A Study of giant cells in inflammatory lesions of the plaice (*Pleuronectes platessa*.L.)

by

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ABSTRACT

The inflammatory process has occupied the attention of physicians since the time of the early Greeks and since then there has been a number of experimental studies on various aspects of its development. There are however still some areas of disagreement despite the importance of understanding the inflammatory process in relation to the rational interpretation of disease.

Giant cells are found in certain chronic infections. They are particularly conspicuous in tuberculous lesions. They also occur in syphilitic lesions, tuberculoid leprosy and fungus infections, while inhalation of a variety of particulate materials e.g. silica (quartz), fibrous silicate (asbestos) and dust or fumes of beryllium compounds produces chronic granulomatous lesions within the lungs which also contain giant cells. Similarly, giant cells may occur in the granulomatous lesions which result when these and other particulate irritants e.g. talcum powder, gain entrance to the tissues, especially following surgical interference.

Fish farming is developing rapidly, and fishes are being raised extensively for both food and sport. One of the major limiting factors on the successful expansion of intensive aquaculture systems is disease, so that the study of fish diseases is becoming a subject of considerable importance. In spite of their importance in the inflamatory process, the literature contains little information concerning giant cells in fish, probably because in the past the study

of fish diseases has been largely undertaken by aetiologists and as a result is dominated by descriptions of the life cycles or biochemical properties of parasites or bacteria.

The present work was carried out in an attempt to redress this balance slightly, with regard to one aspect of fish histopathology, namely the giant cells of granulomata. The intention was to determine whether production of giant cells in the inflammatory lesion of teleost fish could be provoked by irritants, such as Freund's complete and incomplete adjuvant, mycobacteria, beryllium oxide, and talcum powder. These irritants induce the classical types of giant cells (Langhans type and foreign body type) in granulomatous inflammatory lesions of homeothermic vertebrates.

Throughout the study, the plaice (Pleuronectes platessa.L.) was utilized as the experimental animal because it was readily maintained in the facilities available, was readily obtainable from the White Fish Authority, Marine Fish Farm, Hunterston, Ayrshire, and there was already a considerable amount of baseline information available on its physiology and anatomy.

The main histological study was carried out at 10°C but the effect of temperature variation was studied by comparing findings in fish held at 5°C. The results were supplemented by ultrastructural studies at various stages of development of the granulomatous lesion induced by Freund's complete adjuvant at 10°C. In addition to these studies, an in vitro migration inhibition test was carried out in order to demonstrate the release of migration inhibition factor (MIF), a component of the cell mediated immunity response, by lymphocytes of

sensitized fish using piscine Mycobacterium as an antigen. This test confirmed that the delayed type hypersensitivity reaction is a feature of such lesions in teleost fish just as in mammals.

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SECTION I

REVIEW OF LITERATURE

INFLAMMATION IN HOMEOTHERMS

Inflammation comprises the series of changes within a living tissue which take place in response to injury, howsoever caused, provided that the injury is not so great as to destroy the tissue completely (Ogilvie 1967).

Types of injury which initiate an inflammatory response include physical trauma (e.g. heat, mechanical trauma, and ionising radiation), chemical injuries (both organic and inorganic) and damage due to infective agents such as bacteria, fungi etc. Depending on the degree and the duration of the tissue injury, the inflammatory changes may arise subside and resolve within a few hours, or may persist for weeks, or years (Cappell and Anderson 1971).

The changes occurring in inflammation are grouped into two classes, those typical of the acute inflammatory response, and the changes characterising chronic inflammation. The two are not mutually exclusive and may occur together within the same tissue (Morehead 1965; Cappell and Anderson 1971).

Acute inflammation:-

Acute inflammation is an immediate response to tissue injury and the response is characteristically vascular. These vascular changes are responsible for the cardinal clinical signs of inflammation in mammals; heat, redness, swelling, pain, and loss of function. These have been known since the earliest days of medicine.

The redness caused by the inflammatory process is attributed to dilation of vessels, the heat is due to the increased blood flow to the area, the swelling is due to the escape of liquids and cells into the extravascular spaces and the pain is due to the pressure on nerve endings, and/or the action of mediators such as bradikinin on these structures (Ward 1971).

The major process involved include:

1. dilation of blood vessels in the affected tissue due to axonal reflex stimulation of vasodilator nerves and release of vasoactive amines (Spector and Willoughby 1965; Cappell and Anderson 1971).

2. increase of permeability of the walls of the capillaries and venules to larger plasma molecular components (Cappell and Anderson 1971).

3. escape of serous fluids into interstitial spaces (Wright 1958).

4. migration of phagocytic leukocytes from blood vessels into the interstitium of the tissues (diapedesis), (Addison 1843; Clark and Clark 1935; Marchesi and Florey 1960; Marchesi 1961).

The main vasoactive amines operative in the inflammatory response are histamine and serotonin, which ordinarily do not exist free in the circulation but are stored within cells such as mast cells, and platelets (Ward 1971).

Histamine is responsible for capillary dilatation and the vascular permeability of the venules (Cappell and Anderson 1971; Ward 1971). Kinins are produced by the action of proteolytic enzymes (e.g. kallikrein) upon certain plasma globulins. The enzymes are normally present in the plasma in inactive forms, but may become activated by tissue injury. Kinins are capable of inducing increased vascular permeability in the small vessels in various species including man (Cappell and Anderson 1971).

Chronic inflammation:-

Whereas exudative changes of vascular origin are the dominant feature of acute inflammation, chronic inflammation is characterised by the proliferation of tissue cells to produce a vascular fibrous tissue. Whenever acute inflammation fails to resolve an insult to a tissue, chronic inflammation supervenes, so that the two processes are frequently coexistent within a lesion (Cappell and Anderson 1971). However, a chronic inflammatory lesion need not to be produced subsequent to an acute inflammatory process and may present features of the chronic response from the beginning (Willis 1950; Morehead 1965; Cappell and Anderson 1971).

In general chronic inflammatory responses are distinguished from the acute form by the lesser prominence of vascular and granulocytic components and by the greater participation of macrophages, and fibroblasts (Wright 1958). Chronic inflammatory lesions characteristically contain macrophages, lymphocytes, fibroblasts, plasma cells, and may in addition possess giant cells, especially in the presence of foreign bodies (Willis 1950; Wright 1958).

The function of both monocytes and neutrophils once outside blood vessels is of considerable importance. Both types of leucocytes are actively phagocytic (Wright and Dodd 1965; Ward 1971). It is the macrophages which possess the

remarkable capacity to form giant cells. These are found surrounding extraneous particulate matter, such as large dust granules or fragments of ligature. They form a cellular defence against bodies which are too large to be ingested by single cells and against very resistant forms of microorganisms such as tubercle bacilli (Willis 1950; Wright 1958).

Granuloma:-

This is a type of chronic inflammatory response to certain agents. Originally the term granuloma descriptively defined tiny granular white bodies which could be seen in the tissue, as a result of systemic (haemat ogenous) spread of tuberculous bacilli which represented the local inflammatory reaction to Mycobacterium tuberculosis. The term was later redefined to include all lesions showing microscopic criteria of chronic lesions of this type (Morehead 1965; Spector 1969; Ward 1971). Granulomatous chronic inflammation is characterized in the light microscope by the local accumulation of a variety of cell-types, namely macrophages, epithelioid cells, lymphocytes, plasma cells, fibroblasts and giant cells.

In a long standing chronic inflammatory lesions macrophages are transformed into cells showing a different morphology - the epithelioid cells, which result in the production of a tissue composed of groups of large cells with pale staining nuclei, ill defined cytoplasm, and communicating intercellular process. Cells of this type bear a superficial resemblance to the cells of epithelial tissue and for this reason were designated epithelioid cells (Morehead 1965; Carr 1973). When the epithelioid cells assume a nodular arrangement,

as they do in response to certain injurious agents, notably Mycobacteria, the nodules are known as tubercles. These are characteristic features of inflammatory responses in tuberculosis although they may also be found in other conditions e.g. syphilis, sarcoidosis, fungus infections (Lever 1967).

It is well recognised that granulomatous inflammation may be caused by variety of diverse agents. Generally, however, it is accepted that three main groups of causative agents may be implicated.

1. Infective agents
2. Foreign bodies
3. Idiopathic (Unknown agents)

Infective granulomas

Tuberculosis:-

The classical granuloma of homeothermic vertebrates is that seen in tuberculosis where histologically there is central necrosis and a peripheral collar of epithelioid cells, giant cells, lymphocytes, and fibroblasts. In view of its importance it will be described in detail. Experimentally lesions can be induced in mammals by the intravenous or subcutaneous injection of tubercle bacilli (reviewed by Francis 1958). The first stage in the development of the tuberculous granuloma known as the "tubercle" is the migration of neutrophils from the nearby capillaries into the affected area. These rapidly begin to ingest the bacteria but soon the neutrophil reaction becomes obscured by the advent of numerous macrophages. By the end of the first day macrophages become predominant and the polymorphonuclear leucocytes which have been attracted to the site die within a few days (Medlar 1926; Vorwald 1932; Willis 1950; Wright 1958). Almost as soon as

they reach the site, the macrophages begin to phagocytose both neutrophils and free bacilli, so that soon the great majority of the tuberculous bacilli have been engulfed by macrophages. From this stage on, the macrophages continue to accumulate and aggregate in increasing number to form a minute nodule or tubercle, whose cells later become swollen and oval or spindle shaped with foamy cytoplasm and a pale staining nucleus. These are given the name epithelioid cells. It is suspected that they are modified by humoral factors released by specifically sensitized lymphocytes, rendering them immobile and mutually adhesive and enhancing their capacity to destroy tubercle bacilli (Cappell and Anderson 1971).

Small lymphocytes accumulate around the margin of the tubercle. They comprise both lymphocytes of the serum antibody producing plasma cell series with basophilic cytoplasm and "cart-wheel" or clock-face nuclei as well as the small morphologically undifferentiated lymphocytes of the cell mediated immunity series (Montgomery 1967). In the centre of the lesion multinucleated giant cells are formed which are usually Langhans type and occasionally, foreign body type (Warren 1917; Lewis et al 1925; Doan et al 1930; Montgomery 1936-1937; O'Leary and Harrison 1941; Ratcliffe and Wells 1948; Montgomery 1967; Poole and Florey 1970; Walter and Israel 1970).

In the central part of the tubercle the epitheliod cells undergo necrosis, losing their outline and their nuclear staining and become fused into a homogeneous or slightly granular material. The necrotic material is creamy white and resembles cream cheese in appearance and consistency (hence the term caseous material), (Cappell and Anderson 1971). Secondary

enzymatic injury from the lysosomal components of damaged monocytes and the lymphokines released by lymphocytes may be responsible for the necrotic material (McCall 1971).

Syphilis:-

The follicular granuloma of the gumma of the tertiary syphilis is characterized as a tuberculoid reaction, being composed of epithelioid cells, lymphocytes, plasma cells and giant cells, (Tuta and Coombs 1942; Willis 1950; Montgomery 1967). The causative organism is a spirochaete (Treponoma pallidum).

