyses were performed on water samples taken from both systems used for the estimation of bacterial numbers. There was no significant difference between the protein concentrations of water samples from the untreated and the streptomycin treated system (Table 14). This indicates the protein content of the water attributable to bacteria to be negligible and thus no routine correction factor for bacterial growth was included in the main set of experiments. However, the effect of bacteria present in the experimental systems was monitored during the experiments investigating the effects of temperature on mucous secretion (see streptomycin experiment).

The difference between the total protein (in filtered) and the soluble protein (filtered) content of water samples taken at 48 and 24 h periods were similar in both untreated and streptomycin treated systems (Table 14). This indicates that filtration of water samples in the main series of experiments was unnecessary.

Fig. 15

BACTERIAL GROWTH IN EXPERIMENTAL SYSTEM



× Control untreated

⊠ Streptomycin treated

TABLE 14. PROTEIN CONTENT OF FILTERED WATER SAMPLES FROM UNTREATED AND STREPTOMYCIN TREATED (BACTERIA FREE) SYSTEMS.

	Protein Content ($\mu g\ cm^{-3}$)		
	24 h	48 h	48 - 24 h
Untreated System			
Total protein	23.3 ± 7.7	32.7 ± 0.3	9.4
Protein after filtration	14.4 ± 5.2	23.8 ± 2.8	9.4
% soluble protein of total	61.8	72.7	
Streptomycin Treated System			
Total protein	25.6 ± 2.2	35.5 ± 3.6	9.9
Protein after filtration	15.6 ± 0.8	25.2 ± 2.7	9.6
% soluble protein of total	60.9	70.9	

Estimation of the Rate of Mucous Secretion

Estimations of the rate of mucous secretion (RMS) under a number of experimental conditions (Tables 15 - 18, Figs 16, 17) were calculated from protein analyses and presented as μg mucus (dry wt.) per square cm per hour (μg cm^{-2} h^{-1}). Estimations of the RMS were calculated from carbohydrate analyses only when protein determinations were unobtainable e.g. interference due to sea water, as the modified Lowry technique for protein was more sensitive to low levels of mucus than the phenol-sulphuric acid technique used for the determination of carbohydrate.

The effect of temperature on mucous secretion (Table 15) shows an approximate linear relationship in the range 4 - 22 °C, (Fig. 16) (regression $y = 0.64x + 0.361$) (significance of regression, $P < 0.001$, F-test).

Streptomycin added to the experimental systems in an attempt to inhibit bacterial growth (Table 16, Fig. 17) shows no significant change (Student's t-test) in the rate of mucous secretion over respective control values at 4, 12 and 22 °C.

The increase in the rate of mucous secretion seen with agitation of the water (Table 17) ($P < 0.001$, Student's t-test) when compared to control values may be due to increased dissolution as shown by the shear thinning properties of the mucus (see section on rheology and relevant discussion). The effect of atropine added to the experimental systems (Table 17) shows no significant change (Students t-test) in the rate of secretion compared to control values. This suggests the mucous layer is retained on the surface of the fish and released as a result of external influences as the surface mucous cells are prevented by the action of atropine from releasing their contents (Pl. 26C).

TABLE 15

EFFECT OF TEMPERATURE ON MUCOUS SECRETION

Temp. °C		Nº of Fish	Mean Rate µg PROTEIN h⁻¹	Mean Surface Area CM²	Secretion Rate µg PROTEIN cm² h⁻¹	Secretion Rate µg MUCUS cm² h⁻¹
4		6	79·5	160·4	·49 ± ·07	2·19
12	a	3	202·5	162·3	1·25 ± ·12	5·51
	b	6	208·0	167·7	1·24 ± ·15	5·48
14		3	165·0	136·0	1·21 ± ·12	5·36
15		6	190·0	143·7	1·32 ± ·08	5·85
22	a	4	250·0	140·3	1·78 ± ·13	7·87
	b	1	204·5	120·5	1·69	7·50

Fig.16 EFFECT OF TEMPERATURE ON MUCOUS SECRETION

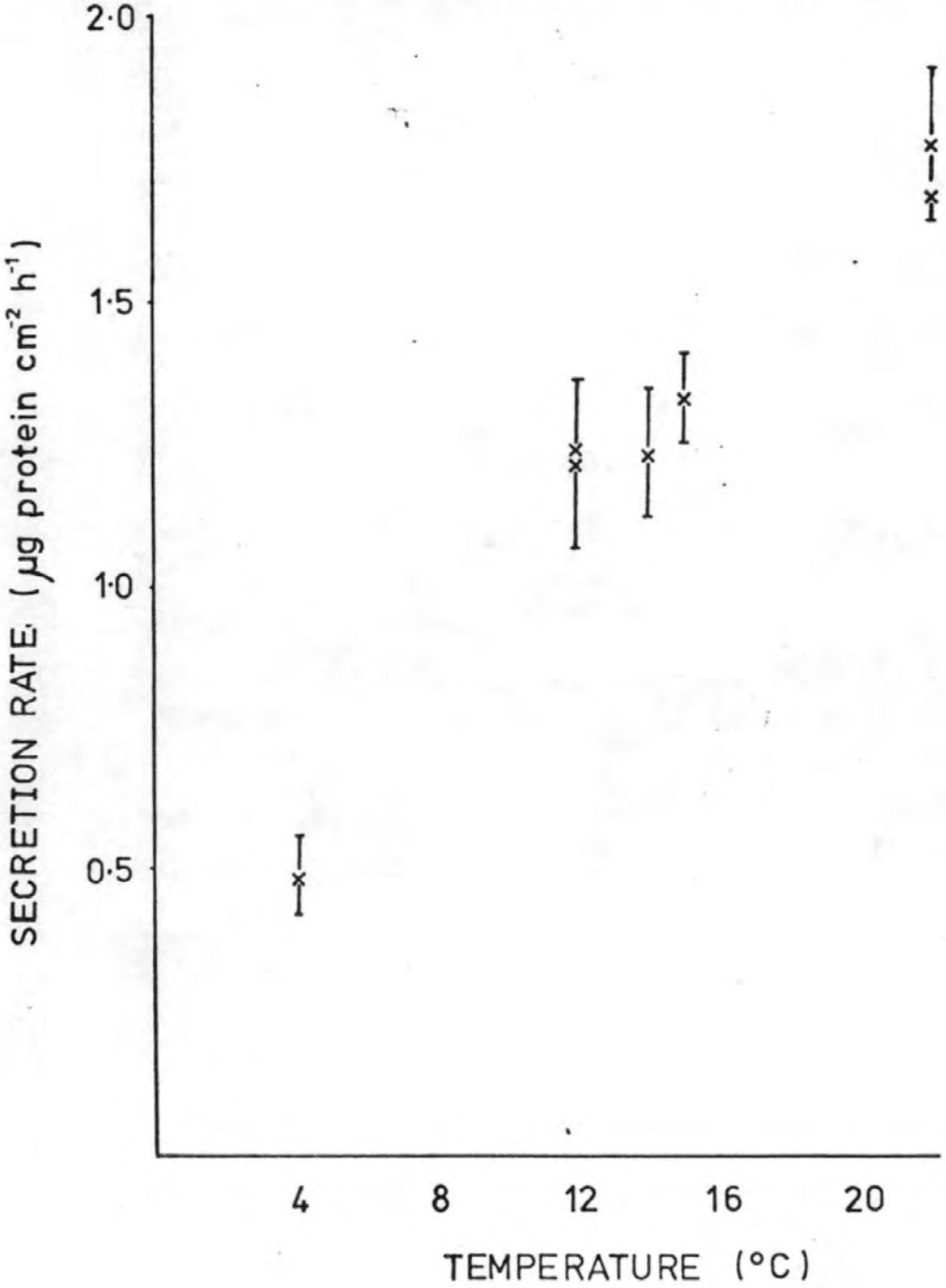


TABLE 16

EFFECT OF STREPTOMYCIN ON MUCOUS SECRETION

Temp °C	N° of Fish	Mean Rate µgPROTEIN hr^{-1}	Mean Surface Area CM^2	Secretion Rate µg PROTEIN $cm^{-2}hr^{-1}$	Secretion Rate µg MUCUS $cm^{-2}hr^{-1}$
4° Streptomycin	6	112·0	160·4	0·69 ± ·10	3·08
4° Control	6	79·5	160·4	0·49 ± ·07	2·19
12° Streptomycin	3	133·0	123·4	1·07 ± ·09	4·77
12° Control	6	208·0	167·7	1·24 ± ·15	5·48
22° Streptomycin	4	256·0	162·1	1·58 ± ·14	7·04
22° Control	4	250·5	140·3	1·78 ± ·13	7·87
	1	204·5	120·5	1·69	7·50

Fig. 17 EFFECT OF STREPTOMYCIN ON MUCOUS SECRETION

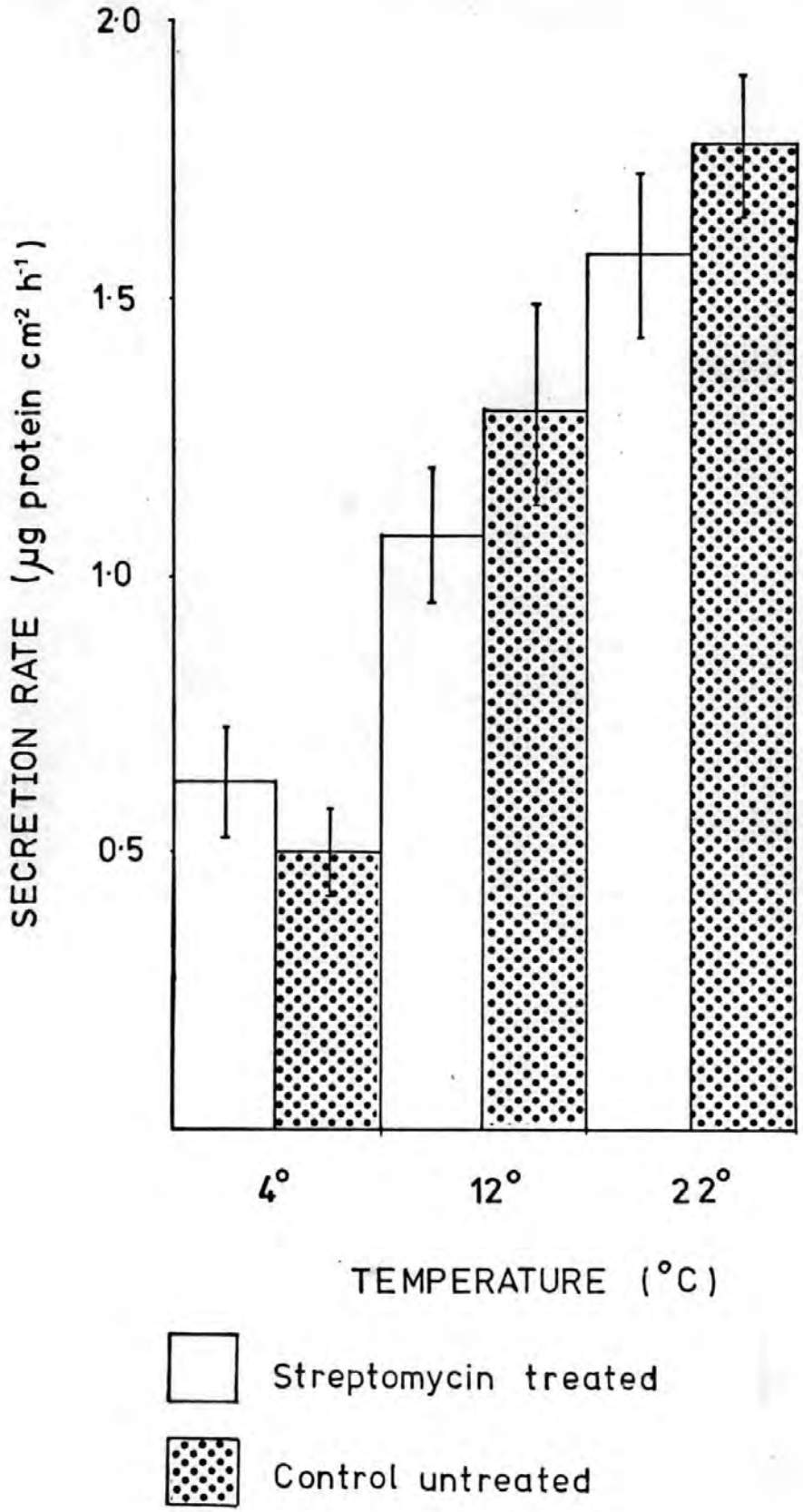


TABLE 17

EFFECT OF AGITATION & ATROPINE ON MUCOUS SECRETION

	No. of Fish	Mean Rate μg PROTEIN hr^{-1}	Mean Surface Area CM^2	Secretion Rate μg PROTEIN $cm^{-2}hr^{-1}$	Secretion Rate μg MUCUS $cm^{-2}hr^{-1}$
12° AGITATION	3	437·5	162·3	2·69 ± ·10	11·91
12° ATROPINE	6	152·0	140·5	1·08 ± ·09	4·77
12° CONTROL	6	208·0	167·7	1·24 ± ·15	5·48

TABLE 18

EFFECT OF SEA WATER ON MUCOUS SECRETION

	Nº of Fish	Rate* μg CARB hr^{-1}	Mean Surface Area cm^2	Secretion rate* μg CARB $cm^{-2} hr^{-1}$	Secretion rate μg MUCUS $cm^{-2} hr^{-1}$
12° D.W. Cont.	6	35·5	167·7	·21±·09	3·11
12° S.W. Imm. tfr.	3	39·3	149·6	·26±·08	3·85
12° S.W. Slow adp.	3	25·1	146·2	·17±·05	2·51

*Sea water interferes with protein analysis

D.W. = Distilled Water Control

S.W. Imm. tfr. = Sea Water Immediate transfer

S.W. Slow. adp. = Sea Water Slow adaptation

The effects of immediate transfer from freshwater to sea water and of adaption to sea water over a period of three weeks show no significant change (Student's t-test) in the rate of mucous secretion (Table 18) when compared to control values. The rate of secretion in this experiment is measured by the phenol-sulphuric acid method for carbohydrates as there is interference with the protein assay by the sea water.