Tuberculoid Leprosy:-

The causative organism of Leprosy is leprosy bacillus, (Mycobacterium leprae) an acid-fast organism. Tuberculoid leprosy displays an infiltrate composed largely of epithelioid cells and giant cells with a variable number of lymphocytes, around the periphery (Cochrane 1957: Willis 1950).

Fungal infections:-

Many fungal infections are marked by the formation of follicular granulomata composed of epithelioid cells and giant cells. Examples include histoplasmosis (Histoplasma capsulatum) (Curtis and Grekin 1947); coccidioidomycosis (Coccidioides immetes) (Trimble and Doucette 1956; Moore 1945; Levan and Huntington 1965); North American Blastomycosis (Blastomyces dermatidis) (Moore 1945); South American Blastomycosis (Paracoccidioides brasilienses) (Moore 1945) in man.

Foreign body granulomas

The causative agents of this class of granulomas include many organic and inorganic substances such well known examples as silica, beryllium, aluminium hydroxide, starch, lipid, Freund's adjuvants, glass fibre, nylon, tattoos and many others.

Silica reactions:-

These result from the contamination of wounds with particles of silica derived from soil or glass which contain silicon dioxide. Such wounds appear to heal but many months or years later, nodules develop in the skin or subcutaneous tissue (Epstein 1955; Shelly and Hurley 1960). Histologically the silica granuloma has been of great interest because it closely resembles the granuloma seen in sarcoidosis (infra); however the constant distinguishing feature of silica granulomata is the appearance of birefringent crystals seen when polorized light is used. The granuloma consists of islands of epithelioid cells, as well as many giant cells and lymphocytes and it is a noncaseating tuberculoid granuloma (Sommerville and Milne 1950; Epstein 1955; Shelly and Hurley 1960). The appearance of many plasma cells and their precursors during the development of the silicotic nodules was reported by Vigliani and Pernis (1959). A similar lesion was described following dusting of wounds with talcum powder (Tye et al 1966; McCallum and Hall 1970). Roberts (1947) described the talc granulomata which developed in operation wounds or in the Fallopian tubes of some patients after previous appendicectomy operations. Histologic examinations showed that multiple follicles containing giant cell and epithelioid cells were present which when examined with polarising microcrope, revealed numerous small anisotropic particles intimately related to the giant cells and, in the main, grouped around them.

Beryllium reactions:-

Beryllium granulomata may arise after particulate beryllium inhalation or may develop following a laceration through which beryllium enters the skin directly (Dutra 1948; Helwig 1951; Williams et al 1967). Beryllium is used in

the manufacture of fluorescent lamps and beryllium copper alloy casting for precision instruments for aircraft. Workers in these industries may suffer from acute pneumonitis or from delayed beryllium poisoning. In delayed poinsoning, the chronic granulomatous lesions may resemble miliary tuberculosis or sarcoidosis, being characterized by giant cells of the foreign body type or Langhans type surrounded by epithelioid cells, lymphocytes and plasma cells (Dutra 1948; Helwig 1951).

Aluminium reactions:-

Aluminium hyroxide is a type of adjuvant used in certain vaccines, particularly toxoids, to enhance antibody production. Protein antigen mixed with any of a variety of aluminium compounds forms a precipitate which contains antigen absorbed to the aluminium. This mixture is normally injected subcutaneously or intramuscularly and the resulting antibody response is usually higher and more prolonged than that resulting from injection of soluble antigens alone (Abromoff and La Via 1970). Erdohazi and Newman (1971) however, have pointed out the dangers of alum adjuvants in descriptions of aluminium hydroxide skin granulomata in two individuals. In both cases, a metal salt adjuvant (aluminium hydroxide) used in a vaccine was the probable cause of the reaction. The histological picture was dominated by epithelioid cells and numerous multinucleated giant cells. Moreover, Gaafar and Turk (1970) found giant cells in the granuloma induced by intradermal injection of aluminium hydroxide in albino guinea pigs (Cavia spp.)

Starch reactions:-

Starch glove powder is used extensively by surgeons in Britain and is generally considered inocuous. However, some cases of starch granulomatosis of the peritoneum have been reported

in Britain and Australia. Neely and Davies (1971) described cases of starch granulomatosis in the peritoneum which developed after surgery. Microscopical examination of the peritoneal nodules showed granulomata composed of epithelioid cells, giant cells, lymphocytes, fibroblasts, eosinophils, and neutrophils. Starch granules were found within the giant cells.

Freund's Adjuvant reactions:-

Freund's adjuvant is used to aid production of high titres of serum antibody in experimental immunology studies (Freund et al 1948). Freund's incomplete adjuvant consists of mineral oil and an emulsifying agent.

Freund's complete adjuvant contains in addition killed Mycobacteria (Freund 1956). Both oil and Mycobacteria produce a specific cellular reaction at the site of injection. If the adjuvant is inoculated intraven ously, specific cellular reactions develop in a variety of organs (such as spleen, liver, kidney and lymph-nodes) containing the usual components of the chronic granuloma (White et al 1955; Freund 1956; Rupp et al 1960; Steiner et al 1960; Spector and Lykke 1966; Spector and Willoughby 1968).

In efforts to determine the origins of the reacting cells, Spector and Willoughby (1968) induced chronic inflammatory reactions to complete and incomplete Freund's ajuvant in rats in which the bone marrow and lymphoid tissue had been destroyed by irradiation. Transfused homologous lymph-node cells or thymus cells did not lead to a detectable restoration of the cellular inflammatory response, but transfused bone marrow cells, migrated into the inflamed area, proliferated

there and produced all the histological features of chronic inflammatory granulomata including macrophages, epithelioid cells histiocytes, giant cells and cell mediated immunity lymphocytes.

Lipid reactions:-

This type of granuloma is formed as a reaction to lipids (xanthoma). The Touton type of giant cell is exclusively found within this inflammatory granuloma. In man there are numerous clinical types of xanthoma such as Xanthoma tuberosum, Xanthelasma, Eruptive xanthoma, (Milne 1972). All of those lesions show a similar histological picture of foamy macrophages and scanty multinucleated giant cells. These are predominantly of the Touton type but giant cells of either Langhans or foreign body type may also be encountered (Montgomery and Osterberg 1938; Montgomery 1967).

Granulomas of Unknown Aetiology

There are a number of chronic granulomata whose aetiology is obscure. The most common is sarcoidosis.

Sarcoidosis:-

Sarcoids are focal granulomas characterized by the presence of epithelioid cells, and giant cells (Langhans type). The giant cells are large and irregular in shape, occasionally containing inclusions (Schaumann bodies or asteroid inclusion bodies). Although the epithelioid cells and the giant cells are the most prominent histological elements in the sarcoid granuloma, there may be a narrow zone of lymphocytes and, at times, plasma cells at the periphery of the epithelioid tubercle (Longcope and Freiman 1952; Wanstrup and Christensen 1966; Hirsch et al 1967; Montgomery 1967).

GIANT CELLS IN INFLAMMATORY LESIONS IN HOMEOTHERMS.

Giant cells were described as early as 1855 by Rokitansky, and his work and subsequent reports by Virchow (1858), Langhans (1868), and others were reviewed by Haythorn (1929). Langhans was the first to describe accurately the giant cells of the characteristic tuberculosis lesion. He worked with fresh tissue, teased out or crushed, from miliary tubercles of pleura or peritoneum. He noted considerable variations in giant cell size and outlines, and variation in nuclear number from two or four in small cells to 100 or more in larger ones. The cytoplasm of the cell was pale, homogeneous or finely granular with the centre usually clear. The nuclei were generally round or oval, with sharp outlines and contained nucleoli. The arrangements of nuclei was characteristic. Some of the cells had nuclei which were arranged around the periphery. These cells have been subsequently designated Langhans type giant cells (Morehead 1965; Montgomery 1967; Milne 1972). Langhans also described giant cells which had diffusely distributed nuclei throughout the cell. These cells were subsequently designated foreign body type giant cells (Morehead 1965; Montgomery 1967 and Milne 1972).

Another type of giant cell, the Touton giant cell is found in xanthomatous conditions of the human skin. According to Unna (1896); Touton first described (1885) Touton giant cells in his article on "Xanthoma, its histology and histogenesis" (J A Milne pers. comm 1975). Touton cells characteristically possess a ring of nuclei situated mid way in the cell between periphery and centre.

Giant cells have engaged the attention of many famous pathologists, especially with regard to their relationship to tuberculosis. There were originally two distinct schools of thought as to the origin and the significance of the Langhans giant cells. According to Hektoen (1898), (Baumgarten (1885) and Weigert (1885)) found that the giant cell was a degenerating structure showing slow necrobiotic changes from its first appearance, whereas Metchnikoff and his co-workers (1881) were convinced that the giant cell of tuberculosis were in fact living active defensive cells.

Hektoen (1898) induced foreign body type giant cells in the anterior chamber of rabbit's eye in response to the introduction of coagulated blood serum. He concluded that the giant cells which developed subsequently subdivided again into small mononuclear cells. Metchnikoff (1891) observed very characteristic degeneration of the bacilli within epithelioid and giant cells after inoculating marmots (marmota spp.) with avian or human bacilli. Faber in 1893 demonstrated digestion of agar by giant cells and Maximow (1924) followed the formation of tubercles in cultures of mammalian tissue and observed the phagocytosis and subsequent digestion of bacilli. He stated that the intracellular digestion of bacilli in epithelioid cells was probably significantly increased after their fusion into giant cells, which explained why the number of microorganisms in the multinucleated cells was never great.

The phagocytosis of particulate irritants such as silica, talcum powder and starch powder by giant cells in spontaneously or experimentally induced granulomatous lesions has been

described by a number of investigators (Roberts 1947; Shelly and Hurley 1960; Tye et al 1966; McCallum and Hall 1970; Neely and Davies 1971; McDougal and Azar 1972).

In the earlier literature there were conflicting reports as to the mode of formation of giant cells. The first theory was that of Langhans (1868). He raised the question as to whether the giant cell existed ab initio or was derived from the surrounding tissues. He considered that giant cells originated from the surrounding cells and that the spindle cells played the principal part. He thought that these cells might arise by division of the nuclei without division of the cell or by fusion of several cells to form a single large cell (reviewed by Haythorn 1929). Metchnikoff (1891) carried out extensive studies of phagocytosis in tubercles, describing epithelioid cells and their transformation into giant cells. Metchnikoff considered giant cells were defensive phagocytic cells arising from cell division of or from fusion of epithelioid cells.

The origin and formation of multinucleated giant cells have been frequently observed in tissue and organ cultures of organs such as the spleen (Lambert 1912; Weil 1913; Smyth 1916), lymph-nodes (Lewis and Webster 1921; Maximov 1924) and blood and buffy coat (Lewis and Lewis 1925-1926; Sutton and Weiss 1966). Lambert (1912) observed the production of foreign body giant cells in vitro by the addition of foreign material such as Lycopodium spores or cotton fibres to cultures of chick embryo spleen. He considered these giant cells to be formed by the fusion of large mononuclear wandering cells. The large giant cells sometimes spread out over the cover glass in cultures of chick embryo spleen and Lambert suggested

that the cover glass might have been acting as a foreign body. However Weil (1913), in repeating these experiments found that the formation of giant cells was by the division of the macrophage nucleus without division of cytoplasm, and did not obtain evidence of fusion of cells to form multinucleated ones.