Autoradiography of Eel Epidermis

Counts of the percentage labelled mucous cells in the lower, mid- and upper regions of the epidermis at varying times after injection of H^3 galactose are presented in Figure 18. Only mucous cells containing four or more silver grains per cell were taken to be positively labelled and the mean and standard deviation (SD) of six counts are presented. The results are expressed as a percentage because of the variation in mucous cell numbers between fish. Labelled mucous cells were apparent in the lower epidermis (Fig. 18C) after 6 h and after injection reached a maximum after 9 h. After 16 h few cells of this region appeared to be labelled. In the mid-epidermal region (Fig. 18B), the maximum labelling occurred 16 h post-injection when over 60% of mucous cells were labelled. This level subsequently dropped but was maintained throughout the remainder of the experimental period with approximately 35% of the cells labelled. Mucous cells of the upper regions of the epidermis (Fig. 18A) showed a maximum labelling at 36 h after injection and these levels of labelled cells (approx. 60%) were maintained throughout the remainder of the experimental period.

Fig18 AUTORADIOGRAPHY OF LABELLED MUCUS CELLS IN LOWER MID & UPPER REGIONS OF EPIDERMIS

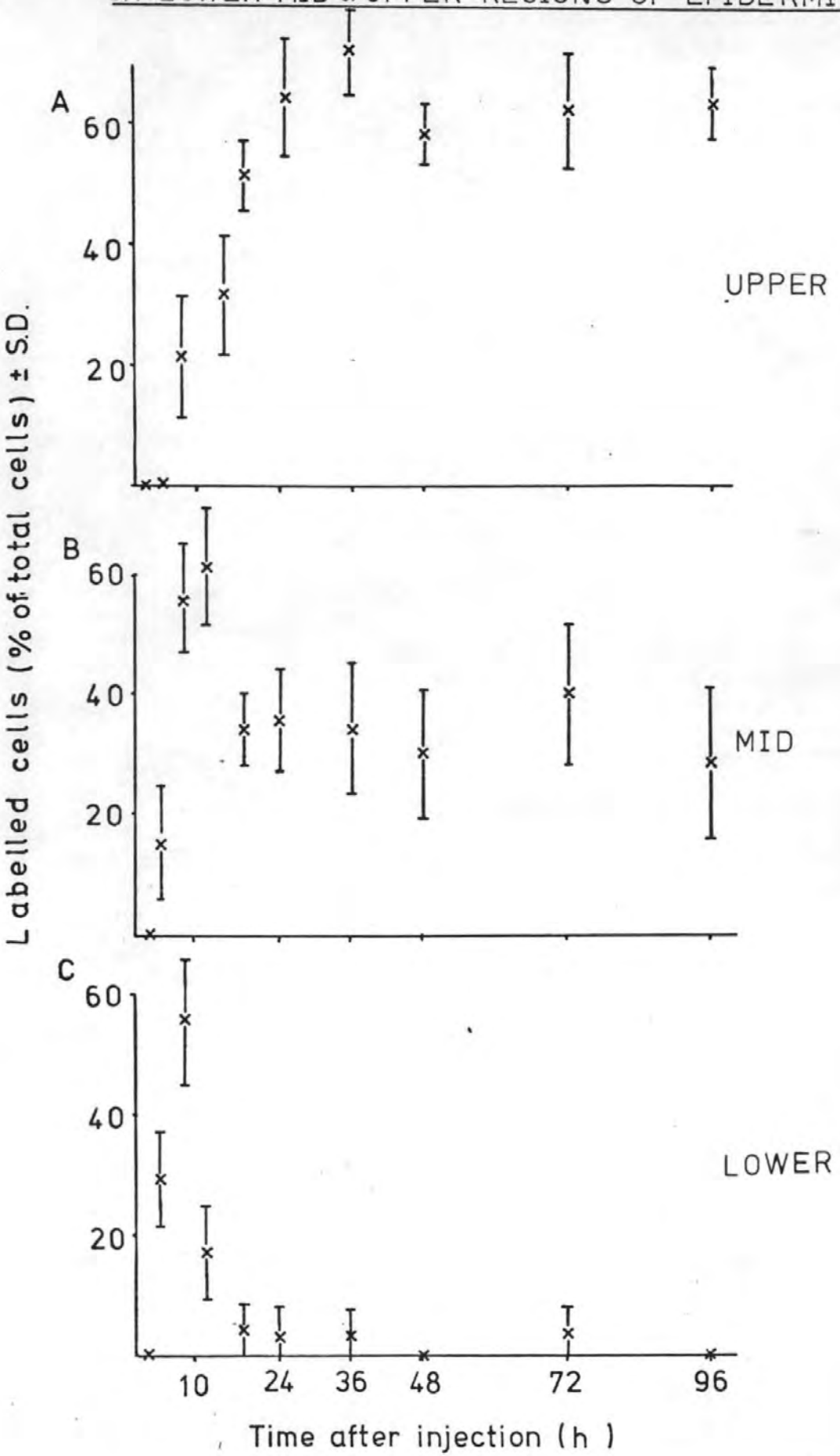


PLATE 28

A. Light autoradiograph of epidermis 6 h after injection of H^3 galactose. Labelling of mucous cell (arrowed) is apparent in lower region of epidermis. Alcian blue staining (x 3,200).

D Dermis

M Mucous cell

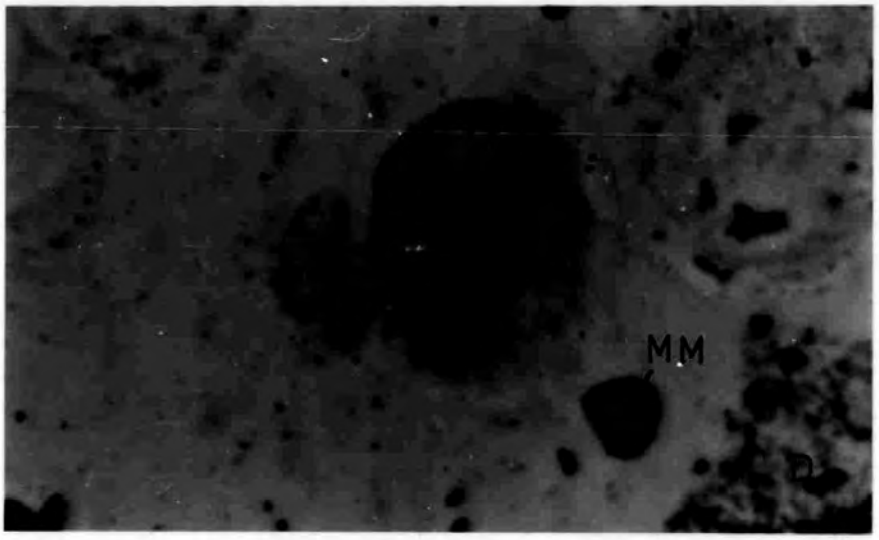
MM Melanin containing macrophage

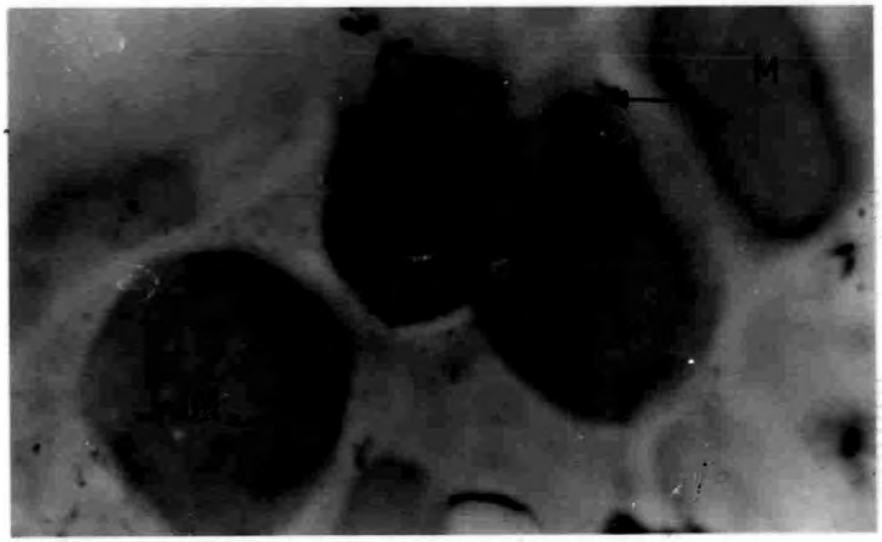
B. Light autoradiograph of epidermis 24 h after injection of H^3 galactose. Labelling of mucous cells (arrowed) is apparent in mid-epidermal region. Alcian blue staining (x 3,200).

M Mucous cell

C. Light autoradiograph of epidermis 72 h after injection of H^3 galactose. The mucous layer is labelled (arrow). Alcian blue staining (x 3,200).

M Mucous cell


MM


M

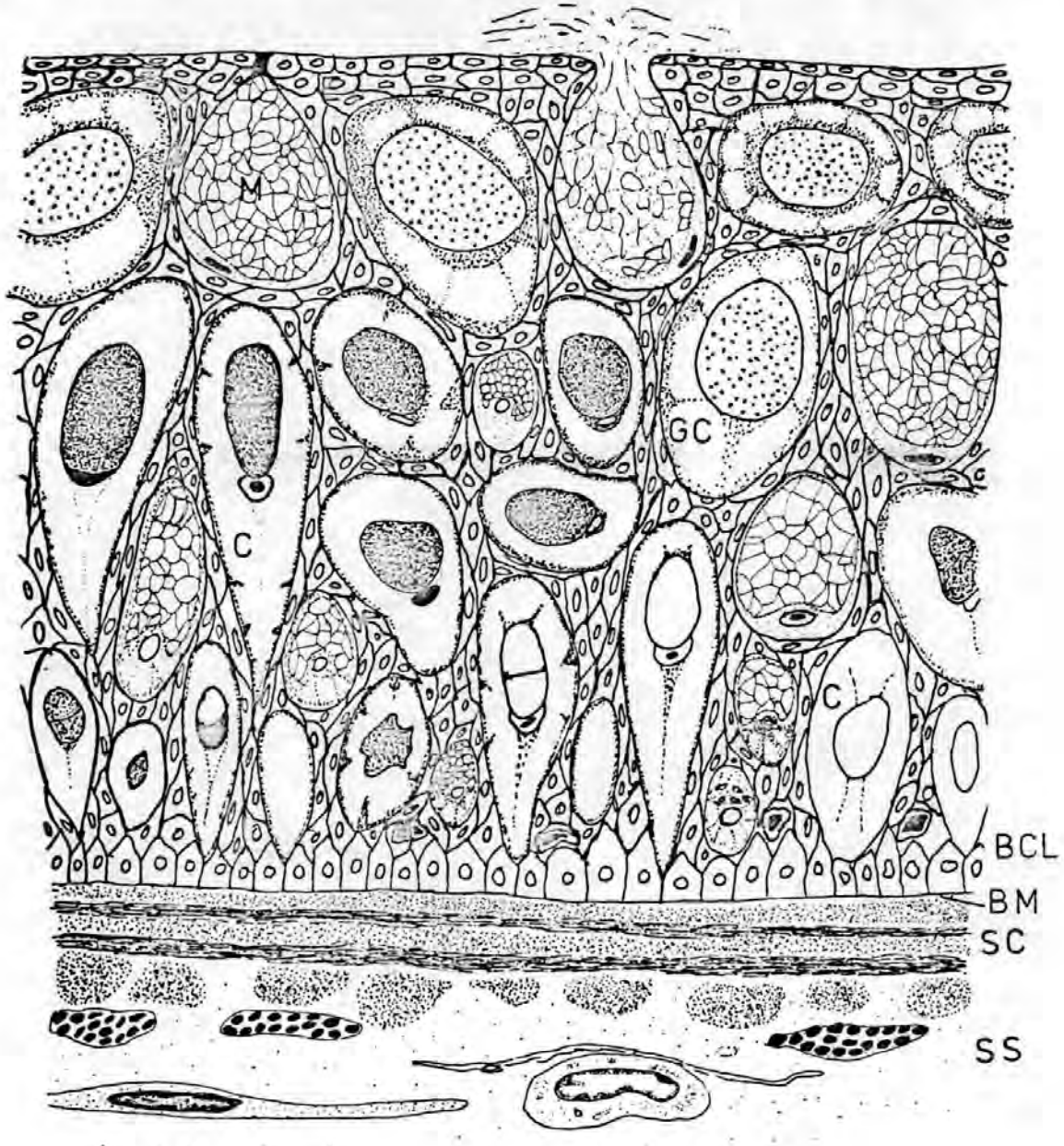
DISCUSSION

HISTOLOGY, HISTOCHEMISTRY AND ULTRASTRUCTURE OF THE EPIDERMIS

The epidermis of the European eel comprises three main types of cell: filament or epidermal cells, goblet or mucous cells and clavate or club cells (shown diagrammatically in Fig. 19). A similar cytological picture is found in the epidermis of both A. rostrata (Leonard and Summers, 1976) and A. japonica (Asakawa, 1972; Yamada and Yokote, 1975). These three cell types were found in a variety of teleosts by Burgess (1954) and Bremer (1972), although few teleosts posses club cells with a similar structure to those of the Anguillidae.