This disagreement as to the ontogeny of the giant cell continued with the studies by Smyth (1916) of cultures of chick spleen with Mycobacteria. He found that the large epithelioid cells produced, when they came into contact with one another, tended to fuse and form giant cells. Lewis and Webster (1921) found that giant cells frequently occurred in cultures of normal and tuberculous human lymph-nodes in human plasma. Only after a great many attempts did they succeed in observing division (amitotic) of the nucleus without division of the cytoplasm in one epithelioid cell, allowing them to conclude that the giant cells in such cultures were probably formed by amitotic division of the nucleus without division of the cytoplasm, as they saw no indication of the production of giant cells by fusion. However, Maximow (1924-1925), in his studies on lymphoid tissues and blood leukocytes (buffy coat) with tubercle bacilli in vitro, considered that the giant cells arose by fusion of the mononuclear, epithelioid cells. He believed that amitosis played an inconspicuous part in the formation of giant cells, and he concluded that the epithelioid and giant cells of the tuberculous lesions have the same double origin as the polyblast or mononuclear exudate cells in common or purulent inflammation. They arise partly from local fixed elements - the histiocytes of the respective tissue (resting wandering cells or clasmatocytes, reticular cells, cells of Kupffer,

"endothelial" cells of the sinuses in the spleen, etc.), partly from the migrated (or local, if available), nongranulated, i.e. lymphoid white blood corpuscles - both monocytes and lymphocytes.

Lewis and Lewis (1925-1926), described transformation of mononuclear blood cells into macrophages, epithelioid cells, and giant cells in hanging drop cultures of whole blood from mammals, birds and lower vertebrates. Many of these giant cells of the Langhans type similar to those found in tuberculous lesions, were formed.

Sutton and Weiss (1966) added a further dimension to the study of giant cells by their study of the sequential transformation of chicken monocytes into macrophages, epithelioid cells, and multi-nucleated giant cells in tissue culture, by electron microscopy. Their conclusion was that the plasma membrane of the epithelioid cells broke down allowing fusion to form giant cells. This latter finding was supported by Gillman and Wright (1966) in their study on the origin of the giant cells around implanted foreign bodies (Millipore filters or polyvinyl sponges) in rats. Their experiments indicated that multinuclear cells were probably derived from fusion of emigrated blood mononuclear cells.

Thus the presently accepted thesis of the origin of giant cells is that they are formed either by the fusion of mononuclear cells or by multiple nuclear division of one mononuclear cell without the division of the cytoplasm. The concept that giant cells take their origin from mononuclear cells is supported by a number of investigators (Spector and Lykke 1966; Spector 1969; Spector and Willoughby 1968; Volkman and Gowans 1965 a-b) all of whom have recently published work on the origin of the inflammatory cells.

DELAYED TYPE HYPERSENSITIVITY

The phenomenon of delaved type hypersensitivity has been recognised since 1800. Edward Jenner (1749-1823) described it but did not use that term (reviewed by Ebert 1965). Koch (1890) demonstrated that subcutaneous injection of tuberculin (tubercle protein) into a tuberculous animal caused a delayed allergin reaction (Levy et al 1973), the development of swelling and redness within 48 hours at the site of injection implying allergy to the tuberculin and indicating previous contact with Mycobacterium tuberculosis.

An understanding of the importance of this type of immunologic repsonse in diseases, and perhaps more significantly in health, has only recently begun to emerge, and delayed type hypersensitivity is found to occur in individuals following injection with some but not all bacterial, mycotic and viral agents (Bloom and Bennett 1970).

Histologically, delay type hypersensitivity is characterized by a perivascular infiltrate, rich in both lymphocytes and macrophages (La Via 1971; Roitt 1972). The mechanism of delayed type hypersensitivity is not yet completely understood but both sensitized lymphocytes, (thymus derived), and macrophages (Roitt 1972; Boros and Warren 1973) participate in the response.

The pathogenesis of tuberculosis appears to involve delayed hypersensitivity (Spector 1967; Crowle 1962). The Mycobacterium tuberculosis organism itself is apparently innocuous producing no important toxin and multiplying slowly, so that it is apparently physically harmless per se. However, even non-pathogenic or killed organisms are nevertheless highly antigenic and per se can call forth tuberulin allergy in the

body or if this is maintained artifically by repeated injections, initiate severe destructive allergic reaction for the body (Crowle 1962).

The nature of the allergy in tuberculosis was studied in vitro, as early as 1932 by Rich and Lewis and they reported inhibition of the migration of cells from buffy coat or spleen explants of hypersensitive animals in the presence of specific antigen. This technique was later simplified by George and Vaughan (1962), who studied the migration of peritoneal exudate cells placed in capillary tubes and cultured in vitro. More recent works (David 1966; Bloom and Bennett 1970) has indicated that the lymphocytes from hypersensitive animals, when incubated with specific antigen elaborate a soluable substance into the tissue culture media which inhibits migration of macrophages. This substance is called migration inhibition factor (MIF) (Bloom and Bennett 1970; Morley et al 1973; Roitt 1972). It seems probable with this factor which along with others released from sensitised lymphocytes, which are called lymphokines (Roitt 1972; Morley et al 1973), play a significant part in the pathogenesis of the granulomatous lesions.

THE MORPHOLOGY OF CELLS IN INFLAMMATORY LESIONS OF HOMEOTHERMS

Neutrophil leukocytes (polymorphonuclear leucocytes):-
Neutrophils contain numerous, minute, evenly distributed granules. Although in humans the granules are poorly staining, and truly basophilic the term heterophil may be a better term for the neutrophils of many other species since these are more markedly basophilic or eosinophilic. The nucleus has several lobes (2-5) which may overlap, and show variation in size and shape (Ogilvie 1967; McDonald et al 1970)
Electron microscopic studies show that there is a thin ectoplasmic layer of cytoplasm that is devoid of granules and pseudopodia are found. This is probably important in the ameboid locomotion of the cell. It contains large vesicles, characteristic granules and occasional mitochondria in the cytoplasm (Watanabe et al 1967; Bloom and Fawcett 1968).

Macrophages:-
These vary in size from about 10 microns to several times this size and their shape is rounded to elongated, the excentrically situated nucleus being less convex or even concave towards the main cytoplasmic mass (Evans 1915; Fedorko and Hirsch 1970). Under the electron microscope, the nucleus may have up to three nucleoli and nuclear pores are usually obvious. The cytoplasm is characterized by phagocytic inclusions (phagosomes), long slender pseudopodia and mitochondria. In the cytoplasm lysosomes are very num erous, appearing most commonly as dense small granules. Both smooth and granular endoplasmic reticulum are abundant and they show large and complex Golgi apparatus and intracytoplasmic filaments (Spector 1969; Fedorko and Hirsch 1970).

Epithelioid cells:-

Epithelioid cells are large and irregular with poorly defined cytoplasmic margins that merge with those of neighbouring cells. The abundant cytoplasm is eosinophilic and amorphous or finely granular. The nucleus appears large, ovoid, and palely stained, with a sharp delicate nuclear membrane (Epstein 1967; Papadimitriou and Spector 1971).
Under the electron microscope, the epithelioid cells show many mitochondria, lysosomes and large vacuoles. Granular endoplasmic reticulum (ER) is present in varying amount (Sutton and Weiss 1966; Hirsch et al 1967). The cell membranes show fingerlike processes interdigitating with those of adjacent cells (Hirsch et al 1967).

Giant cells:-

The morphology of giant cells is the main criterion for their differentiation. All are around 70 μm in diameter and their shape is oval or rounded. The number of nuclei may vary from single figures to over a hundred and it is the disposition of these which allows their definition.

The nuclei of the Langhans giant cells are distributed in an interrupted circle around the edge of the cell, enclosing central cytoplasm.

Ultrastructurally they show all the organelles possessed by individual macrophages (Dumont and Sheldon 1965). These workers also found dense granules and frequently the multiple Golgi apparatus was located in the central zone while the endoplasmic reticulum was disposed outside the ring of nuclei.

Sutton and Weiss (1966), in a study of the sequential changes of macrophages by electron microcopy found the nuclei of such cells to show a thin rim of marginal chromatin with two or three nucleoli. The cytoplasm of the epithelioid cells contained long interlocking filaments which passed between cells prior to the fusion to produce giant cells. Mitochondria increased in number with the edge of the lesion but they did concur with Dumont and Sheldon's (1965) findings on the distribution of endoplasmic reticulum which in their case was present throughout the cytoplasm.
The ultrastructure of the giant cells of the sarcoid granuloma was investigated by Hirsch et al (1967). The cytoplasm of the giant cells contained multiple foci of Golgi complexes, abundant mitochondria and strips of endoplasmic reticulum. The giant cell nuclei were peripherally placed and were elongated in shape with an irregular margin.

Foreign body giant cells have their nuclei scattered throughout the cytoplasm. Ultrastructurally, Cliff (1963), found in a study in the rabbit, that the nuclei frequently showed a grid pattern of the nucleolus. They possessed all of the components of macrophages and had regular, "ruffled" pseudopodia.

The Touton type giant cell is characterized by a complete circular arrangement of the nuclei situated mid-way between the centre of the cell and the periphery. The central portion of cytoplasm within the ring of nuclei has a homogeneous appearance, while the cytoplasm at the perihpery of the cell is granular and often vacuolated (Morehead 1965; Montgomery 1967; Milne 1972).

Lymphocytes:-

Lymphosytes have a thin rim of clear, non-granular slightly basophilic cytoplasm and a round or oval nucleus with a characteristically coarse chromatin net-work (Ham 1969; McDonald et al 1970).

Under the electron microscope, the nucleus contains one or more nucleoli and the nuclear chromatin shows dense clumps. In the cytoplasm, mitochondria are few, the endoplasmic reticulum is absent or barely represented and free ribosomes are abundant. The golgi apparatus is rudimentary or absent (Spector 1969).

Plasma Cells:-

Plasma cells resemble lymphocytes in being non-granular but differ in that they are larger and have more definitely basophilic cytoplasm. They are round, oval and contain a spherical, usually eccentric nucleus with its chromatin masses characteristically arranged in radial fashion about a minute, central nucleolus - the cart-wheel or clock-face nucleus (Movat and Fernando 1962; Ogilvie 1967).

Under the electron microscope, there is slight or marked condensation of the chromatin at the periphery of the nucleus, giving it the well known cart-wheel appearance. The Golgi apparatus is pronounced in most cells, consisting of small vesicles and parallel membranes or tubules. Mitochondria are prominent and occur between the ergastoplasmic sacs. The ergastoplasm is more highly developed than in any other connective tissue cell. There is no clear peripheral zone as in fibroblasts, the ergastoplasm reaching to the periphery of the cytoplasm. In immature cells there are only a few ergastoplasmic sacs, but as the cells mature they

become more numerous to reach their full development and become dilated at maturity (Movat and Fernando 1962).

Fibroblasts:-

At the light microscope level fibroblasts are characterized as spindle-shaped or stellate cells with oval or reniform nuclei (Movat and Fernando 1962; Chapman 1961-1962). At the electron microscope level, the mitochondria of fibroblasts are less conspicuous than those of those mesenchymal cells but ergastoplasm is well developed especially in the proliferating cells. Resting fibroblasts or fibrocytes are thin, elongated cells; proliferating fibroblasts are also usually elongated but are plumper. The nucleus of the fibroblast is usually elongated (Chapman 1961-1962; Movat and Fernando 1962).