Mucous Cells. The appearence and development of the mucous cells of the eel epidermis are comparable with those of other teleosts. Only one type of mucous cell, however, is found in the eel epidermis, whereas, in some other teleosts two or three types of mucous cell have been observed e.g. Esox americanus (Merrilees, 1974) and Protopterus annectens (Kitzan and Sweeny, 1968). The mucous cells of the eel epidermis show the characteristic development of secretory vesicles found in goblet mucous cells of mucosal membranes of the alimentary, respiratory and reproductive systems of higher vertebrates (Freeman, 1966; Neutra and Leblond 1966; Rhodin, 1972). The formation of the secretion appears to involve free ribosomes, endoplasmic reticulum and the Golgi apparatus. The mucus is formed in vesicles, the membranes of which are thought to be derived from the Golgi complex. The membranes form the principal lipoprotein in mucus and this has been implicated as a possible cross-linking agent for glycoprotein molecules(Lewis, 1976).

Fig.19 DIAGRAMMATIC REPRESENTATION OF EEL EPIDERMIS



BCL	BASAL CELL LAYER
BM	BASEMENT MEMBRANE
C	CLUB CELL
E	EPIDERMAL CELL
GC	GRANULAR CLUB CELL
M	MUCOUS CELL
SC	STRATUM COMPACTUM
SS	STRATUM SPONGIOSUM

As the mucous cells enlarge they move from the lower and mid-epidermal regions of the epidermis to the more superficial layers. The mucous vesicles within the cell increase in size and number, displacing the nucleus and organelles to the base of the cell. At the surface of the epidermis, the cells open at their apex. The membranes surrounding the mucus 'packets' disintegrate and their contents coalesce prior to release from the cell on to the epidermal surface.

The controlling mechanism governing secretion of mucus from the goblet cell is not fully understood. The process appears to involve a neural or neurohormonal pathway as indicated by the blocking action of atropine (Pl. 26B). Atropine is a parasympathetic nerve blocking compound that prevents the release of the mucus from the mature goblet cell but has no inhibitory effect on the growth, development or migration of these cells to the periphery of the epidermis. Furthermore, electrical stimulation of the fish (Pl. 26C) causes the epidermal mucous cells to elongate towards their apex. This may be due to movement of the epidermal (filament) cells rather than a direct stimulatory effect on the mucous cells themselves. The superficial epidermal cells appear to become cuboidal, after electrical stimulation, when compared to the more flattened cells of the unstimulated epidermis. Bereiter-Hahn (1971) suggests that the epidermal cells are capable of considerable movement within the epidermis and migration of epidermal cells has been reported during wound closure in the plaice (Bullock, Marks and Roberts, 1978). In addition Bereiter-Hahn (1978 pers. comm.) has shown the surface layers of the epidermal cells to contain actin fibres. The mucous cells do not appear to have direct innervation and the apparent lack of contractile fibres may indicate that the mucus is not expelled by pressure from within the cell. Slight lateral flattening of superficial

mucous cells (Pl. 2B) may indicate, however, that pressure exerted by surrounding epidermal cells could play an important role in the release of the mucous cell secretion.

It is interesting to note that in a recent scanning electron-microscope study on fish epidermis (Hunter and Nayudu, 1978) the appearence of the mucous cells is seen to differ depending on the fixation methods employed. Epidermis fixed in 5% glutaraldehyde have mostly closed mucous cell orifices that appear as small domes at the junction of two or three epidermal cells. The specimens that had been fixed in 10% formalin showed mucous cells open at their surface. Although these findings confirm those of Lanzing and Higginbotham (1974) and Dobbs (1975), samples of skin fixed in 3% glutaraldehyde in the present study show a large number of open mucous cells (Pls 3A,B). There was no explanation for the differences between fixatives although formalin is known to to penetrate tissues at a faster rate than glutaraldehyde (Hayat, 1972). The skin samples in this study were thoroughly washed to remove all traces of the mucous layer and this procedure may have encouraged the opening of mucous cells. This is discussed in detail later.

Histochemically, the mucous cells of the eel, in common with those of many other teleosts, contain sulphated, non-sulphated and sialic acid containing mucosubstances (see Appendix I). It is not possible to determine, histochemically whether these staining characteristics are produced by separate glycoprotein molecules or by sulphated and sialylated side chains on the same protein backbone. Variation in staining of the mucous 'packets' seen with lead citrate and uranyl acetate or PTA under the electron microscope (Pl. 19B) and also in thick Epoxy sections stained with methylene blue (Pl. 2B), may indicate that different molecules are synthesised within the same cell. Mucous

cells do not stain positively for proteins (Table 9) even though eel mucus has been shown to contain a high proportion of protein and it is assumed that the strongly charged groups of the carbohydrate side chains effectively mask the staining of protein. The membranes of the mucous 'packets' do, however, stain for protein and it is interesting to note that when mucus is separated by electrophoresis (Fig. 7) only one component is positive for protein.

Positive staining reactions are seen with Sudan black B and Nile blue sulphate both of which are thought to be specific for lipid material. Pearse (1968), however, mentions the positive reaction of mucosubstances to Sudan black B and also the non-specificity of Nile blue sulphate. Other staining procedures for lipids all proved to be negative, in contrast to the findings of Mittal, Rai, Banerjee and Agarwal (1976) who described strong reactions for phospholipids in the mucous cells of Heteropneustes fossilis.

Epidermal Cells. The superficial epidermal cells of the eel in common with most other teleosts are not keratinised (Spearman, 1972). The surface of these cells is folded into a series of microridges (Pls 3A,B, 4A,B). Microridges have also been found in other teleosts (Yamada, 1966; Bereiter-Hahn, 1971; Hawkes, 1974a), amphibian skin (Whitear, 1975, 1977) and also respiratory cervical and vaginal surfaces of mammals (Elstein, 1973). They are present wherever there is a mucous layer and it would appear likely that at least one of their functions is to aid the attachment of mucus. Bereiter-Hahn (1971) showed that these microridges were capable of movement and suggested they played a role in primary wound closure; a claim supported by Bullock, Marks and Roberts (1978) in the epidermis of P. platessa. Other possible functions of the microridges may be to allow for flexing

of the epidermis during swimming and to increase the surface area available for gaseous exchange. Recently Kirsch and Nonnotte (1977) have shown that although the epidermis of the freshwater eel does not act as an accessory oxygen exchange epithelia for the benefit of other tissues and organs, it does have a high oxygen consumption which it satisfies with oxygen absorbed directly from its aqueous surroundings. In the marine eel and freshwater eel adapted to sea water over a period of four weeks, Nonnotte and Kirsch (1978) do report a net inward transcutaneous oxygen flux, although they could give no explanation for the differences occurring between sea and freshwater eels. The micro-ridges may, in addition, help to retain an 'unstirred layer' close to the epidermal surface which could maintain ionic integrity (Dainty and House, 1966).

The epidermal cells vary in shape (Pl. 1A) but those of the basal cell layers are mostly cuboidal and in electronmicroscope preparations are more electron-dense than those of higher layers of the epidermis. Cells of the basal cell layer appear to differentiate into the other cell types and dividing cells may be seen both in the basal cell layer and in the layers immediately above it (Pls 2A,B). Autoradiography with H^3 thymidine has shown, however, that cells in all layers of the epidermis are capable of division in the goldfish (Henrickson, 1967) and plaice (Bullock, Marks and Roberts, 1978a).

Ultrastructurally, the epidermal cells are characterised by the presence of intracellular filaments (approx. 8 nm in diameter) usually found in the peripheral regions of the cells which appear to form connections with desmosomal junction (Pl. 17A). These filaments are thought to provide mechanical strength to the epidermal cell. Complex inter-cellular interiigitations with desmosomal junctions occur not

only between adjacent epidermal cells but also between epidermal and club cells (Pl. 9A). Junctions between cells may be important in the transport of nutrients for club, mucous and other epidermal cells (Staehelin and Hull, 1978).

In addition to the functions outlined above, the peripheral epidermal cells can also perform a secretory function (Pl. 13A). Secretion of a mucosubstance from the epidermal cells of A. rostrata was suggested, but not established, by Leonard and Summers (1976) but has been reported in other teleosts (Whitear, 1970). The secretion does not appear to contain sulphated mucosubstances but does contain carboxylated mucosubsubstances (Table 8). Whitear (1970) believes this secretion to comprise the mucous layer or 'cuticle', the secretion of the mucous cells providing only emergency lubrication. A more detailed discussion of the 'cuticle' occurs later.

Under certain conditions e.g. complete removal of the surface mucous layer, the epidermal cells of the mid-layers may undergo transformation to a secretory cell type similar to a mucous cell (Pls 14 - 16). This transformation has not previously been demonstrated in fish epidermis although Downing and Novales (1971) have suggested that undifferentiated epidermal cells may retain the potential to develop into filament-containing cells or mucous cells until a fairly late stage of development. Clearly this would appear to be the case in the eel although the transformed cells still retain characteristic 8 nm filaments. This transformation appeared only after the mucous layer had been removed by compressed air and suggests the possibility of a 'feedback mechanism' to encourage transformation to the secretory cell type when mucous cell numbers are reduced.

Histochemically, the epidermal cells show staining reactions for proteins. At the surface layers they fequently show staining reactions for carbohydrates and mucosubstances confirming the observation, under the electron microscope, that the epidermal cells may secrete material into the mucous layer. The surface epidermal cells also show strong staining reactions for cholesterol and its esters by the Schultz method although this was not confirmed by the Ökamoto method. Cholesterol or cholesterides may be incorporated into the cellular membrane (Keele and Neil, 1971).

Club Cells. In the Anguillidae, club cells are thought to produce the 'filaments' that may be found in the mucus (Reid, 1894; Henrickson and Matolsty, 1968b). These filaments are visible with the naked eye and are thus distinct from the 8 nm filaments characteristic of epidermal cells. Although these club cell filaments may be found in mucous smears and in mucus removed from the body surface, the club cells have not been found at the epidermal surface or been observed to secrete filamentous material directly into the mucous layer.

At the electron microscope level, the cells do not show the characteristic, well developed, rough-surfaced endoplasmic reticulum or Golgi apparatus usually associated with cells secreting either protein or protein-polysaccharide complexes. Their cytoplasm does, however, contain numerous spherical or U-shaped bodies (Pls 9A,C) which appear in some sections as helically coiled filaments 25 - 28 nm (Pl. 9B). Aggregates of these filaments seen in some sections where the cells have ruptured (Pl. 10B) appear identical to filamentous material observed in mucous smears (Pl. 24B) and this is presumably the filamentous material which is visible to the naked eye. In addition to the coiled filaments, the club cells possess a large central membrane-bound

vacuole, the contents of which become increasingly granular as the cell reaches the upper layers of the epidermis (Pls 2A,B, 10A).

Club cells may occasionally be seen to rupture and release their contents beneath the epidermal surface (Pls 11A,B). Several club cells usually appear to be involved and their contents spread horizontally through the epidermis. Dark-staining filaments seen in thick Epoxy sections (Pl. 11A,B) appear under the electron microscope as aggregates of finer filaments. Sections of filaments are also apparent which would suggest that the filaments do not necessarily align themselves in any particular direction. The release of club cell contents in this way has not been previously reported but it may account for the extreme slipperiness of the eel, when handled, as the whole upper part of the epidermis may be lost. This action may be of importance in a burrowing animal to prevent the complete loss of the epidermis and also to provide an emergency lubrication to prevent damage to deeper layers. Evidence to support this claim is found with the increased numbers of club cells in the lateral regions of the fish (Table 5): the fish flexes laterally when burrowing. Similar club cells are found in a loach, Misguranus anguillicaudatus (Satô and Sannohe, 1968) but are not found in the lungfish, Protopterus annectens (Kitzan and Sweeny, 1968) or in the plaice, P. platessa (Roberts et al., 1972) both of which burrow.

In some teleosts, the club cells release a substance that can initiate a 'fright' reaction in other fish (Pfeiffer, 1963a, b). In lampreys the club cells contain a substance that is found to be distasteful to other fish (Pfeiffer and Pletcher, 1964). The fright reaction has not been shown in adult Anguillidae but Leonard and Summers (1976) suggest either function could be of survival value to vulnerable glass

and early pigmented elver stages especially when they occur in vast numbers during upstream migration.