INFLAMMATION IN FISHES

Inflammation in general:-

There have been a limited number of general studies on the inflammatory response in fish. The earliest in this field was made by Metchnikoff (1905) who observed intraperitoneal phagocytosis of guinea pig erythrocytes in gold fish (Carassium auratus). Jakowska and Nigrelli (1953) reported their observations on the wounds of guppies (Lebistes reticulatus) infected with a species of Mycobacterium, where they found that the initial exudate comprised coarsely granular eosinophils which were replaced in two days by macrophages, the wound later healing with granulation tissue, but their work was characterized by a lack of detailed observation. Prazdnikow and Mikhaliova (1968) studies the reactions of prelarval humpback salmon (Oncorhynchus nerka) to the introduction of cotton threads impregnated with carmine suspension and various

bacteria under skin. They recorded accumulation of leukocytes in the wound but did not extend their observations beyond this.

Klontz et al (1966) observed the sequential pathologic changes in rainbow trout (Salmo gairdneri) following intramuscular inoculation of virulent Aeromonas salmonicida, the cause of furunculosis, noting a marked inflammatory response consisting of lymphocytes, neutrophilic granulocytes, morphages, and fibroblasts. After 40 hours there was a noticeable diminution of this inflammatory element in the area of the injection. By the end of the 3rd day, the kidney, spleen and the site of injection were devoid of granulocytes and macrophages and were undergoing marked liquefaction necrosis.

Finn and Neilson (1971) carried out the first extensive experimental histopathological study of the teleost inflammatory response in rainbow trout. They produced lesions in various ways such as by injecting a suspension of heat killed Staphylococcus aureus, or Freund's complete adjuvant and by means of physical trauma. Fish were held at 15° C and sampled over the period from 3hours to 16 days. They studied the various exudative and cellular changes and concluded that the response to killed Staphylococcus aureus or to adjuvant consisted of polymorphonuclear leukocytes, macrophages, lymphocytes, fibroblasts, giant cells and it was remarkably similar to that of the higher animals but that the onset of cellular changes was delayed and they were probably quantitatively less severe than in mammals, although qualitatively similar. Roberts et al (1973 a-b) studied the pathogenesis of lesions induced by the physical trauma

of insertion of a light plastic identification tag, in salmon parr, (Salmo salar). Microscopic examination revealed that in the early lesion, within two days, the tissue had become infiltrated by polymorphonuclear leucocytes and over the following days damaged muscle fibres replaced fibrogranulation tissue, and consisted of follicular granulomata which contained macrophages and multinucleated giant cells of foreign body type which did not contain doubly refractile material. The macrophages of the granuloma were swollen and many contained melanin pigment and large foamy vacuoles. The extension of the muscle lesion was limited by the perimysium and intermyotomal fascial places. They also described the histopathological features of the chronic tagging lesion in returning adult salmon which were apparent in the tag wound at the end of the one year or two year period which the majority of salmon spent at the sea prior to their return to their native rivers to spawn. The chronic inflammatory lesion consisted largely of highly cellular fibrous tissue, overlaid by an acanthotic epidermis. Two unusual cell types were frequently seen in the wound area: first the eosinophil granule cell (EGC) a large, ovoid cell with numbers of strongly eosinophilic refractile granules, its nucleus flattened and situated close to the cell membrane. The other cell was a melanin containing cell. Such cells were randomly distributed throughout the lesion, as large black cells. Subjacent muscle fibres to fibrogranulation tissue showed regeneration. However, degenerating fibres on the other hand observed frequently closely related to regenerating fibres, showed myophagia.

In the long standing tagging lesion, however, there was a high degree of vascularisation with numerous small capilliaries throughout the tissue. The arterial supply to the area showed severe endarteritis obliterans, indicating that the tissue of the lesion had now reached maturity and its vascular requirements had reduced.

Mawdesley-Thomas and Bucke (1973) described tissue repair in goldfish traumatized by passing a hypodermic needle through the dorsum. The cellular infiltrate comprised plasma cells, macrophages, and lymphocytes. The dermis also showed an increased fibroblastic response resulting in fibroplasia. There was little attempt at regeneration of muscle fibres but this may have been because they did not keep the fish alive sufficiently long after the initiation of the wound.

Timur M (1975), induced chronic granulomatous inflammatory reactions in plaice at $10^{o}C$. He produced lesions by injection of carrageenin, an irritant mucopolysaccharide and described the histopathological features of the chronic granulomatous inflammation which consisted of macrophages, lymphocytes, plasma cells, fibroblasts, epithelioid cells and giant cells. He found that a highly cellular fibrous tissue developed in the lesion of plaice just as in mammals exposed to carrageenin.

Effect of temperature variation on the inflammatory response:- Since teleost fish are poikilotherms it might be expected that their inflammatory responses would vary with temperature just as all other metabolic functions are so affected (Finn and Nielson 1971).

The teleost in normal health has a balance at a given temperature with the potential pathogens in its environment. When changes occur in ambient temperature it is forced to reorient its defensive mechanism to deal with the new situation (Roberts 1974). That the defence mechanism of fish may vary with temperature was shown by Bisset (1946). He reported that goldfish at 10^{o}C could be infected with bacteria normally considered to be saprophytic but when the temperature was raised to 23^{o}C, the same bacteria were rapidly cleared from the fish tissues. Janssen and Waaler (1967) reported that hibernating hedgehogs with a body temperature of 6^{o}C showed no production of antibodies compared with non-hibernating animals with a body temperature of 32-33^{o}C. Hibernating hedgehogs, cod and goldfish at low temperature showed no inflammatory response after injection of silica emulsion. He claimed that the reaction of controls at high temperature was marked but since he gave no detailed description of this reaction it is not possible to draw useful conclusions from this work.

Finn and Nielson (1971) reported that after injection of killed staphylococcal bacteria and Freund's complete adjuvant there was little qualitative difference in the response of rainbow trout, whether the animals were kept at 5^{o}C or at 15^{o}C.

However they reported that the appearance of macrophages within myotomes, the clearance of bacteria and necrotic tissue from the lesions and the advent of the fibroplasia were delayed by as much as 50 per cent in fish kept at $5^{o}C$ compared with the responses in fish kept at $15^{o}C$.

McQueen et al (1973), described the effect of temperature on the inflammatory response to the metacercaria of the digenean trematode Cryptocotyle lingua in plaice. At low temperature ($5^{o}C$) they found no massive degeneration of muscle and replacement fibrosis, as observed in the occasional fish at the high temperature ($15^{o}C$). They found a negligible acute inflammatory response to the infection both at $15^{o}C$ and $5^{o}C$, but considered this to be the mark of a well adapted parasite. The rates of cyst and capsule formation were considerably reduced by the temperature reduction of $10^{o}C$. The host capsule at high temperature consisted of a highly cellular fibroblast layer, three or four cells thick, with abundant melanin containing cells. Even capsules deep in muscle tissue contained melanin containing cells. However, at low temperature, the capsule consisted of a layer of fibroblasts and fibrous tissue, one cell thick, compared with 4 to 5 cells thick in the warm acclimated fish.

Roberts et al (1973) reported that the rate of inflammatory response development was markedly inhibited at the low temperature of $4^{o}C$ in newly tagged salmon parr compared with fish held at $12\text{-}8^{o}C$. The qualitative response at the lower

temperature was also slightly different in that polymorphonuclear leukocytes appeared to play a slightly more dominant role and granulation tissue contained a smaller proportion of fibroblasts and was composed mainly of eosinophilic ground substance with considerable numbers of monocytes, myophagic histiocytes, and in its early stages polymorphononuclear leukocytes whereas at the higher temperature (12-8^{o}C) specimens the fibrogranulation tissue was dominated by fibroblasts and fibrous tissue.

Timur M (1975) observed that in carrageenin granulomata in plaice the appearance of the polymorphonuclear leukocytes was prolonged and the clearance of carrageenin and degenerative muscle fibres from the lesion and the advent of fibroblasia were delayed in plaice kept at 5^{o}C.

GIANT CELLS IN INFLAMMATORY LESIONS OF FISHES

The first reference to the existence of giant cells in fish was in experimentally produced tuberculous lesions in the marine fish Sparus anularis by Betegh in 1921 (reviewed by Alexander (1931)). Fish were infected by intramuscular injection of tubercle bacilli of piscine origin and tuberculous nodules containing giant cells resulted.

Sutherland (1922) however failed to find typical giant cells in the lesions of halibut (Hippoglossus hipoglossus) which contained acid fast organisms and resembled tubercles. Aronson (1926) found tubercles containing acid fast bacteria in various organs of salt water fish in the tanks of Philadelphia aquarium. Nodules found in the liver resembled in some respects the tubercles of homeothermic vertebrates and occasional giant cells with nuclei in the centre of the cell were seen on the perihpery of the necrotic areas. However, Parisot and Wood (1960) reported that the outstanding characteristic of mycobacterial diseases of salmonid fishes was complete lack of inflammatory response to organism.

Nigrelli and Vogel (1963) in reviewing the histopathology of tuberculosis in fishes, suggested that it resembled the tubercular picture in warm blooded animals except that inflammatory reactions were milder, there was greater fibrous tissue development, and there was an absence of typical giant cells with little or no caseation. Amlacher (1970) expressed his view that giant cells occasionally in experimental granulomatous inflammatory ractions; but that in spontaneous piscine tuberculous granulomata lesions these cells did not occur.

The existence of giant cells in the inflammatory lesions due to other agents was reported by a number of workers. Wood and his co-workers (1956-1956) discussed multinucleated giant cells in mycosis-like granulomata of brook trout (Salvelinus fontinalis). Finn and Nielson (1970), reported giant cells

in experimentally induced inflammatory lesions of rainbow trout where killed staphylococcal bacteria and Freund's complete adjuvant were the stimulus and Duncar and Herman (1971), reported that the discrete granulomas of the condition known as visceral granuloma in brook trout, were tubercle like concretions in the intestinal wall and were arranged in whorling patterns. They were generally composed of fibrous tissue and large mononuclear leucocytes many of which had coalesced to form giant cells. Eosinophilic granulocytes were seen in some of areas of the granulomas and their centres often contained small mineralized deposits.

Wolke and Trainer (1971) reported giant cells of both Langhans and foreign body type in granulomata within the submuscosal tissue of the intestine of white suckers (Catostomus commersoni) in response to silicaceous processes of marine diatoms which had penetrated the mucosa and were acting as foreign bodies. Roberts et al (1973a) found foreign body type giant cells in the tagging lesions in newly tagged salmon parr. Timur M (1975) found Langhans and foreign body type giant cells in the carrageenin granuloma in plaice, and Russell (1974) reported that giant cells, occasionally, were seen in lymphocystis lesions in that species.

THE MORPHOLOGY OF CELLS IN INFLAMMATORY LESIONS OF FISHES

The morphological description of most of the chronic inflammatory cells of the plaice (P. platessa) has been given by Russell (1974) in his study of lymphocystis infection. He described macrophages, neutrophils, lymphocytes, plasma cells and fibroblasts. The morphology and histochemistry of plaice blood and leucocytes was studied by Ellis (1974) and he also described free macrophages found mainly in smears of kidney, spleen and thymus. The ultrastructure of blood cells was studied by Ferguson (1975). The following is a summary of their descriptions.

Neutrophil leucocytes (polymorphonuclear leucocytes):-
Neutrophils possess a round or bean or occasionally a lobed "Dumbell" shaped, excentrically placed nucleus occupying about one third or less of the cell and having a greyish, granular cytoplasm when stained with Romanowski dyes. Alkaline phosphatase activity is located in the nucleus. Under the electron microscope, the nucleus of the cell is irregular in outline but not mutilobed. The cytoplasm possesses numerous large, round or elongated granules. The Golgi apparatus is not prominent but the outline is often irregular and pseudopodia are absent.

Macrophages:-
Macrophages are circular in shape with a round or bean shaped excentric nucleus. The abundant cytoplasm is only slightly basophilic. Darkly staining inclusions are sometimes seen and considered to be phagocytosed debris. With PAS - the cytoplasma is moderately positive taking on a reddish colour.

Epithelioid cells:-

They are large, polygonal in shape with large oval nuclei. Their cytoplasm is eosinophilic and they tend to merge with their neighbour cells (McQueen 1974; Roberts 1974 pers. comm.).

Giant cells:-

They have already been discussed in pages 30-32.

Lymphocytes:-

The nucleus of the lymphocyte occupies most of the cell and is surrounded by a rim of basophilic cytoplasm. A small proportion possessed a few fine PAS - positive granules in their cytoplasm. Azurophilic granules are not observed. Under the electron microscope the plasma membrane occasionally shows small pseudopodia. The nuclear chromatin is mainly distributed peripherally. The cytoplasm of the cell contains small vesicles, limited amounts of rough endoplasmic reticulum, scattered ribosomes and extremely large and often elongated mitochondria.

Plasma cells:-

Plasma cells are oval in shape with the eccentric nucleus occupying one half to one third of the cytoplasm. The nucleus is blue, and the cytoplasm is a purple colour when stained by Unna-Pappenheim's staining method.

Fibroblasts:-

They are spindle shaped of a varied size and have densely staining nuclei.

THE PLAICE

Biology:-

The plaice is probably the best known British flatfish. It is a teleost classified, according to Bagenal (1972), thus:

CLASS : PISCES
ORDER : Heterosomata
Family : Pleuronectidae
Species : Pleuronectes platessa .L.

The plaice family (Pleuronectidae) comprises, in addition, the following British fish, the Dab, (Limanda limanda .L.) the Flounder (Platichthys flesus .L.), the Long rough dab, (Hippoglossoides platessoides .L.), the Halibut, (Hippoglossus hippoglossus .L.), the Lemon sole (Microstomus kitt .L.), and the Witch (Glyptocephalus cynoglossus .L.)
The upper side of the plaice is an olive greenish brown with brown red or orange spots which usually have a white or blue halo. There are also a few dark brown spots and some cream ones. The underside is a dull cream white. Plaice spawn from December to April. One of the largest spawning areas is the Southern North Sea, but other smaller areas are located in Rye Bay and elsewhere in the English Channel, off Flamborough Head, in the Firth of the Forth, the Moray Firth and elsewhere in the Northern North Sea, in the Bristol Channel, in Cardigan Bay and on other grounds in the Irish Sea. There are also many small foci of

spawning water round the coast of Scotland and Ireland. The fertilized eggs, about two mm in diameter, float near the surface and hatch after 15 to 21 days. The larvae which are only 6.5 mm long, subsist for some time on the food stored in the yolk sac. This yolk, as in other fish, provides the nourishment for the growing embryo both in the egg and in the larval fish after hatching. In 8 or 9 days the yolk supply is nearly used up and the larva begins to feed, first on diatoms, and then on larger planktonic plants and animals. After about 4 weeks the larval plaice has grown to 10-14 mm and it begins to metamorphose i.e., change its shape. It then goes down to the bottom to start its life as a flat fish. By this stage the larvae have drifted from the spawning grounds to inshore nursery grounds where they feed largely on the palps of bivalve molluscs such as Tellina tenuis. After one summer inshore they move out to deeper water (Wimpenny 1953; Marshall 1965; Begenal 1972).

Brief review of apposite anatomy and physiology:-
The axial firm skeleton of the teleost fish is composed of the skull, the vertebral column, the ribs, and the intramuscular bones. Fishes like all other vertebrates have three types of muscles. These are smooth (gut muscle), cardiac (heart muscle), and skeletel muscle. The trunk muscles are effectively attached to the firm skeleton, and are primarily used in movement of skeletal components and in locomotion. They comprise white and dark (red) muscle fibres (Lagler et al 1962). In contrast to mammals, where the adipose tissue

serves as the main site of lipid storage, fish appear to use the liver and skeletal muscle for a similar storage function. In fish the red and white muscle fibres are more clearly distinct from one another than in mammalian muscle, where the two types of fibres are normally mixed. The red muscle of fish is usually situated under the skin, along the lateral line although some fish have developed deep-seated red muscle (Bilinski 1969).

Fishes have a single circulation from body via heart to gills and on to the body again. Fish blood circulates as in higher animals, through a system of closed vessels, the arteries and veins. In this circulatory route, the heart pumps reduced blood that is relatively low in oxygen and high in carbon dioxide (Lagler 1962).
In fishes as in the other vertebrates lymph is collected from all parts of the body by a system of ducts and sinuses which finally return to the main blood stream. In plaice and probably other teleosts the lymph volume in skin and muscle is much larger than in higher animals (Wardle 1971). The blood cells of the plaice have already been described (*vide supra*).

The skin of the plaice as with the other teleost fish (Henrikson and Matoltsy 1968; Jakubowski 1960) is divisible into epidermis, dermis and hypodermis. A detailed description of plaice skin was given by Roberts *et al* (1971). The epidermis is a nonkeratinized mucous membrane on a regular basement membrane. The epidermis is composed of four major cellular components, filament containing cells, mucous cells, eosinophilic granular cells, and melanin containing macrophages. The dermis is divided into the *stratum spongiosum* and *stratum*

compactum. They correspond to the papillare and reticulare of mammalian skin. The stratum compactum is composed of collegen bundles derived from fibroblasts melanocytes and mast cells around the vessels. The stratum spongiosum contains the scales and the guanophores (irridophores) and melanophores Roberts et al (1971).

SECTION II

(MATERIALS AND METHODS)

General

Fish:-

Plaice of 1^+ or 2^+ year classes were used throughout the work. The majority of fish were obtained from the White Fish Authority Marine Cultivation Unit, Hunderston, Ayrshire. They were generally 19-20 cm in length and weighed 60-70 gr. In the middle of the experiments, it was found that stock from that source had become unsuitable due to infection by the fungus Ichthyophonus hoferi, so that wild specimens were used for the remaining experiments. Their size was 8-10 cm and they were obtained from Traie Bay, Oban, Argyll through the assistance of Dr R Gibson.

Aquaria:-

Fish were normally held in fibreglass tanks (43 cm x 46 cm x 74 cm) in a recirculating filtered salt water aquarium facility. They were held at either $10^{\circ}C$ or $5^{\circ}C$ and were acclimated for 14 days prior to experimentation. Where pathogens were involved e.g. in work with tuberculous infected fish, they were held in closed circuit recirculation facilities in an isolation room.

Fish were fed on a prepared pelleted diet WFA1 kindly provided by Mr C D Anderson of the White Fish Authority.

Experimental Phlogistons:-

In the course of the experiments several inflammatory agents were used.

1. Complete Freund's adjuvant, comprising mineral oil, emulsifying agent, and killed Mycobacterium butyricum. This was obtained from Difco Laboratories Detroit, Michigan U.S.A. It was diluted with an equal volume of physiological saline, before use. (Spector and Lykke 1966; Spector and Willoughby 1968).

2. Incomplete Freund's adjuvant, this was similar to the complete Freund's adjuvant except that it lacked the mycobacteria. It was obtained from Difco Laboratories, Detroit, Michigan U.S.A. It was also diluted with an equal volume of physiological saline before use. (Spector and Willoughby 1968).

3. Mycobacterium sp. NCMB 1484 obtained from Torry Research Station, Aberdeen, Scotland. This strain had been isolated by Mr D Cann at Torry Research Station from a halibut. Freeze dried culture was resuspended in nutrient broth and transferred to Dorset's egg medium, Lowenstein - Jensen medium and Lowenstein - Jensen medium without glycerol and incubated at 25^{o}C according to the method of Hendri (1973 pers. comm.) The mycobacteria were harvested by washing of the medium with physiological saline.

4. Talc fine po der (purified by acids) obtained from BDH Chemicals Ltd, Poole, England. A three per cent talc suspension was prepared in physiologic saline.

5. Beryllium oxide (BeO) obtained from Hopkin and Williams Ltd, Chadwell Heath, Essex, England. A three per cent suspension was prepared in physiologic saline.

Experimental Inoculations:-

Inoculations were made into the superficial myotomal muscle on the aboral side of the fish. The inoculation site was marked by clipping out the lateral fin web in line with the site of injection (Fig. 1)

Experimental fish were sampled at regular intervals for histological examinations. Details of exact sampling times are described for the specific experiments. Fish were killed by a blow on the head and then the wedge of myotomal tissue indicated by the fin clip was excised and fixed in 10 per cent formal saline.

Histologic procedure for light microscopy:-

Tissues were processed in a 24 hour cycle in an automatic tissue processor (Shandon Elliot Ltd). Blocks were passed through ascending grades of methylated spirits, two changes of absolute alcohol, two changes of chloroform followed by two changes of paraffin wax (melting point 56^{o}C). Blocks were cut at 4-5 micron on a Leitz rotary microtome. A variety of staining methods were used to stain the sections of tissues. Sections were stained by haematoxylin and eosin (H & E), for routine general histology; Ziehl-Nielson - Methyhlene blue and Ziehl-Nielson - Tartarzine staining method for acid fast mycobacteria; Unna-Pappenheim's staining method for plasma cells and their precursors; Masson's trichrome staining method for connective tissue. Methods used were those described by Culling (1963) and Drury and Wallington (1967).

Fig. 1. This diagram shows the site of injection of the various irritants inoculum and the fin clipping site which allowed its definition after the lesion had developed.

Histologic procedures for electron microscopy:-

Sampling:

Small blocks of lesion (0.5mm x 0.5mm x 0.5mm) were rapidly excised from the injection site of freshly killed experimental animals. Razor blades were used in order to obtain the thinnest possible slices of tissue, undistorted by mechanical trauma.

Fixation:

The samples were quickly fixed in freshly prepared Karnowsky's fixative as described by Roberts et al (1963) in their ultrastructural study of plaice skin. Samples were fixed at pH.7.4 for from 5 to 12 hours at room temperature. After fixation they were washed in 0.2 M sodium cacodylate buffer for from 2 hours to 12 hours at 4^{o}C, fixed in two per cent osmium tetroxide for one hour and washed in 0.1 M sodium cacodylate buffer for ½ hour. Very little variation in fixation quality was found with the variations of fixation or washing indicated.