Histochemically the club cells show strong reactions to protein (Table 6). A very weak positive reaction with the PAS technique may be seen and this led Asakawa (1970) to suggest the club cell contents of A. japonica contain sulphated mucopolysaccharides. There is, however, no evidence from the present study or from the study by Yamada and Yokote (1975) to support this claim. Although the club cells contain helically coiled filaments (Pl. 9B), these did not stain for either elastin or reticulin fibres or for amyloid material. Mucous smears prepared with the aid or compressed air (which may cause a slight disruption of the epidermis) show the presence of free club cells which appear to possess a spirally extended basal region (Pl. 24C).

The 'Cuticle' or Extracellular Layer. There is still controversy concerning the presence of a 'cuticle' completely overlying the epidermis. Many authors avoid the use of the term 'cuticle' and refer to the covering as a membrane, a 'fuzz' or a filamentous coat depending on its appearance, which is seen to vary according to fixation, embedding and staining techniques employed. Whitear (1970) suggests these are all different aspects of the same phenomenon. She further suggests that this layer is composed of material secreted from the epidermal cells only and is normally present over the epidermis with the secretion from the mucous cells providing an emergency lubrication only. The mucous cell secretion may displace the cuticle on discharge.
In an extensive review of the extracellular coat Luft (1976) describes a layer comprising glycoprotein and glycolipid (approx. 10 - 20 nm thick) surrounding all animal cells. He further suggests that the appearance of this coat is dependant upon the fixation, embedding and

staining procedures employed. Added confusion occurs when a mucous layer is also present. Conventional fixation and embedding techniques usually result in the loss of the mucous layer and, on occasions, the extracellular coat as described by Luft (1976). This may explain why their presence on fish epidermis is often overlooked. The 'fuzz' appearence is likely to be an artefact of fixation (Ito, 1974) and Johnson and Bronk (1977) examining the ultrastructure of rat intestine by cryoultramicrotomy, without the use of fixatives, found this layer to be far more substantial than that described for the cat intestine by Ito (1965), who employed conventional fixation and embedding techniques.

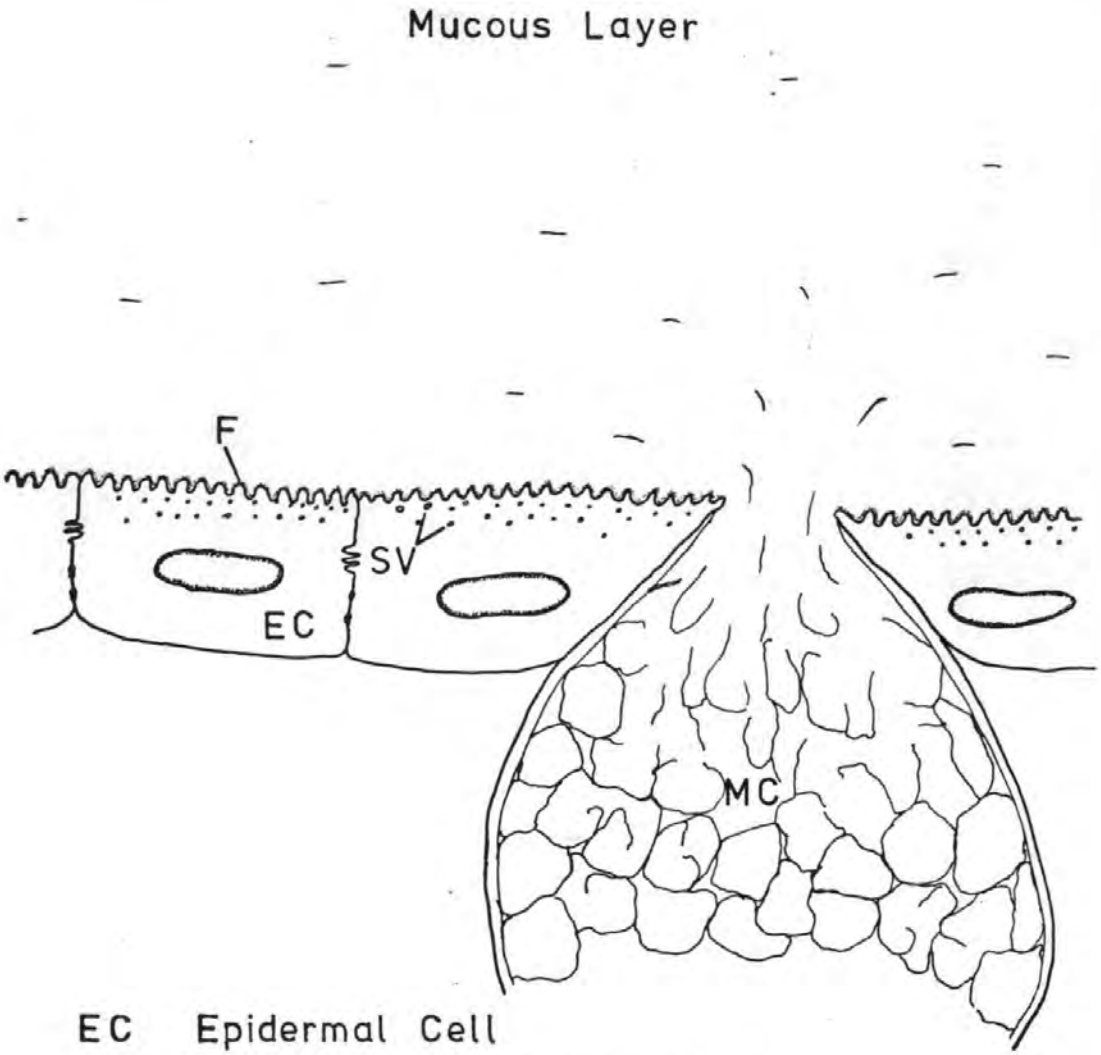
In the eel, in common with other fish, the extracellular layer is frequently lost during preparation for the electron microscope. When and where it preserved (Pl. 7B) a fine layer (approx 20 nm thick) may be seen closely associated with the microridges of the epidermal cells. This layer is similar to the extracellular coat described by Luft (1976) and may be derived from the secretory vesicles of the epidermal cells. The outermost layer (Pl. 7B) appears as filamentous material that may stretch over the microridges and is thought to be derived from the mucous cells. Further evidence to support this claim is seen histologically (Pl. 1B) where the outermost layer shows identical staining to the mucous cells. The measurement of the mucous layer of the pike was attempted by Fedak et al. (1973), who found the layer to be approximately 40 μm thick. There was no account made, however, of the considerable stress that was likely to occur to the fish resulting from the experimental procedure which involved the restriction of the fish and the gradual heating of its surrounding water. The dissolved oxygen, released from solution, became entrapped on the boundary of the mucous layer and was measured optically. No direct

measurements of the mucous layer of the eel were attempted although measurements made from histological preparations (e.g. Pl. 1B) would indicate a thickness similar to that of the pike. A model of the extracellular layer which includes the mucous layer and the extracellular coat or 'fuzz' is shown diagrammatically (Fig. 20).

The role of the secretory vesicles of the epidermal cells is uncertain. These cells usually contain a mucosubstance (although not all are stained by the PTA technique for mucosubstances) and may form the 10-20 nm 'fuzz' or extracellular coat, or may contribute directly to the mucous layer. Cholesterol and its esters, present in surface epidermal cells (Table 10) are known to be important in both the structure and formation of cell membranes (Levin, 1969; Keele and Neil, 1971). It is these surface membranes that are important for the attachment of the glycoproteins and glycolipids of the cell surface coat (Luft, 1976).

Although Whitear (1970), believes that the mucous cells produce a secretion for emergency lubrication only, surface staining reveals that a considerable number of mucous cells (approx. 300 - 400 per sq. mm) (Table 4) are open at the epidermal surface of apparently unstressed fish. This observation, together with histological evidence to show continuation of the mucous layer with the contents of the mucous cells (Pls 1B, 4B), suggests a more permanent presence of the mucous layer which is presumably replaced as it is lost from the epidermal surface. Further evidence to support the claim that the mucous cell secretion is not simply an emergency lubrication but is produced more regularly has been shown by experiments involving the radioactive labelling of precursors of mucous cell components and then measuring the labelled surface mucus (Pickering, 1974). The increase in protein and carbohydrate levels in water (Tables 15 - 18) would also indicate that the surface mucous layer is continually dissolving from the fish.

Fig. 20 DIAGRAM OF EXTRA-CELLULAR LAYERS



EC Epidermal Cell
F 'Fuzz' or Extra-cellular coat
MC Mucous Cell
SV Secretory Vesicles

1μm

Other Cell Types. Lymphocytes frequently occur in the epidermis of the eel (Pl. 22A). Generally, they are found in the lower regions of the epidermis, usually between the apices of the cells in the basal layer. Bullock and Roberts (1975) suggest that immunoglobulins of the IgM class (detected in the surface mucus by Fletcher and Grant , 1969 and Bradshaw et al., 1971) may be derived by local production from the lymphocytes of the epidermis.

Also observed in the lower regions of the epidermis, occasionally in large numbers, are small granular cells (Pls 21A,B,C) which appear similar to neutrophil cells of mammals (Rhodin, 1972) with their lobed nucleus, tubular smooth endoplasmic reticulum and round or ovoid granules. However, Percy (1970) did not find neutrophils in goldfish epidermis and neither did Leonard and Summer (1976) in the epidermis of the American eel. Similarly, Henrickson and Matolsty (1968c) did not report the presence of neutrophils in their ultrastructural study of the epidermis of Anguillidae. Small rounded cells (which may be neutrophils) were seen, however, in large numbers between cells of the basal layer at the light microscope level only in P. platessa, P. flesus and Murostomus kitt (Roberts, Young and Milne, 1972). In mammals, granular neutrophils contain a high concentration of lysozyme (Hansen, Karle and Anderson, 1974) and the presence of lysozyme has been detected both in the external mucus and in the intercellular spaces of the epidermis of P. platessa (Fletcher and White, 1973; Murray and Fletcher, 1976). It is suggested that these neutrophil-like cells may be the source of lysozyme in the epidermis and mucus (see also the later discussion on lysozyme).

Macrophage cells may also be found in the epidermis of the eel (Pl. 22B). They are characteristically larger than other blood cells

and contain both membranous whorls and larger numbers of membrane-bound vesicles which have been interpreted as secondary lysosomes. Macrophages may also be packed with melanin granules (Pl. 23B). These melanomacrophages have been observed by Leonard and Summers (1976), and Roberts (1975) presented evidence of their migration through the epidermis and of their subsequent release at the epidermal surface in the mucous layer. In common with the present study, Roberts (1975) also observed macrophages crossing the basement membrane. It would appear likely, therefore, that the melanin granules originate in the pigmented layers of the dermis.

Unmyelinated nerve fibres are often found in the intercellular regions of the eel epidermis (Pl. 23A). Henrickson and Matolsty (1968a) reported a close association between nerve fibres and the epidermal cells in which the cells were occasionally seen to envelope the nerve fibre. Myelinated nerve fibres are also found in the eel epidermis (Pl. 23A), an observation which is not in agreement with the findings of Whitear (1971) who observed that myelin sheaths were lost as the nerve fibres entered the epidermis. Cells resembling the Merkel cells of Ictalurus melas and Phoxinus phoxinus (Lane and Whitear, 1977) were not observed in the eel epidermis.

Blood capillaries (Pl. 23A) were also seen in the eel epidermis. These are not generally found in the epidermis of teleosts but have previously been reported in the eel (Jakubowski, 1960) where they form loop-like vessels supplying sense organs in the skin. Recently Nonnotte and Nonnotte (1978, pers comm.) have investigated vascularisation of the eel integument. They reported an increase in vascularisation in the skin of eels adapted to sea water when compared to that of fresh-water eels. This finding further substantiates their claim of an

increased role of the skin as an accessary respiratory surface in sea water adapted eels.

Cell Distribution

The epidermis varies in both thickness and in the proportion of the specific cell types at different regions of the body (Pl. 1A,B). The thickness of the epidermis (Table 6) is greater than that reported by Jakubowski (1960). Yamada and Yokote (1975), however, found the thickness of the epidermis of A. japonica increases during the winter months, and they suggest this might provide added protection during this period, when the eel frequently becomes quiescent whilst buried in mud. They also reported that the relative numbers of club and mucous cells remains constant. The distribution of mucous cells as determined from sections and by the surface staining method are consistent with the observations of Pickering (1974) who found that the mucous cells of the head region of both S. trutta and Salvelinus alpinus were more numerous than those of the tail region. He postulated that as the fish swims, the mucus might move posteriorly, and hence the high concentration of anterior mucous cells would maintain an even distribution of mucus over the body surface. In the eel, significantly fewer mucous cells are found in the lateral regions when compared to the dorsal and ventral areas (Table 4, 5). The lateral areas moreover contain higher numbers of club cells. In view of the possibility that the club cells are held under tension (Pl. 24C), this may act as a buffer to reduce mechanical damage during burrowing movements.

The epidermal mucus of fish belongs to the class of protein-polysaccharide complexes known as mucous glycoproteins (see Appendix I). Other epithelial glycoproteins with similar properties are found in the cervical canal, respiratory tract, gastric and intestinal systems and also in the saliva of higher vertebrates (Clamp, 1977).

Spectroscopic analyses are largely confirmatory to other experimental procedures. On this basis, the low extinction coefficient for eel mucus at 280 nm (Fig. 3) is probably due to the paucity of aromatic amino acids (tyrosine, tryptophan, phenylalanine) whereas the peaks of absorbance at lower wavelengths are probably due to carbohydrates and are characteristic of mucous glycoproteins in general (Masson, 1973).