Dehydration and embedding:

The dehydration was carried out by passing the fixed tissues through increasing grades of ethanol, then the tissues were processed as follows:- 50/50 propylene oxide and alcohol; 100/100 propylene oxide; 50/50 propylene oxide and resin (Epon embedding mixture) and; 100/100 in resin. The resin was polymerised for 48 hours at 60^{o}C to obtain maximal hardening.

Cutting and staining:

Resin embedded tissues were first trimmed with a razor blade under a light microscope. Such trimmed blocks were then cut at one micron with a glass knife on an LKB III automatic ultra-microtome. These thick sections were picked up with a single hair brush and floated on hot, one per cent toluidine blue, stained for one minute and washed twice in distilled water. They were examined under light microscopy to enable orientation and correct sampling for cutting of ultrathin sections. Samples which showed, in the light microscope, inflammatory areas of interest, were cut at 100 A and carefully mounted on to the dull face of copper grids or on to carbon film coated grids and left to dry in air.

Two methods were used for staining the sections.

1. Uranyl acetate. The sections were stained by floating them on the drops of the two per cent freshly prepared uranyl acetate within the petri dishes for 30 minutes. They were washed with distilled water, 0.02 M sodium hydroxide, and distilled water and later left to dry in air.

2. Uranyl acetate-lead citrate (Double staining). The sections were stained by floating them on the drops of staining solution of the two per cent. Uranyl acetate for 30 minutes and then washing with 0.02 M sodium hydroxide and staining again by floating on the drops of the staining solution of lead citrate for 45 minutes. After staining sections were washed with distilled water, 0.02 M sodium hydroxide and distilled water and later left to dry in air.

Microscopy:

Stained sections were examined in an A.E.I. Corinth 275 electron microscope (magnification varied between 600 x 100000). Resolution was down to 0.9 A and accelerating Voltage 60 KV. Photographs of areas of interest were taken on roll film (Agfa T sip) and enlargements made as required.

Immunology:

Migration Inhibition Test (M.I.T.):-

Experimental animals were sensitized by an intramuscular injection of piscine Mycobacteria in physiological saline. Thirty days post innoculation, blood samples were taken from the renal portal vein of the unanaesthetized fish as described by Wardle (1971), and quickly introduced into heparinized tubes. The heparinized blood was then sucked into microhaematocrit capillary tubes and the end of the capillary tubes sealed in a bunsen flame. The capillary tubes were centrifuged in a microhaemotocrit bench centrifuge at 1500 r.p.m. for 5 minutes. The capillary tubes were then cut at the surface of the buffy coat layer and placed in a petri-dish containing 5-6 ml. of tissue culture fluid at room temperature. The tissue culture medium was prepared according to Wood (1973) as follows:-

100 ml. of chilled distilled water
10 ml of Earles BSS (Balanced Salt Solution)
2 ml of MEM AA (Minimal Essential Medium-Amino Acids)
1 ml of NE AA (Non-essential Amino acids)
1 ml of MEM Vit (Vitamins)
2 ml of Penicillin/Streptomycin
2 ml of Sodium bicarbonate

To each 6 ml of this medium was added ½ ml of Glutamine and 5 ml of Feotal Bovine Serum (FBS)* before use.

Piscine Mycobacteria was collected from culture medium by washing with physiological saline, a thick suspension of bacteria which had been broken down in an ultrasonic disintegrator (M.S.E. Ltd England) for 20 minutes. 1 ml of this crude antigen was added to the medium and the result was read after 4-5 hours. A control sample of blood from the same fish, without added antigen was always used.

A positive result was indicated by failure of the buffy coat of the tube to move out into the surrounding medium, due to release into the medium of lymphokines released by the reaction between the sensitized lymphocytes and their specific antigens. The negative control result was indicated by a fan-like head on the capillary tube consisting of uninhibited leucocytes migrating into the medium.

* Flow Laboratories

SECTION III

RESULTS

Experiment 1.

The development of giant cells and granulomata in response to inoculation of complete Freund's adjuvant at 10°C.

Freund's complete adjuvant is a mineral oil - water emulsion containing killed Mycobacterium butyricum. It is well known as a stimulus to giant cell production when used as an irritant to enhance the immune response in experimental studies in mammals (Roberts, R.J. Pers. comm.) and for this reason it was chosen, in the present study, to define the generalized chronic inflammatory response, and specifically to investigate the giant cell response in the plaice. The study was carried out at both light microscope, and electron microscope levels.

a) Light Microscopy:-

Materials and Methods:

Initially, a pilot experiment was carried out to determine the ideal dose level of irritant, the optimal sampling times and to determine if the generally accepted histological staining and fixation methods could be applied to this specific study.

The fish were inoculated with 0.05ml of adjuvant intramuscularly on the aboral side of the lateral line and the site of inoculation was marked by clipping the lateral fin level with the site of injection.

A fish was sacrificed at 3, 8, 16, 21, 28, 35, 42 days after the inoculation, and the blocks from the tissues to be examined were removed from the fish with scalpel and forceps. The processed tissue sections were examined by light microscopy and the observation of multinucleated giant cells in the inflammatory area on certain sampling days encouraged the establishment of the main experiment as follows.

In the main experiment 18 fish were used. Their size measured from 19-20 cm snout to tail fork. Inoculations were carried out in the same way as in the pilot experiment except that the inoculum volume was increased to 0.1 ml to evoke a greater cellular reaction in a larger lesion. Two fish were sacrificed at 3, 8, 16, 21, 28, 35, 42, 49, 56 days after inoculation. The tissues were sampled as blocks 0.3 cm thick and two blocks were removed in each case. This size of block fixed very adequately and good staining was achieved with all stains used.

Results:-

The inoculum had by 3 days after inoculation, induced focal muscle changes at the injection site. Many muscle fibres had degenerated, and showed fragmentation of their sarcoplasm, with centrally placed muclei, and damaged sarcoplasm was regularly invaded by macrophages (myophagia). Some of the blood vessels within the inflamatory area were surrounded by brown-black coloured melanin granules. There was evidence of diapedesis of many leucocytes. A small number of adjuvant

lacunae were also observed within the inflammatory area, among the degenerated muscle fibres; they were seen as empty vacuoles in the Haemotoxylin and Eosin stained sections. Similar sections stained by the Ziehl-Nielson method showed the adjuvant lacunae to contain aggregations of mycobacteria. These were observed best under high power and were usually sited at the edge of adjuvant lacunae as bright red coloured bacilli. The cellular inflammatory reaction consisted of a few neutrophil leucocytes (polymorphonuclear leucocytes), occasional lymphocytes and the major cellular component of the exudate, the macrophages. The neutrophils were circular in shape with an eccentric kidney shaped nucleus and pink coloured cytoplasm and their size was approximately 4 microns in diameter. Lymphocytes had a peripheral rim of cytoplasm and a circular mucleus, the diameter of the cell being 3µm. The nucleus of the macrophages was eccentrically placed and round shaped within pink coloured cytoplasm. The macrophages measured from 6-9 micron in diameter. A few were laden with acid-alcohol fast bacilli.

A similar histopathological picture was seen by the 8th day in a rather larger inflammation area. The inflammatory cells at this stage were increased in number and, in addition to the above described cells, occasional plasma cells were seen among them. Plasma cells were circular or oval in shape, 3-4 micron in diameter and possessed dense eccentric nuclei and intensely basophilic cytoplasm. In sections stained by Unna-Pappenheim's specific plasma cell method their cytoplasm stained a bright purple colour and their nuclei stained blue.

By the 16th day, the loose cellular reaction contained a high proportion of macrophages. Within the inflammatory area, the degenerative muscle fibres had already been cleared by macrophages. Adjuvant lacunae at this stage were large and numerous and again contained collections of mycrobacteria (Fig 2). Epithelioid cells first appeared at this stage, and they surrounded some of the adjuvant lacunae as a rim up to 3 or 4 layers thick. They were polygonal in shape with a circular to oval shaped nucleus, and indistinct cell margin. They measured 6-10 micron in diameter. They appeared to merge with their neighbouring cells and their cytoplasm was eosinophilic. Other cells seen were lymphocytes, plasma cells, fibroblasts and occasional Langhans type multinucleated giant cells. The fibroblasts were spindle shaped with an oval mucleus. Langhans type giant cells were circular or oval and their multiple nuclei were distributed at the periphery, usually in a horse shoe configuration with eosinophilic pink coloured cytoplasm. Their size varied between 16-23 microns and their nuclei number from 3-10.

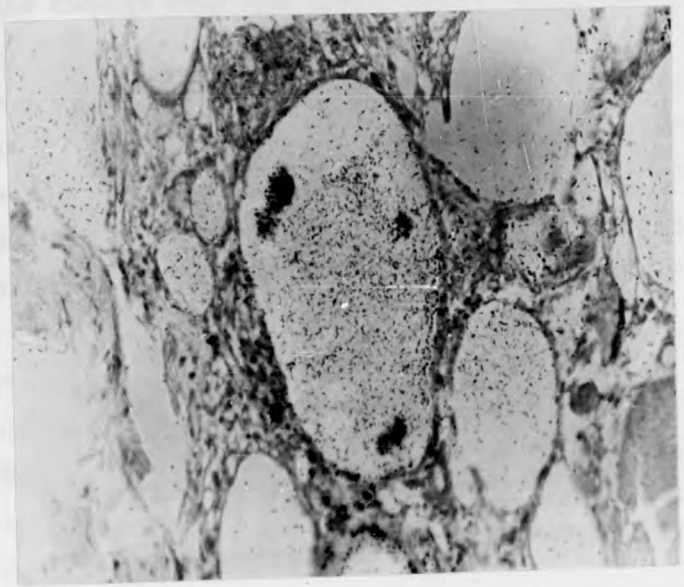
After the 21-28th days, the cellular reaction of the inflammatory response was more pronounced, and comprised macrophages, epithelioid cells, plasma cells, fibroblasts, lymphocytes, and multinucleated giant cells (both Langhans type and foreign body type by this time) (Fig 3). Whereas the Langhans type giant cells were circular to oval in shape and had their nuclei disposed at the periphery of the

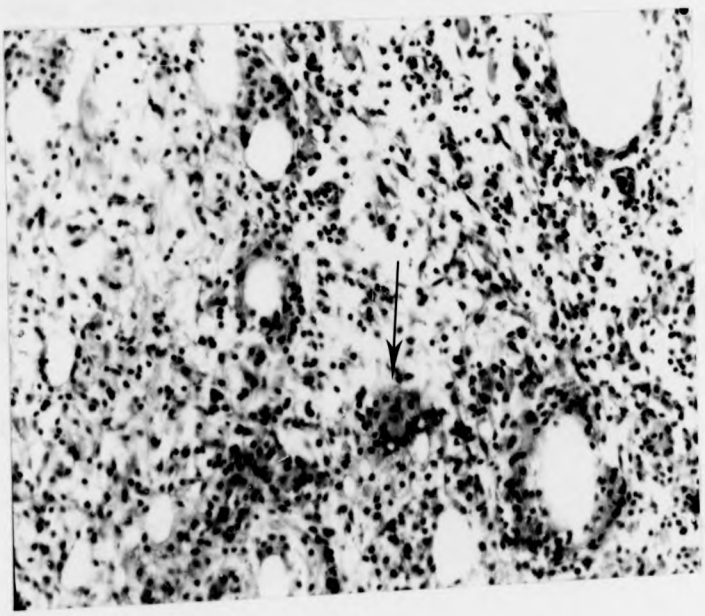
Fig (2) Adjuvant lacunae containing aggregations of Mycobacteria. Both macrophages and epithelioid cells are present around the adjuvant lacunae, and these also possess Mycobacteria within their cytoplasm. The plane focus is on the Mycobacteria and hence the cellular background is less distinctly focussed. Freund's complete adjuvant 16 days.

Ziehl-Nielson - Methylene blue x 500

Fig (3) Inflammatory area showing cell types 21 days after complete Freund's adjuvant injection. Cells present include epithelioid cells, around the adjuvant lacunae, giant cell (arrowed), macrophages, plasma cells, fibroblasts and lymphocytes.

H & E x 500





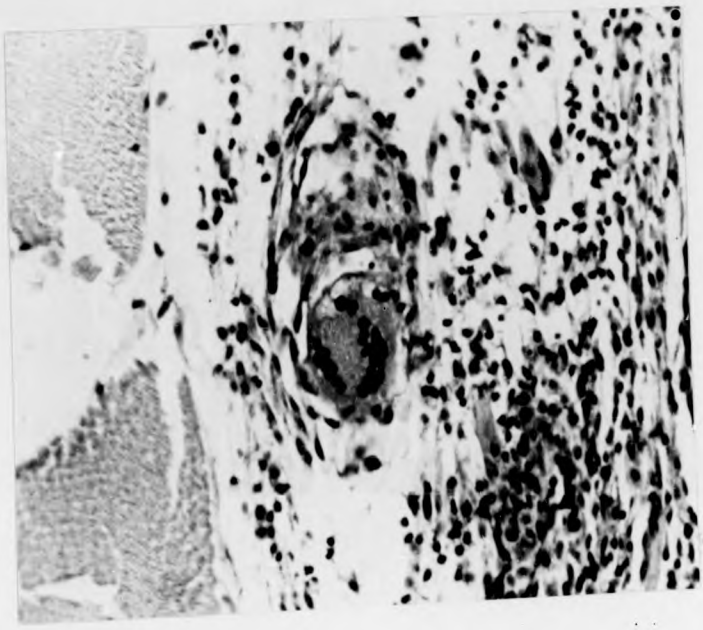
cell, the foreign body type giant cells, which had not been seen previously, were irregular in shape or elongated and were usually seen around the adjuvant lavunae. Their multiple nuclei were scattered randomly throughout the cytoplasm (Figs 4, 5, 6). The number of nuclei in the Langhans and foreign body type giant cells varied, apparently depending on the plane of section between 4 and 20 for the nuclei of the Langhans type giant cells and between 4 and 30 for the foreign body giant cells. Both types of multinucleated giant cells contained apparently empty vacuoles within their cytoplasm, but they did not appear to contain any phagocytosed acid-alcohol fast bacilli. Their size varied considerably. Langhans type giant cells measured 18-43 micron in diameter whereas the bigger foreign body giant cells measured 40-60 microns. Their nuclei were stained very densely basophilic while their cytoplasm was stained pink coloured in H & E stained sections. Among the inflammatory cells focal collections of epithelioid cells were often seen. By this stage fibroblast proliferation had become very prominent and new capillary vessels were apparent revascularising the area of the granuloma. Plasma cells and their procursors were seen in large numbers (Fig 7). The plasma cells measured 3-4 µm in diameter, but their procursors, the large pyroninophilic cells measured 5-6 µm. Lymphocytes were seen as small collections at different sites of the inflammatory lesion.

Fig (4) The large Langhans type giant cell containing multiple nuclei distributed in horse shoe configuration. Freund's complete adjuvant 28 days.

H & E x 800

Fig (5) Langhans type and foreign body type giant cells within the inflammatory area of plaice inoculated with complete Freund's adjuvant 28 days.

H & E x 800



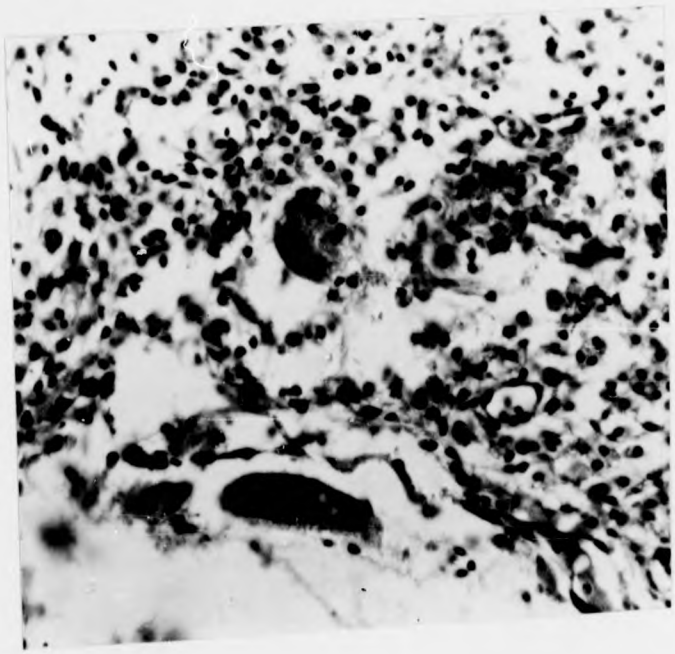
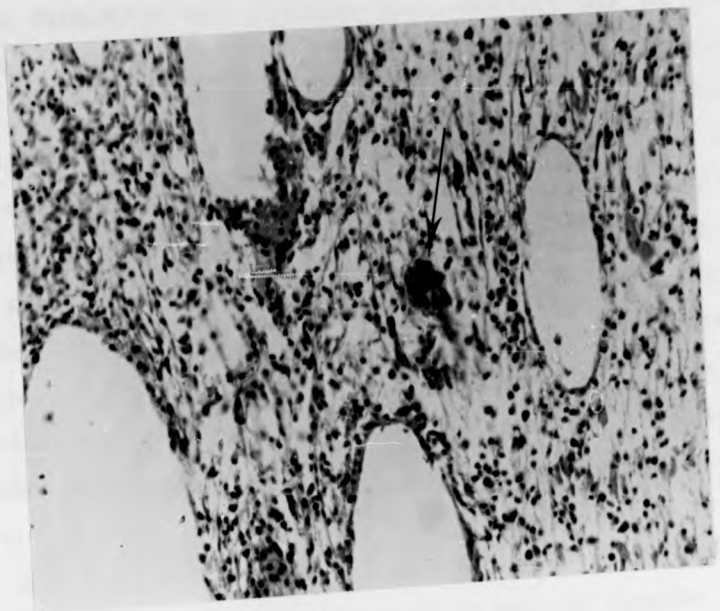


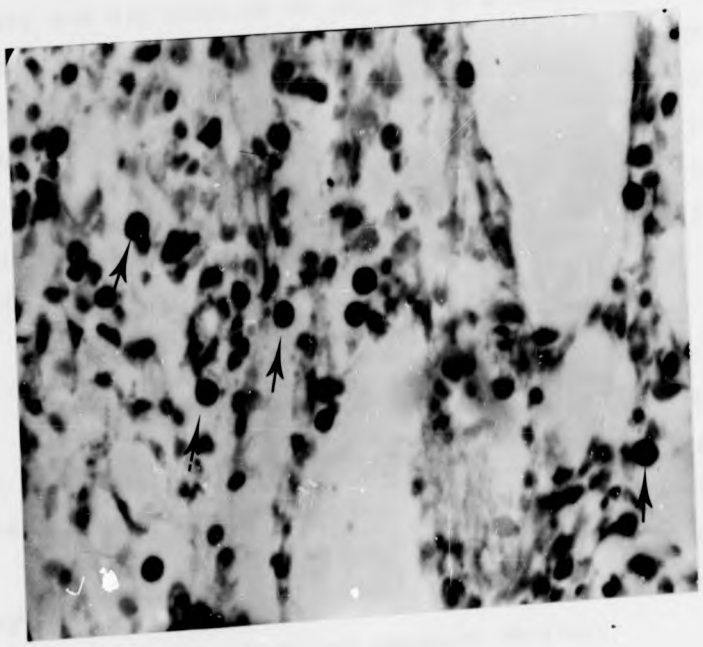
Fig (6) The foreign body type giant cell (arrowed). Complete Freund's adjuvant 28 days

H & E x 500

Fig (7) Plasma cells (arrowed) within the inflammatory area of plaice inoculated with complete Freund's adjuvant 28 days.

Unna-Pappenheim x 800





By the 35th-42nd days although the granuloma was more cellular, multinucleated giant cells were rare. Langhans type giant cells were still occasionally observed among the inflammatory cells. Epithelioid cells were the predominant cells rather than macrophages and were well developed around the adjuvant lacunae (Fig 8). They were also arranged, in a whorl like pattern, as discrete focal accumulations among the inflammatory cells (Fig 9). The presence of collections of lymphocytes at different sites of the granuloma was very striking (Fig 10). Many blood vessels showed the changes of developing endarteritis obliterans, while medium size vessels showed medial hypertrophy both suggesting degeneration of a vascular supply which had outlasted its usefulness (Fig 11). Instead of isolated fibroblasts as in earlier samples fibrous tissue per se was developing into the granuloma from the periphery and appeared to be derived to a large extent from the interstial tissue of the muscle septae. Also many fibres of the muscle at the periphery of the granuloma were showing evidence of regeneration. The Masson trichrome method served to define this feature very well, the collagen fibres, even the finest, staining green while the small regenerating muscle fibres stained bright red.

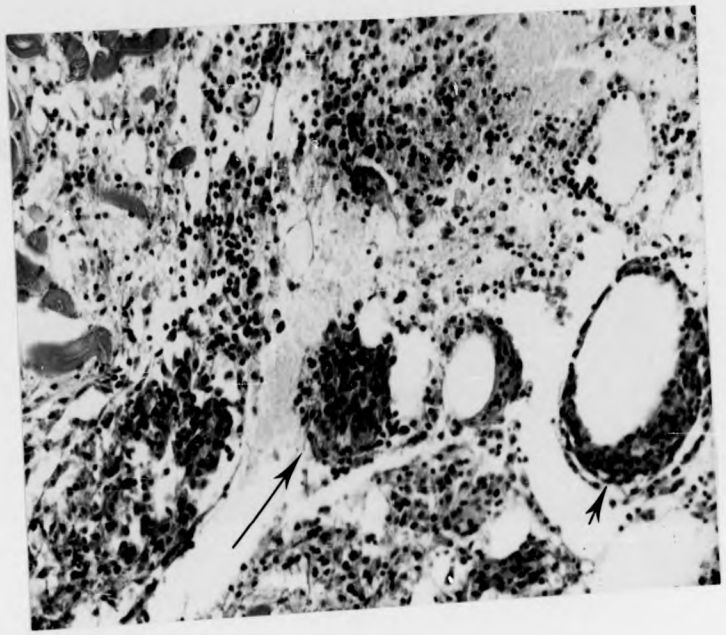
Resolution of the granuloma was apparent by the 49th - 56th days. It was considerably less cellular although there was little increase in fibrosis or regeneration of muscle fibres. Occasionally giant cells (Langhans type only) were still seen. Acid-alcohol fast bacilli were still to be seen but in very reduced number and often occurred as single bacilli instead of clumps, as they had appeared earlier.