Infra-red spectra form a characteristic series of bands (Fig. 4) corresponding to the functional groups present in the compound. Thus, in eel mucus, the presence of primary and secondary amide and also alkene groups are presumably from the protein fraction whilst carboxyl, methyl, alcohol and sulphate groups are probably derived from the carbohydrate portion of the molecule(s) (Scheinmann, 1970). Infra-red spectra of other samples of fish mucus are, at present, unavailable for comparison but that of the eel indicates marked differences in the proportions of functional groups when compared to the mucus secreted by the snail, Otella lactea L. (Pancake and Karnovsky, 1970).

The analysis of the soluble mucous fraction of eel mucus is biochemically consistent with the findings from a previous study of fish mucus (Wessler and Werner, 1957). Both studies indicate the presence of low levels of both fucose and uronic acids. By contrast, however, the mucus of P. platessa contains relatively little sialic acid but is rich in fucose and also in hexosamine (Wessler and Werner, 1957; Clamp, 1977).

The amino acid composition of fish mucus does not vary markedly between species. Most samples are rich in aspartic acid, leucine and glutamic acid but contain relatively low levels of tryptophan, tyrosine, histidine, cysteine and methionine (Enomoto et al., 1961; Chaikovskaya, 1970; Harris and Hunt, 1973).

Eel mucus separated on Sepharose 4B (Fig. 5) in common with that of char (Wold and Selset, 1977), revealed two major components, both containing protein and carbohydrate material. Sialic acid was only found in the first component of the eel mucus. Wold and Selset (1977) reported that the first fraction of char mucus contained more carbohydrate, especially hexosamine, fucose and sialic acid, whereas the second fraction contained more protein. The amino acid protions of both proteins were characteristic of fish mucus in general. In the eel a number of minor fractions that were not observed in the separated char mucus were also apparent (Fig. 5). These fractions appeared to be consistent in both position and concentration in five separations of different mucus samples. This suggests that the minor fractions are an integral part of the mucus and not simply breakdown products of some of the components.

Five fractions of mucus may be separated on D.E.52 ion-exchange cellulose (Fig. 6) and five bands are also seen for mucus separated on polyacrylamide gel (Fig. 7). It is interesting that one of these bands on polyacrylamide gel stains for protein with Coomassie blue, a dye which will not stain the mucous cell contents in wax sections. It does however, stain the membrane of the mucin 'packets' and is thus in agreement with Lewis (1976) who suggests this membrane may be a cross-linking agent of mucous glycoprotein molecules. Five fractions were also separated electrophoretically from mucus of A. japonica (Asakawa,

1972). Asakawa (1972, 1973, 1976) also separated and characterized the sialic acid containing mucous glycoprotein of A. japonica which he found to have a molecular weight of 7×10^5 daltons and contained N-acetylgalactosamine, sialic acid and hexose (galactose and mannose) in a ratio 1:1.01:0.14. He also found that the carbohydrate portion was attacked by an O-glycosidic linkage, presumably to serine and threonine of the peptide chain.

Fish mucus thus appears to comprise several chemical species although the relative proportions, biochemical and structural nature of each, in common with most other mucous glycoproteins, have not yet been elucidated.

It has been established that eel mucus shows properties of shear thinning and elastic recoil (Figs 8, 9). These phenomena are also observed in gastric, cervical and respiratory tract mucus of mammals and also in many long chain polymers e.g. hydroxycellulose and polyacrylamide (Fig. 8B). The ability of mucus to remain on a fish has been suggested (Lewis, 1976) to be largely due to its viscosity. Fish mucus may also reduce the turbulence of water flow over the body during swimming movements (Rosen and Cornford, 1971). As mucus dissolves from the fish, a laminar flow of water over the fish is produced which reduces turbulence. This reduces the drag and permits efficient swimming with low expenditure of energy. Further drag reduction occurs if inert long chain polymers are added to water. Experiments indicate that, in this solution, the fish are thus able to swim up to twice as fast as their normal swimming speeds (Breder, 1976). The property of shear thinning could explain the observation of Rosen and Cornford (1971) described as the 'reluctance' of mucus to dissolve in water. The mucus will dissolve, however, when stirred (i.e. a shearing force is applied). Shear thinning of the mucus would suggest that as

the rate of shear increases (as would occur during swimming and also in strong currents) the mucus would become thinner in consistency and hence more likely to be dissolved from the body. An analogous situation occurs when a strong sugar syrup is added to cold water; it dissolves readily only when a shearing force e.g. stirring is applied. On the other hand, a fish stationary in still water (i.e. no shear stress) would have mucus thicker in consistency and therefore likely to be retained on the surface. Thus it would appear that mucus dissolves from the fish only when required to aid swimming, however, there is presumably a small fraction of mucus which dissolves when there is no shear stress and the rate of this dissolution is likely to be proportional to the temperature, viscosity being indirectly proportional to temperature. An approximate conversion from shear rate, measured on the rheogonimeter, to swimming velocity appears in Appendix III. This indicates that the reduction in viscosity seen with increasing shear rate is likely to be of use under normal environmental swimming speeds.

The factors which determine the viscosity and also the molecular structure of mucus in general are largely unknown but Gottschalk (1972) suggests the molecular shape of certain glycoproteins is due to electrostatic repulsion by anionic groups. Thus, sialic acid and sulphate groups of fish mucus which are negatively charged at environmental pH would cause rigidity of the molecule by electrostatic repulsion. Certainly sialic acids are important in maintaining viscosity. When they are removed with neuraminidase the viscosity of the mucus is greatly reduced (Fig. 13). The addition of divalent ions Ca^{++} and Mg^{++} (Fig. 10, 11) produces an increase in the viscosity of eel mucus. This has also been reported for pig gastric mucus (Deman, Mareel and Bruyneel, 1973) and may be due to binding of the ions to carboxyl groups of

adjacent chains thus increasing cross-linkage between molecules (the more cross-linkage the higher the viscosity). Monovalent ions K^+ and Na^+ (which have only one binding site) are thus unable to increase cross-linkage and hence have no effect on viscosity (Fig. 10).

Divalent ions Pb^{++} and Cd^{++} did not produce an increase in viscosity (cadmium in fact produced a decrease) which may be related to the larger size of these metallic ions. The cadmium ion may be similar to copper in its ability to disrupt disulphide bonds and thus reduce the viscosity of mucus (Oster, 1971). Irreversible structural changes in electron spin resonance were also observed by the addition of low concentrations of lead and mercuric ions to trout mucus and it has been suggested (Varanasi et al., 1975) that possible changes in rheological properties occur with these ions. Coombs, Fletcher and White (1971) showed that the mucus of P. platessa chelated both copper and zinc ions and the mucus might, therefore, act as an additional protective mechanism against metal pollutants.

The reduction in the viscosity of mucus seen with the addition of a solution of DDT (and also with Cd^{++}) may be important because any weakening in the defensive barrier of mucus may allow colonisation by potentially pathogenic organisms. Furthermore, pathogenic organisms may incorporate a lytic action to breakdown the mucous barrier so that colonisation can occur. For example, bacteria of the Vibrio species are known to contain neuraminidase, this enzyme completely destroys the shear thinning behaviour of mucus, as mentioned above. It was noted that during a confirmed outbreak of Vibrio anguillarum, fish infected with the disease had a sparse mucous layer that was extremely thin in consistency.

No apparent change was seen in the viscosity of eel mucus after treatment with hyaluronidase or glucuronidase (Figs 13, 14) indicating that uronic acids are relatively unimportant to the mucus structure (similar to other mucous glycoproteins) or that the molecule is resistant to enzymic action. The viscosity of eel mucus was reduced by pronase (Fig. 13), a non-specific proteolytic enzyme, indicating either lysis of the glycoprotein molecule or lysis of a cross-linking protein molecule.

The possibility of a separate molecule providing the cross-linkage of mucous glycoproteins has been suggested by Gibbons (1969) although later (Gibbons and Selwood, 1973) it was suggested that mucous structure was maintained by hydrogen, electrostatic and hydrophobic bonds. Allen et al., (1973) suggest that pig gastric mucus was composed of mucoprotein sub-units (Mol. Wt. 30,000) linked by disulphide bonds. Disulphide bridges are important in the maintenance of structure in eel mucus. When they are removed by the action of mercaptoethanol (Fig. 12) the viscosity is drastically reduced, a property also characteristic of other mucous glycoproteins (Iacobelli, Garcea and Angelouis, 1971; Davis, Scobie and Inglis, 1975). It is interesting to note that mucous glycoproteins are generally inexplicably low in cysteine, the amino acid involved in disulphide bridges. It may be, however, that only relatively few bonds are required to maintain the molecular structure.

In contrast, Deman et al., (1973) believe the cross-linkage of mucus to be entirely due to physical entanglement of the molecular network (mechanical cross-linkage). Other authors (Schmacher and Pearl, 1968; Elstein, 1970) have speculated on the possible involvement of soluble proteins, such as plasma proteins and lysozyme as cross-linkage agents. Lewis (1976) proposed the linkage component to be the mucous

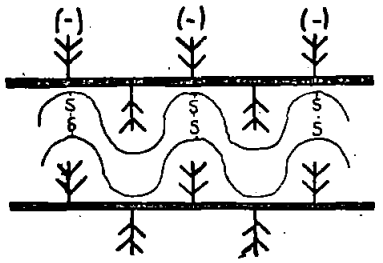
globule membrane which contains both neutral lipid and phospholipid components and can be removed from the solution by centrifugation at 20,000 g. Centrifugation of eel mucus (24,000 g for 40 min) did not, however, significantly reduce the viscosity of the solution (Fig. 8).

Models illustrating the possible molecular structure of fish mucus are shown in Fig. 21. In A, a cross-linking agent contains intramolecular disulphide bridges and the protein is bound by hydrogen or electrostatic bonds to the main glycoproteins. Although this is consistent with the low levels of cysteine in mucous glycoproteins, the presence of a cross-linking agent is, as yet unconfirmed. Model B contains a cross-linking protein which is linked by disulphide bridges to the mucous glycoproteins. This model is favoured by Clamp (1977) and in view of the two major components seen in eel mucus (separated on Sepharose 4B) and also in char mucus (Wold and Selset, 1977), both of which contain glycoprotein, the cross-linking agent (if present in fish mucus) may well be a glycoprotein. Model C contains intermolecular disulphide bonds that link the glycoproteins directly, without a cross-linking agent. This model is favoured by Meyer (1976) because the presence of a cross-linking agent has, at present, not been established.

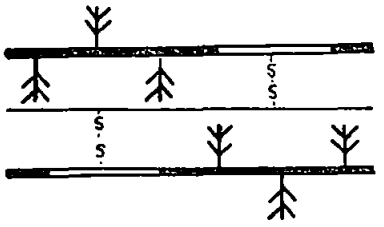
The surface mucus varies in physical consistency between different species of fish. Generally, those species that swim slowly have a more viscous mucus than faster swimmers. In addition the mucous coating appears to be thicker on 'naked' or sparsely scaled fish compared to those which are heavily scaled (Van Oosten, 1957; Bullock and Roberts, 1974). The mucus of fast-swimming fishes is efficient in reducing the fluid friction between thebody and water, The mucus from slow-swimming fish, however, does not generally reduce the fluid friction as effectively (Rosen and Cornford, 1971). As the retention of mucus on the fish is a

Fig.21 MODELS FOR THE MOLECULAR STRUCTURE OF MUCUS

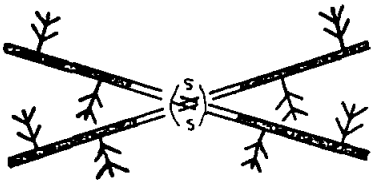
A



B



C



CARBOHYDRATE SIDE CHAIN

s-s DISULPHIDE BONDS

PROTEIN BACKBONE

function of its viscosity (Lewis, 1976), 'thick' (i.e. viscose) mucus is more likely to remain on a slower-swimming fish than 'thin' mucus, which would dissolve more readily and thus need replacement if the fish were to continuously maintain its mucous layer. A 'thick' mucus secretion would, however, be unnecessary in fast-swimming fish as the mucus would need to be constantly dissolving from the fish (to enable efficient swimming). A 'thick' mucus could actually be an impediment as it would be less likely to dissolve from the fish surface when it is needed for locomotory purposes.

The difference between 'thick' and 'thin' mucus has been reported to be the result of amino acid composition of the protein portion of the glycoprotein molecules (Chaikovskaya, 1954; Uskova and Chaikovskaya, 1960; Uskova et al., 1970). Notable differences are observed, however, between carbohydrate fractions of mucus e.g. eel mucus (which is thick in consistency) contains high levels of sialic acid and is low in fucose (Table 11). By contrast the 'thinner' mucus of plaice is rich in fucose and low in sialic acid (Clamp, 1977). Lewis (1976) believes, however, the difference in the consistency of mucus is due to interaction between phospholipid and glycoprotein. He observed a high proportion of phospholipid in high viscosity mucus and suggested this was due to cross-linkage of mucous glycoproteins by the mucous globule membranes. Mucus of low viscosity contained relatively lower quantities of phospholipid.

PROTECTIVE NATURE OF MUCUS

In addition to the mechanical protection afforded by the epidermis and mucous layer to prevent injury or colonisation by bacteria fungi and other organisms (Pickering, 1974) mentioned above, other mechanisms of defence are apparent.

Lysozyme is an enzyme capable of lysing the cell walls of Gram +ve bacteria and has been demonstrated in the mucus of P. platessa (Fletcher and White, 1973). Murray and Fletcher (1975) found lysozyme to be present in the intercellular spaces, however, it was not associated with the mucous cells but it was found in the surface mucous layer. They also noted a dramatic increase in lysozyme levels when the fish were under stressful conditions. Unfortunately they (Murray and Fletcher) were unable to determine whether the lysozyme originated in the serum or was produced locally in the epidermis. In the unstressed eel, lysozyme was found to be associated only with the precipitated fraction from whole mucus after ultrasonication. This fraction may contain, in addition to mucus, cells and other cellular debris that is released from the epidermis by the possible slight disruptive action of the compressed air method used to collect the mucous sample. Granular neutrophils (Pls 21A,B,C) may be released in this way and in mammals the neutrophils are known to contain high levels of lysozyme (Hansen et al., 1974). If the granular neutrophils are the source of lysozyme in the fish epidermis their small size (5 μm) might explain the failure of Murray and Fletcher (1975) to pin-point the exact source of lysozyme in the epidermis.

Lysozyme is also known to be a highly positively charged molecule (Keuttner, Eisenstein and Sargente, 1974). Thus electrostatic bonding with negatively charged sulphate and sialic acid groups would favour

the retention of lysozyme within the mucous layer. The possible electrostatic linkage with glycoprotein molecules has also led to the implication of lysozyme forming cross-linking bridges between molecules to maintain the mucous structure (Elstein, 1970).

Further confirmation of the presence of lysozyme in fish mucus has been made by Rodsaether and Rao (1977) who also discovered the presence of an enzyme capable of lysing Gram -ve bacteria. This enzyme is proteolytic, has an optimum pH 8.0, M.Wt. 17,000 daltons and has two isoenzymes. It is also inhibited by a factor present in human, rabbit and fish sera.

The appearance of antibodies in eel mucus after injection of Salmonella H antigen ^{a} was not observed and this may be due to the high dilution of the mucus even after some concentration. The repeated sampling of mucus may have also presented an addition diluting effect. Antibodies of the IgM class have been detected in fish mucus (Fletcher and Grant, 1969; Bradshaw et al., 1971) although the exact source of these antibodies is, at present, unknown. Roberts et al., (1970) suggest that the lymphocytes of the epidermis are the source of immunoglobulins. Smith (1977) and Mawdesley-Thomas (1975) also favour the suggestion that immunoglobulins are produced locally in the epidermis. The presence of serum proteins found in the mucus (O'Rourke, 1960, 1961a) may suggest that the immunoglobulins of mucus are derived from the serum. O'Rourke (1961b) further suggests that some fish parasites could recognise and be attracted to their correct host by chemoreceptors sensitive to mucus.

The mucus has also the ability to chelate copper and zinc ions (Coombs, Fletcher and White, 1969) although they could find no significant complexing with mercuric, cadmium and lead ions. The accumulation of

lead and mercury was, however, observed by Varansi et al., (1975) and has also been reported by McKone, Young, Bucke and Lisk (1971) and Chow (1974). Precipitation of gill mucus by heavy metal toxins may cause asphyxia (Jones, 1938, 1962). Fish mucus may also have a precipitating action on fine suspended particulate matter e.g. mud (Hora, 1933).

THE DYNAMICS OF MUCOUS SECRETION

The dynamics of mucous secretion are, at present, uncertain. Whitear (1970), from purely observational studies at the electron microscope level, believes the goblet cells of the epidermis only secrete their contents as an emergency response to stress. She suggests the mucus normally present on the skin surface is that of the 'cuticular layer' which is produced by the surface epidermal cells. This layer may be seen overlying the mucous cells and may even be displaced by discharge of the mucous cells.

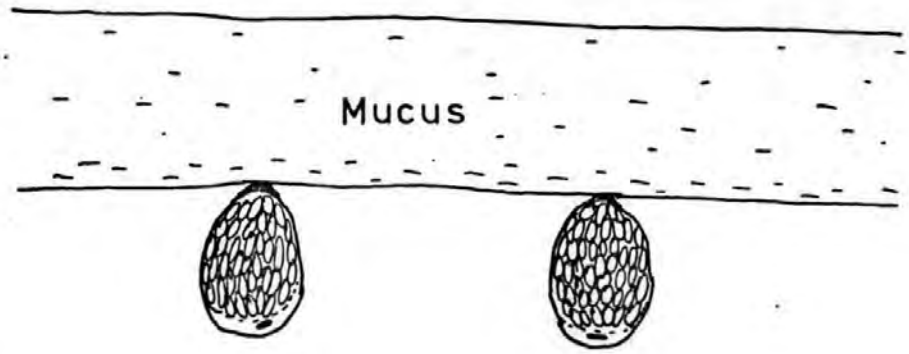
On the other hand, Pickering (1976) who measured radioactively labelled sialic acid appearing at the epidermal surface of brown trout after injection of C^{14} labelled glucose, suggested continual replacement of the mucous layer i.e. a layer covering the body derived mainly from the mucous cells. In a previous study (Pickering, 1974) he showed that the sialic acid content of the epidermis was directly proportional to the numbers of mucous cells present. Further evidence to support his claim is the apparent lack of an increase in mucous cells open at the epidermal surface immediately after a handling stress (Pickering and Macey, 1977).

Autoradiography of eel skin after injection of H^3 galactose revealed incorporation of labelled material initially into the mucous cells of the lower and middle layers of the epidermis (Fig. 18). Maximum labelling of surface mucous cells occurred after 36 h and was maintained at a high level (approx. 60% of cells labelled) throughout the experimental period (4 days). The interpretation of these results is as follows:- initially, incorporation of galactose occurs in mucous cells of both lower and middle regions of the epidermis (maximum labelling in lower epidermis 8 h post injection, 12 h post injection in mid-

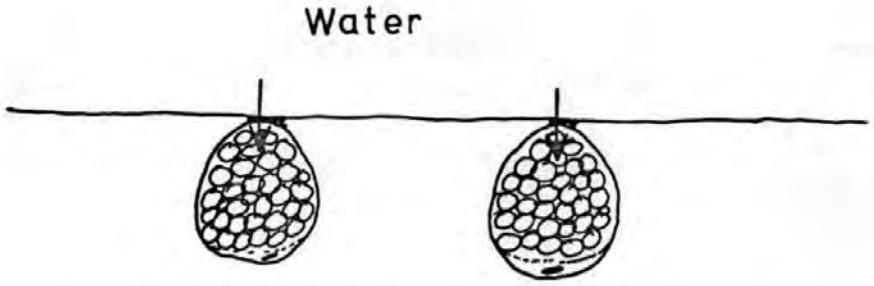
epidermis). Over the next 24 h labelled mucous cells migrate upwards so that a maximum labelling of the upper region of the epidermis is seen after 36 h. Meanwhile the label disappears from mucous cells of the lower epidermis. Cells in the mid-epidermis then migrate to the surface layer to replace those at the surface that have released their contents. The initial high incorporation may be indicative of handling stress during anaesthesia and injection of the fish. Pickering and Macey (1977) noted an increase in the numbers of open surface mucous cells, in the char, approximately one week after a severe handling stress.

It is suggested that the turnover of labelled mucous cells in not necessarily continuous but that release of secretion from the mucous cells occurs when it is required (presumably when the surface mucus has dissolved from the fish). This view is consistent with the rheological properties of shear thinning observed for the mucus (Fig. 8). It is thus proposed that the mucus is retained on the surface of the fish and replaced only when an increase in shear stress 'thins' the mucus and allows some to dissolve from the fish. Thus when there is no removal of surface mucus none is released from the surface mucous cells. This is likely to occur rarely under normal environmental conditions as any movement or current would 'thin' the mucus. Thus the release of mucus is likely to be almost continuous with a number of factors influencing the rate of mucus loss from the fish. This then suggests the possibility of a feedback mechanism to control release of the mucus. This system need not, however, be complex and one possible model based on a simple osmotic mechanism is shown in Fig. 22. For the purposes of this model it is assumed that the mucous cell contains a greater concentration of mucous molecules than the mucous layer. There appears

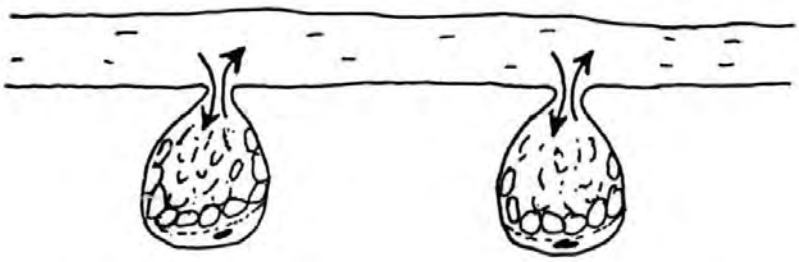
Fig.22 A MODEL FOR MUCUS SECRETION



A High mucus conc. at epidermis surface.



B Mucus removed, water enters cell as osmotic gradient is large.



C Cell ruptures and contents are forced out to replace mucaus layer.

to be no evidence to either endorse or refute this claim but mucous cells do bind certain dye substances very strongly and under the electron microscope the cells do appear to be packed with secretory material. Furthermore, mucus collected from the skin surface is extremely dilute (approx. 99.8% water according to Rosen and Cornford, 1971) and if, as suggested by Fedak et al., (1973), the mucus layer is 40 μm thick then it would appear highly likely that the mucus is synthesised in a concentrated form and then becomes considerably diluted presumably when the cell opens to the surface. In A, an unopened surface mucous cell is seen covered by a mucous layer containing a concentration gradient of mucus (more concentrated at the epidermal surface). If the membrane between the mucous cell and the exterior is semi-permeable, the concentration gradient between the mucous cell contents and surface mucus is small and there is no net flow of solvent i.e. water. If this mucous layer is removed or diluted (Fig. B) the water-proofing effect of the mucus is lost (Negus, 1953), the concentration gradient is increased and there is a net flow of water into the cell. The cell then swells and ruptures (Fig. C) forcing its contents out until the osmotic pressures inside the cell and the exterior are equal. This model is consistent with observations that mucus can be removed from the fish with compressed air until the fish no longer feels slippery i.e. all surface mucus has been removed. If the fish is then returned to water the fish immediately forms a slippery layer which, can in turn, be removed by compressed air.

The findings of the study by Pickering and Macey (1977) can be interpreted with respect to the proposed model. If the mucous layer covering the fish remains intact during the handling stress there will be no increase in the mucous secretion from mucous cells (the mucous

layer was not reported to be stripped from the fish during the handling procedure). They do report an increase in open mucous cells after approximately 7 days and these may represent an increase in the differentiation of mucous cells at the basal cell layer level due to the handling stress.

The effect of chloroform may also be interpreted with respect to the proposed model. Chloroform precipitates mucous molecules and thus structural changes caused by denaturation of the molecules are likely to alter the waterproofing nature of the mucous layer. Access of water to the surface mucous cells is thus likely to cause the release of mucus from the cells (Fig. 22). A further effect may be due to the ability of chloroform to diffuse quickly across membranes, due to its small molecular size, and this may increase the speed of water entry into the mucous cells by codiffusion (Levin, 1969).

The action of atropine may be explained by the following model. Atropine has a cholinergic blocking action which is thought to be in competition with acetylcholine at the receptor sites. It prevents a response to acetylcholine throughout the range of parasympathetically innervated effector organs plus the sympathetically innervated sweat glands (Beckman, 1961). Thus the action of atropine suggests there is a part played by nerve fibres in the secretion of mucus. Mucous cells, however, do not appear to be directly innervated and have no apparent mechanism (e.g. contractile fibres) for forcing out their contents. Nerve fibres are found running between epidermal cells and may be enveloped by them (Whitear, 1970) and thus may be important in the control of mucous secretion as they do show movement and do migrate themselves (Bereiter-Hahn, 1971; Bullock, Marks and Roberts, 1978). If atropine can inhibit the movement of surface epidermal cells by

direct absorption from the surrounding water the mucous cells are unable to reach the epidermal surface and are protected from osmotic change. Epidermal cells beneath the surface layers would not be affected as diffusion of water through eel skin is slow (Bentley, 1962) and thus migration in the lower and mid-epidermal regions would be unaffected.

An attempt to measure the rate of mucous secretion under a number of experimental conditions has been made in this study (Tables 15 - 18), by measuring the rate of entry of dissolved mucus into the surrounding water. It is clear, however, that only when the thickness of the mucous layer remains constant does the rate of dissolution of the mucus (measured in the experiments) equal the rate of mucous secretion. This is assumed for if it were not the case the fish would either over produce mucus and become very thickly covered or be left 'naked' without a mucous layer. Furthermore, if as suggested by Lewis (1976), the retention of the mucus on the body of the fish is a property related to viscosity, then factors which influence the viscosity of mucus are likely to also affect the rate of mucous secretion. Thus, an increase in the shear rate (by increasing swimming or current velocity) and also the temperature will both decrease the viscosity and hence 'thin' the mucus. This would then allow it to dissolve more easily, reducing the thickness of the mucous layer and hence stimulating increased secretion. In addition, increased temperature may also affect behavioural pattens as many fish are more active at higher temperatures and as a result increased swimming activity (increasing shear stress) would increase mucous secretion.

The rates of dissolution of mucus (assumed to be the rate of mucous secretion) (Tables 15 - 18) are consistent with the above proposals. The relationship between the rate of mucous secretion (RMS) with temperature could be a result of a change in viscosity or an increase in

swimming activity. When shear stress is artifically increased, by incorporation of a stirrer into the system, the mucus is 'thinned', the rate of dissolution of mucus and hence the RMS is increased (Table 17). Although addition of atropine, an inhibitor of mucous secretion indicates that there is no change in the rate of dissolution of the mucus from the fish, compared to non-treated control fish, cells are prevented from releasing their contents. This suggests the mucous layer is sufficiently thick enough to allow mucus to dissolve at a rate similar to that of control fish, over the experimental period.

There is no observed significant change in RMS on transfer of the fish from fresh water to full strength sea water although physiological stress is apparent as an immediate increase in opercular rate is noticeable. This would indicate that relatively little mucus is derived from the gills compared to that from the epidermal surface (if this were not the case an increase in RMS is likely to occur as the mucus would be 'thinned' by increased shear stress caused by the increase in opercular rate). With reference to the proposed model and the rheological properties of mucus an increase in dissolved mucus would not be expected on transfer to sea water. Calcium and magnesium ions present in the sea water are likely to increase the viscosity (Figs 10, 11) and thus increase the retention of mucus on to the fish (Lewis, 1976). Similarly the RMS of eels adapted slowly to sea water shows no significant change compared to the control (a small decrease in the rate of secretion was noticed but this was not statistically significant). It is interesting to compare these findings with the increase in cellular turnover of gill tissue of salmonids after adaptation to sea water (Conte and Lin, 1967) and also the increase in the NANA content of the eel epidermis noticed after the progressive transfer of the fish from fresh to sea water (Olivereau and Lemoine, 1972).

SUMMARY AND CONCLUSIONS

1) The mucous layer of the eel is derived mainly from the mucous cells of the epidermis although the epidermal cells may contribute to the secretion. The other major cell type, the club cells, do not normally add to the mucous layer although they may release, under certain circumstances, a filamentous material. Lymphocytes and neutrophils also occur within the epidermis and may be present in relatively large numbers especially in the lower levels of the epidermis. These are considered to be the most likely origin of immunoglobulins (unconfirmed in the eel) and lysozyme found in the mucus.

2) The mucous cells contain both sulphated and sialylated glycoprotein material and there is evidence that different molecules may be produced within the same cell. The mucous cells occur in larger numbers in the anterior regions compared to the posterior regions. The dorsal and ventral areas of the body contain more mucous cells than the corresponding lateral area. Information on the distribution and thickness of the mucous layer is, however, severely limited.

3) The epidermal cells provide an extracellular layer at the epidermal surface which is composed of glycoprotein and lipid material. This layer has previously been termed the 'cuticle' or 'fuzz' and has frequently been confused with the mucous layer produced by the mucous cells. On continual removal of mucus from the surface the epidermal cells may transform into a cell type similar but ultrastructurally distinct from a mucous cell. Further study of this phenomenon is necessary to pinpoint the precise nature of the stimulus needed for transformation.

4) The club cells contain coiled filaments not generally released into the mucous layer. They may, however, be released beneath the epidermal surface allowing the upper part of the epidermis to be lost. This feature together with the greater numbers of club cells in the lateral areas, may indicate a protective mechanism, used for example during 'burrowing'. Further study in this direction may elucidate the nature, chemical composition and function of the coiled filaments, the stimulus needed for there release and also the precise mechanisms involved in their secretion.

5) Mucus is composed of glycoprotein material which comprises a number of distinct fractions when separated without the use of disruptive agents. In common with many other mucous glycoproteins the exact biochemical structure of the carbohydrate and protein portions of individual molecules is yet to be determined, and the type of cross-linkage (if any) between molecules is uncertain.

6) The rheological properties of eel mucus include shear thinning behaviour, an important consideration in the lubricative role of fish mucus. The attachment of mucus to the skin surface may also be explained with respect to its rheological properties. A model has been proposed in an attempt to clarify the kinetics of mucus loss from the epidermis. Addition of a number of substances to mucous samples has pointed to the importance of several molecules in maintaining the integrity of the mucous structure. The use of rheological methods, previously neglected in the study of fish mucus, are likely to be extremely important in the determination of the functional aspects of the mucous molecular structure and also factors which may affect the physical consistency of the mucus. This latter consideration may be of particular relevance in the assessment of disease-producing substances since the mucus provides part of the primary defense mechanism against pathogenic organisms.

7) An attempt has been made to study the kinetics of mucous secretion and some of the factors which affect the secretion. These are discussed with regard to the shear thinning property of mucus. Further study into the action of atropine and its inhibitory effect upon the release of mucus may provide useful information about the mechanisms controlling mucous cell behaviour. The consideration of the rheological properties of mucus is essential to fully understand the process of mucous secretion.

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APPENDIX I

TERMINOLOGY OF MUCOSUBSTANCES

The terms used to describe mucus and mucus components have often led to much confusion in biology; a number of terms being used by both histologists and biochemists with no agreement as to their precise meaning. For example the term mucopolysaccharide is generally used synonymously for mucus or mucosubstance by a histologist, a biochemist, however, would use the term for a specific group of protein-carbohydrate complexes, characterised by carbohydrate chains of alternating sugar residues one of them usually a uronic acid and generally found in connective and structural tissue. Attempts to improve the classifications of mucosubstances by the introduction of new systems has only led to further confusion and the adoption of a universal classification for protein-polysaccharide complexes is well overdue. Recent advances in biochemical sequential analysis of the carbohydrate side chain has brought in a standardised system described below, but there is also a separate classification based on histochemical staining reactions (Spicer, Leppi and Stoward, 1965). An attempt based on that by Clamp (1977) to explain the use of previous terminology is presented below:-

Mucus	The total secretion from mucous membranes.
Mucin	1. Synonymous with mucus.
	2. The nitrogen-containing fraction from whole mucus.
	3. Synonymous with mucous glycoprotein.
	4. Histological term usually a suffix (fucomucin).

Mucoid	1.	Any glycoprotein fraction (not mucus) that is not denatured by the usual techniques.
	2.	Any carbohydrate rich glycoprotein.
	3.	A mucous glycoprotein fraction of high molecular weight.
	4.	Non-purulent sputum.
	5.	Mucus-like material from non-mammalian sources.
Mucopolysaccharide	1.	Synonymous with mucus.
	2.	Synonymous with mucin.
	3.	Synonymous with mucous glycoprotein.
	4.	Glycosaminoglycan component of proteoglycan.
Mucoprotein	1.	Synonymous with mucin.
	2.	Synonymous with mucous glycoprotein.
	3.	Synonymous with glycoprotein.
	4.	Synonymous with proteoglycan.

Classification of Mucosubstances (based on Barrett, 1962)

The terms mucus and mucosubstance have been used for the entire group of molecules that comprise a protein and a carbohydrate moiety. This group may be subdivided into the proteoglycans (mucopolysaccharides or glycosaminoglycans) and the glycoproteins (see Fig. I). Both have a polypeptide backbone to which carbohydrate side chains are attached, although the linkage monosaccharide is different in the two molecules. The other important differences between the carbohydrate fractions of the molecules are summarised in Table I.

Proteoglycans or mucopolysaccharides occur mainly in skeletal and supporting tissue and consist of long largely unbranched polysaccharide chains compossd of a repeating disaccharide structure; one carbohydrate unit usually being D-glucuronic acid. The other component is an amino

sugar which may or may not contain a sulphate group. The monosaccharide components of some of the important proteoglycans are shown in Table III.

Glycoproteins are of two main types, either plasma glycoproteins or mucous glycoproteins. Both types of molecule contain less than 25 monosaccharides in the carbohydrate chain, that of mucous glycoproteins comprising typically eight to ten units. The main differences between plasma and mucous glycoproteins are presented in Table II. The carbohydrate chains of glycoproteins contain fucose, galactose, N-acetyl galactosamine and N-acetyl neuraminic acid. N-acetyl galactosamine is important in mucous glycoprotein, not generally occuring in plasma glycoproteins, whereas, mannose is often present in plasma glycoproteins and rarely found in mucous glycoproteins. N-acetyl neuraminic acid, and also fucose where present, normally occurs terminally. An example of the carbohydrate structure of gastric mucous glycoprotein is presented in Fig II.

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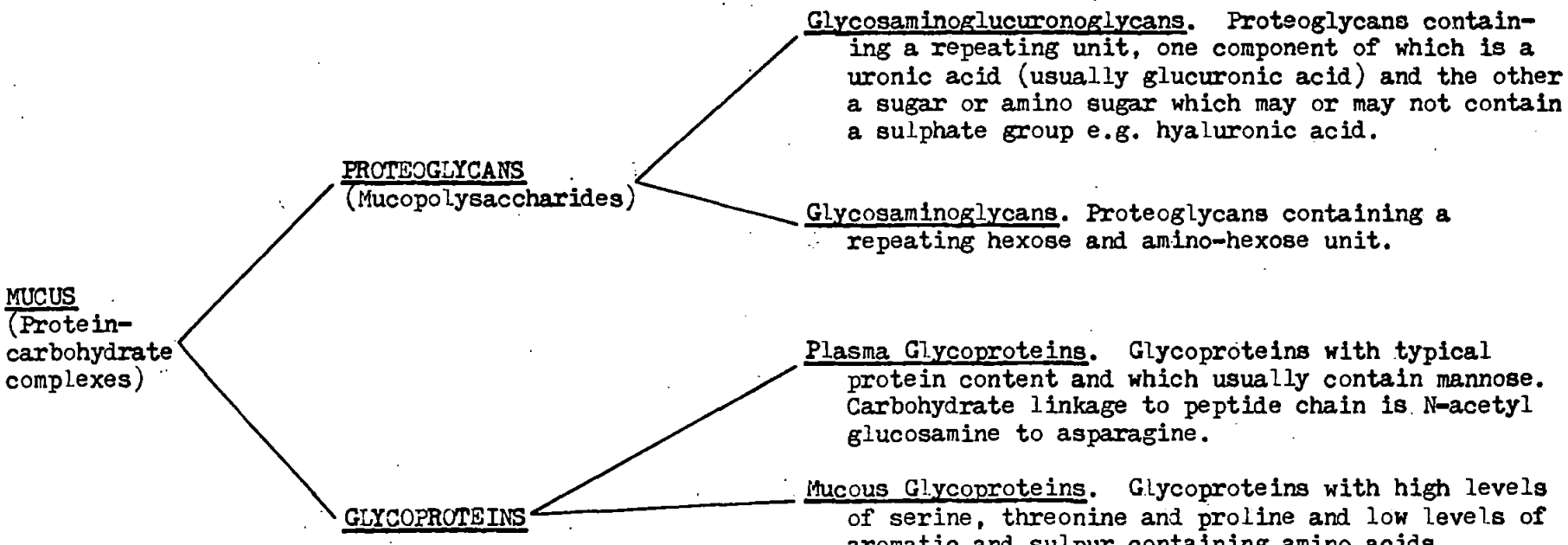
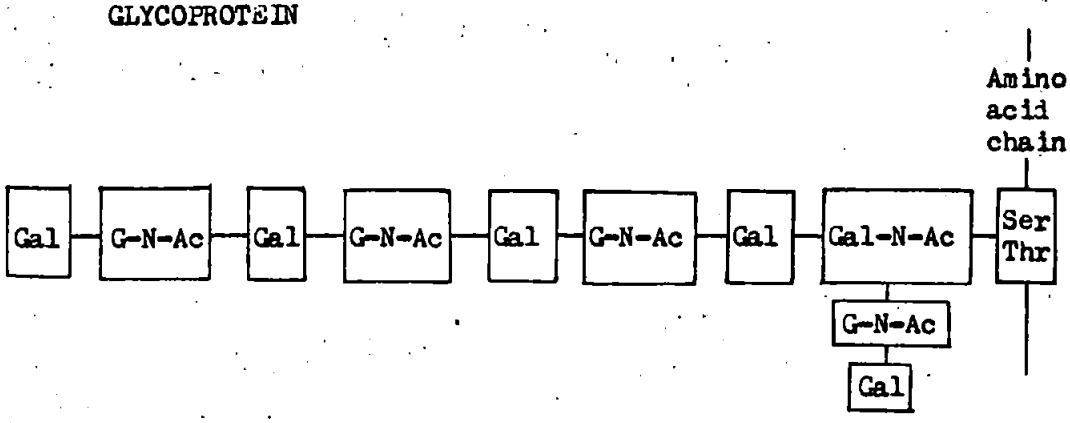


FIG. I. CLASSIFICATION OF MUCOSUBSTANCES

Fig. II AN EXAMPLE OF A CARBOHYDRATE SIDE-CHAIN SEQUENCE OF A MUCOUS GLYCOPROTEIN



Ser	Serine
Thr	Threonine
Gal	Galactose
Gal-N-Ac	N-Acetylgalactosamine
G-N-Ac	N-Acetylglucosamine

In this example of the carbohydrate side chain of a gastric mucous glycoprotein, blood group antigenic determinants A, B, H or Le when present, occur as additional fucose or N-Acetylgalactosamine groups attached to the long chain terminal galactose or N-Acetylglucosamine. Where N-Acetylneuraminic acid (NANA) is present, it is found attached to the long chain terminal galactose.

In sulphated glycoproteins some of the amino sugar residues adjacent to the peptide chain are sulphated.

TABLE I. THE MAJOR CHARACTERISTICS OF THE CARBOHYDRATE COMPONENTS OF PROTEOGLYCANS AND GLYCOPROTEINS

	Proteoglycan	Glycoprotein
Occurrence	Skeletal and supporting tissue	Blood, body secretions
Linkage monosaccharide	Xylose	N-acetyl hexosamine
Size of individual oligosaccharide unit	More than 50 monosaccharides	Less than 25 monosaccharides
Repeating structure	Repeating sequence of disaccharides	No repeating sequence
Shape	Linear, unbranched	Branched
Sialic acid	Absent	Often present
Uronic acid	Present	Absent
Fucose	Absent	Often present
Sulphate	Normally present	Usually absent

TABLE II. DIFFERENCES BETWEEN MUCOUS GLYCOPROTEINS AND PLASMA GLYCOPROTEINS

	Mucous Glycoproteins	Plasma Glycoproteins
Amino acid content	High levels of serine threonine and proline. Low levels of aromatic and sulphur-containing amino acids	Typical protein
Carbohydrate content	More than 50%	Less than 25%
Linkage	O-glycosidic. N-acetyl galactosamine to serine/threonine	N-glycosidic N-acetyl glucosamine to asparagine
Monosaccharides: Fucose, galactose, N-acetyl glucosamine, N-acetyl neuraminic acid	Present	Present
Mannose	Low levels or absent	Present
N-acetyl galactosamine	Present	Low levels or absent
Presence	Mucous secretions e.g. cervical canal respiratory tract, saliva, gastro-intestinal tract	Plasma

TABLE III. PROTEOGLYCANS AND THEIR MONOSACCHARIDE COMPONENTS

Proteoglycan	Monosaccharides
Chitin	2-acetamido-2-deoxy-D-glucose
Hyaluronic acid	D-glucuronic acid, 2-acetamido-2-deoxy-D-glucose
Chondroitin	D-glucuronic acid, 2-acetamido-2-deoxy-D-galactose
Chondroitin Sulphate A	D-glucuronic acid, 2-acetamido-2-deoxy-4-O-sulpho-D-galactose
Chondroitin Sulphate B (dermatin sulphate)	L-iduronic acid, 2-acetamido-2-deoxy-4-O-sulpho-D-galactose
Chondroitin Sulphate C (chondroitin-6-sulphate)	D-glucuronic acid, 2-acetamido-2-deoxy-6-O-sulpho-D-galactose
Heparin	D-glucuronic acid, 2-deoxy-2-sulphoamino-D-glucose
Heparin Sulphate	D-glucuronic acid, 2-deoxy-2-sulphoamino-D-glucose, 2-acetamino-2-deoxy-D-glucose
Teichuronic acid	D-glucuronic acid, 2-acetamido-2-deoxy-D-galactose

APPENDIX II

AN INTRODUCTION TO BIORHEOLOGY

The study of the rheological properties of biological systems is comparatively new and most aspects have been comprehensively reviewed by Scott Blair (1974). An attempt to explain the terms used in the rheological study of mucus is presented below.

Shear Stress (F)

The shearing stress (F) acting through a fluid is defined as the shearing force (P) divided by the Area (A) over which it acts (Fig. I).

$$\text{i.e. } F = \frac{P}{A} \text{ dyn cm}^{-2}$$

Rate of Shear (D)

The mean rate of shear (D) equals the velocity difference (v) between the planes divided by the distance between the planes (h) (Fig. I).

$$\text{i.e. } D = \frac{v}{h} \text{ sec}^{-1}$$

Viscosity (η)

Newton's law of viscosity proposes that the relationship between shearing stress (F) and the rate of shear (D) is one of direct proportionality,

$$F \propto D.$$

For all Newtonian fluids (those which comply to Newton's proposals),

$$F = \eta D$$

where eta (η) represents the viscosity of the fluid and is defined as shearing stress (F) divided by the rate of shear (D). It is measured in dyn sec cm^{-2} which is known as the Poise (P).

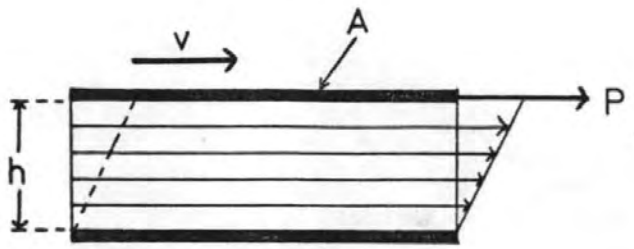
$$\eta = \frac{F}{D}$$

Some viscometers, however, give a direct measurement not of viscosity (η) but of the ratio of the viscosity to the density (ρ) of the fluid. This ratio η/ρ is known as the kinematic viscosity (ν) the unit of which is the stoke. The viscosity of water at normal ambient temperature is .01 stoke or 1 centistoke.

Not all fluids, however, have a simple relationship between shear stress and rate of shear, i.e. some fluids (mucus included) are non-Newtonian in behaviour. These fluids have a more complex (non-linear) relationship between shear stress and rate of shear and examples of these relationships are seen in figure IB. Line A represents a Newtonian fluid and lines B and C represent non-Newtonian behaviour. The relationship between viscosity and rate of shear is seen in figure IIA. The fluid represented by line A is Newtoniam in behaviour and the viscosity remains constant with an increase in the rate of shear. The fluid represented by line B, however, becomes less viscose as the rate of shear increases and this property is known as 'shear thinning'. Line C represents a fluid becoming thicker with increasing rate of shear and this is known as 'shear thickening'.

Fig I

A) SIMPLE SHEAR



Simple shear

B) RELATIONSHIP BETWEEN SHEAR STRESS AND RATE OF SHEAR

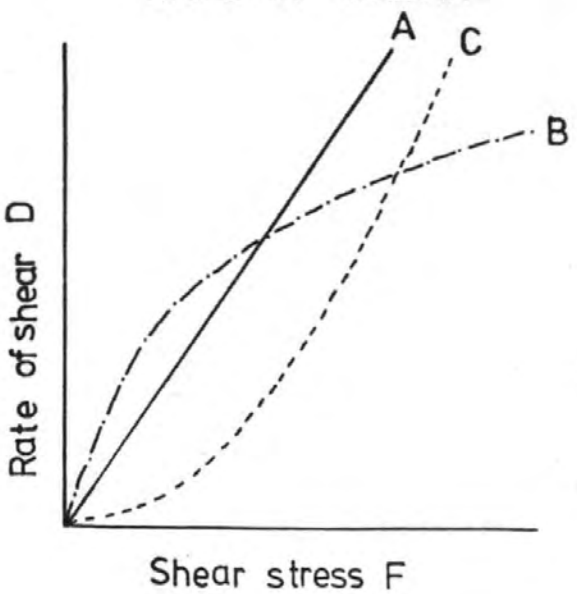
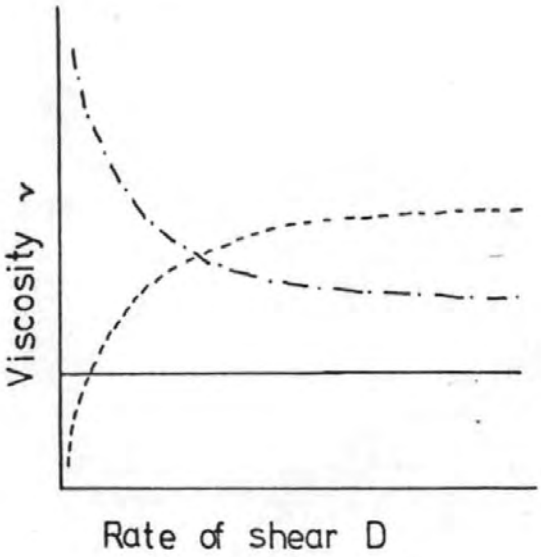
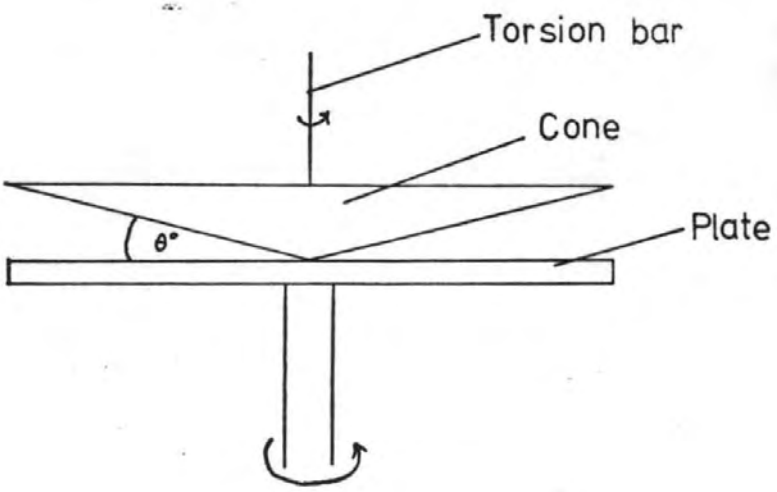


Fig. II

A) RELATIONSHIP BETWEEN VISCOSITY AND RATE OF SHEAR



B) A CONE-PLATE RHEOGONIMETER



Measurement of Viscosity

Fluids that show Newtonian behaviour may be measured in a simple capillary viscometer. There is, however, a variation in the rate of shear (and consequently the shear stress as the viscosity is given as their ratio) between the capillary wall, where it is zero, and the maximum value at the centre of the capillary. It is thus difficult to study the effect of varying shear rate on the viscosity of a fluid. It is for this reason that a cone plate viscometer is used for viscosity measurement of non-Newtonian fluids. The sample is placed between two plates (Fig. IIB). The lower plate is driven by an electric motor via a gearbox and the upper cone-plate attached to a transducer for measurement electrically. In this apparatus the rate of shear is constant throughout the sample at any given speed at which the lower plate is driven and hence study on varying the shear rate is simplified. The Weisenberg rheogonimeter is a machine of this type and was used for all rheological measurements made in this study.

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APPENDIX III

A COMPARISON OF SHEAR RATE WITH SWIMMING SPEED

The Weissenberg rheogonimeter measures the viscosity of a fluid at a predetermined rate of shear. The rate of shear may be altered by changing the ratio of the gearbox that governs the speed of the lower, driven plate. Assuming that the depth of the mucous layer is, for example, 40 μm for an eel 30 cm in length (this figure is quoted by Fedak et al for the pike and observations on the mucous layer of eels would suggest a similar value). Then as

$$\text{rate of shear} = \frac{\text{velocity difference between planes}}{\text{distance between planes}}$$

and the distance between planes is 40 μm, then

rate of shear x 0 04 = velocity difference between planes

thus conversion of measured rate of shear (sec^{-1}) to velocity ($cm\ sec^{-1}$) is possible.

rate of shear	4·5	9	22	45	71·5	112
velocity	0·18	0·36	0·88	1·8	2·86	4·48

This gives, however, a measurement of the velocity difference between the water and the body surface; the actual swimming speed of a fish is likely to be lower than the velocity calculated above as the fish flexes during swimming. Nevertheless, it is apparent that, although only an approximate conversion is possible the shear thinning properties of eel mucus occur at comparatively low swimming speeds.

The approximate cruising swimming speeds of fish (i.e. the

speed which the fish can maintain for several minutes) have been calculated by Bainbridge (1960) and Alexander (1974) to be approximately 2-4 body lengths per second. Thus the cruising speed of an eel of approximate length 30cm is likely to be at least 60cm sec^{-1} and at this speed the mucus is likely to be at its least viscous (Fig.8). Only at rest and low swimming speeds does the mucus become thicker in consistency.

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PLYMOUTH POLYTECHNIC

"THE SKIN AND MUCOUS SECRETION OF THE EUROPEAN EEL, Anguilla anguilla L."

by

G.C. ARCHER, Ph.D. 1979

ABSTRACT OF THESIS

The epidermis of the eel A. anguilla L. secretes a mucous layer mainly from its mucous cells although there may also be a contribution from surface epidermal cells. Under certain conditions, the epidermal cells may transform to a secretory cell type that resembles, but is structurally distinct from a mucous cell. This observation has not previously been reported in fish epidermis.

Specialised club cells, characteristic of the Anguillidae contain filamentous material that may be secreted beneath the epidermal surface. This previously unobserved phenomenon, together with an increase in cell numbers around the lateral region, may suggest an additional protective mechanism which may be of use, for example, during 'burrowing'.

The quantitative distribution of mucous cells over the body was also determined and the cellular contents of the main cell types characterised histochemically. Lymphocytes and previously unseen granular neutrophil-like cells may function in more specific protective mechanisms against pathogenic organisms.

The mucus has been examined biochemically and separated by chromatography and electrophoresis to reveal several components. Rheological study has established shear thinning properties of the mucus and functional groups essential to maintain the integrity of the mucous structure.

The dynamics of mucous secretion have been observed autoradiographically and a model for the mechanism of secretion proposed. Attempts have been made to measure the rate of mucous secretion under a number of experimental conditions which are discussed in relation to the viscous nature of mucus and the proposed model for secretion.

The epidermis and its mucous secretion thus provide an effective lubricative barrier to the environment extremely important for locomotion and protection from dessication, osmotic stress and disease organisms.