Fig (8) The inflammatory response to complete Freund's adjuvant at 10°C, 42 days inoculation. The predominant cell type around the lacunae in the epithelioid cell and these areas also present in whorls amid the other inflammatory cells (arrowed). A Langhans type giant cell and plasma cells are also prominent.

H & E x 500

Fig (9) Non-caseating sarcoid like lesions (arrowed) in a fish from the same samples as figure (8).

H & E x 200



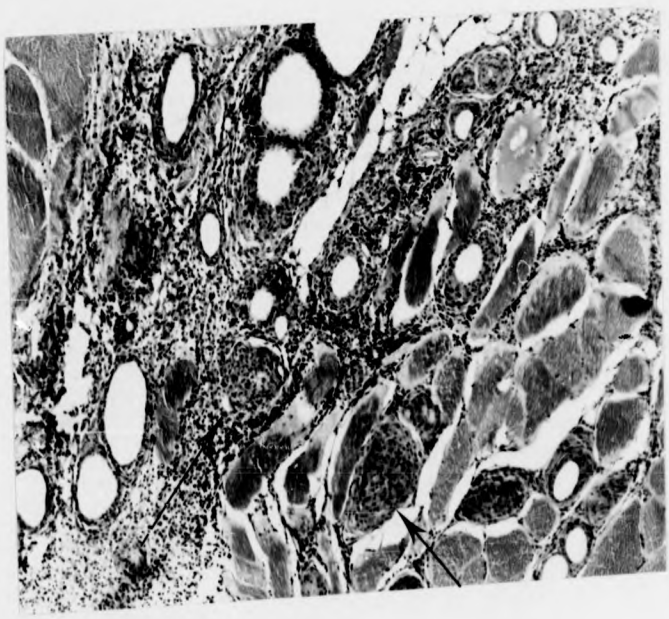


Fig (10) Focal lymphocytic aggregations (arrowed) in a lesion from the same fish as figure (8)

H & E x 500

Fig (11) Complete Freund's adjuvant, 42 days at 10°C. Obliterative changes in a degenerating blood vessel with a perivascular lymphocytic infiltrate. Note the extensive collar of melenocytes around the vessel.

H & E x 500

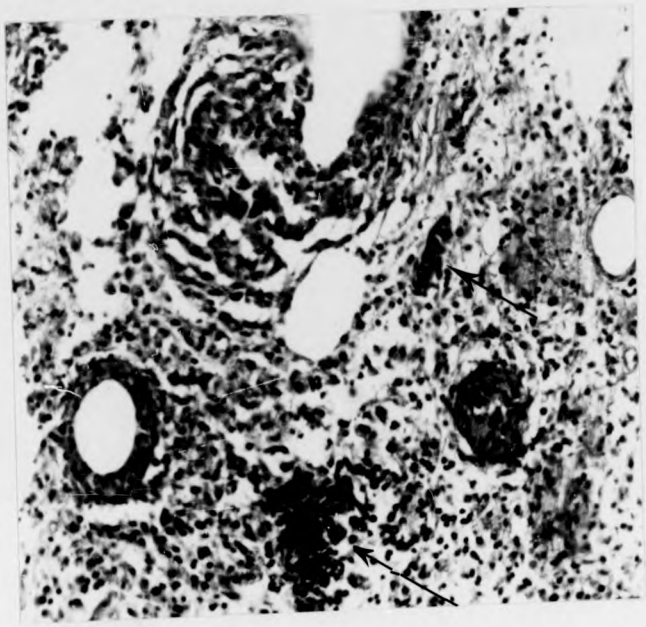
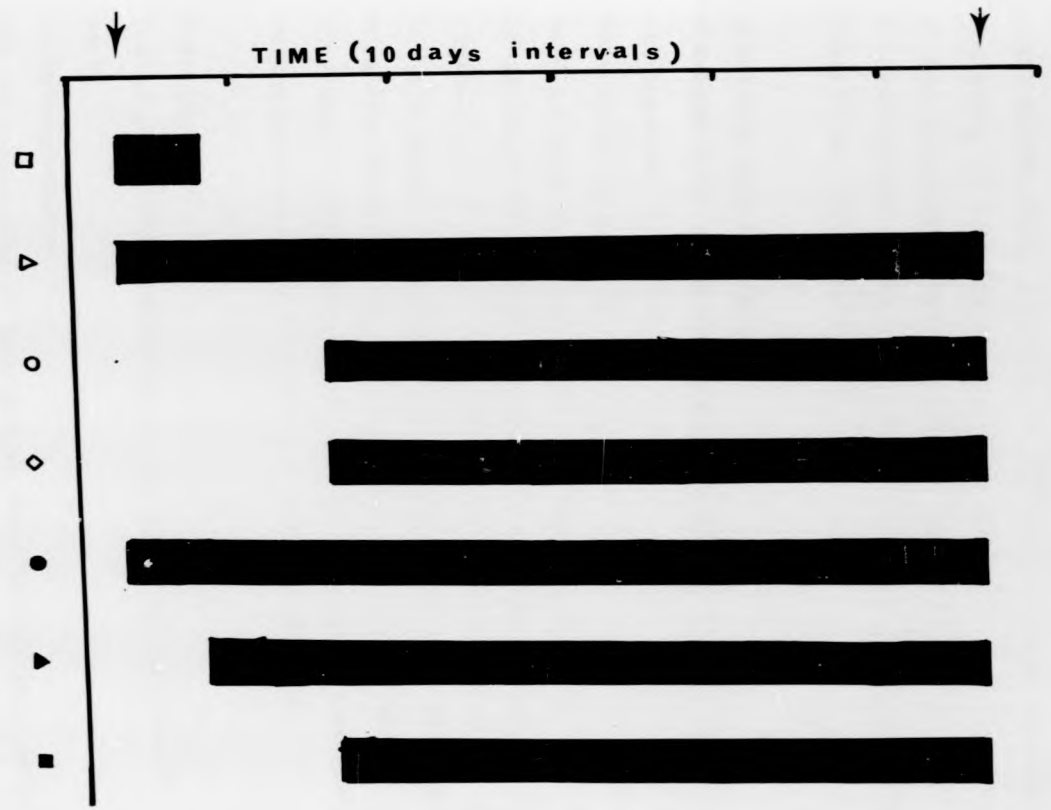


Fig (12) Duration of the inflammatory cell response within the complete Freund's adjuvant granuloma at 10°C. Commencement and termination of sampling days are shown by arrows.

- □ Neutrophils
- △ Macrophages
- ○ Epithelioid cells
- ◇ Giant cells
- ● Lymphocytes
- ▲ Plasma cells
- ■ Fibroblasts


TIME (10 days intervals)

b) Electron Microscopy:-

The sequential morphology of the granulomatous inflammation evoked by complete Freund's adjuvant as observed under the light microscope in plaice at 10°C has already been described. The study of the lesions, and specifically the giant cells of the granulomatous inflammation was extended to the ultrastructual level on a limited number of experimental animals as described below.

Materials and Methods:

The time over which the most obvious giant cell response was apparent in the light microscopy study was the period from 21-28 days after injection at 10°C. Six fish were used in this experiment and they were inoculated with 0.1 ml of adjuvant which was diluted with an equal volume of physiologic saline. Two fish were sacrificed at 21, 28 and 30 days after the inoculation since these were the times when the various cellular component were most readily observed. Electron microscopy was carried out as described in the main section of the materials and methods.

Results:

The ultrastructual details of the cells of granulomata were:

a) Macrophages:-

The cell membrane of macrophages were thrown into pseudopodia. The nucleus, eccentric with a regular margin, had its chromatine dispersed marginally around the nuclear membrane.

Lysosomes appeared as numerous small or larger dense granules within the cytoplasm. The golgi apparatus was well developed, occupying the perinuclear area and consisting of lamellar and vesicular elements. Mitochondria were numerous, their shape and size varying somewhat. The rough endoplasmic reticulum was scattered at the periphery of the cell or arranged in stacks at one pole of the cell (Fig 13).

b) Epithelioid cells:-

Epithelioid cells possessed round to oval shaped nuclei with a smooth nuclear membrane again with marginally dispersed chromatin. In some of the epithelioid cells, the cell membranes were not thrown into pseudopodia but showed fingerlike processes interdigitating with those of adjacent cells. Their golgi complexes were variable in their prominence and the mitochondria varied in number. Rough endoplasmic reticulum was present but the amount varied considerably. Some epithelioid cells showed polar accumulations of rough endoplasmic reticulum as in the macrophages. Again, in resemblance to the macrophages, the cytoplasm of epithelioid cells contained varying numbers of lysosomes, although these were never as numerous as in the macrophage. (Fig 14).

Fig (13) Macrophage within inflammatory lesion Freund's complete adjuvant 28 days at $10^{o}C$.

E & M x 6000

N : Nucleus

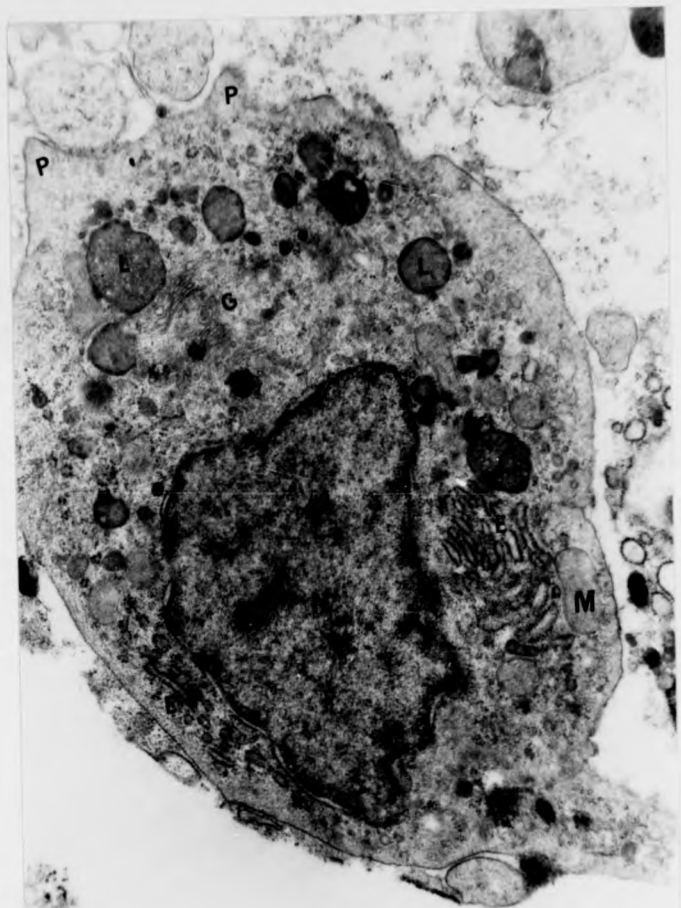
L : Lysosome

G : Golgi apparatus

M : Mitochondria

E : Endoplasmic reticulum

P : Pseudopodium


P
P
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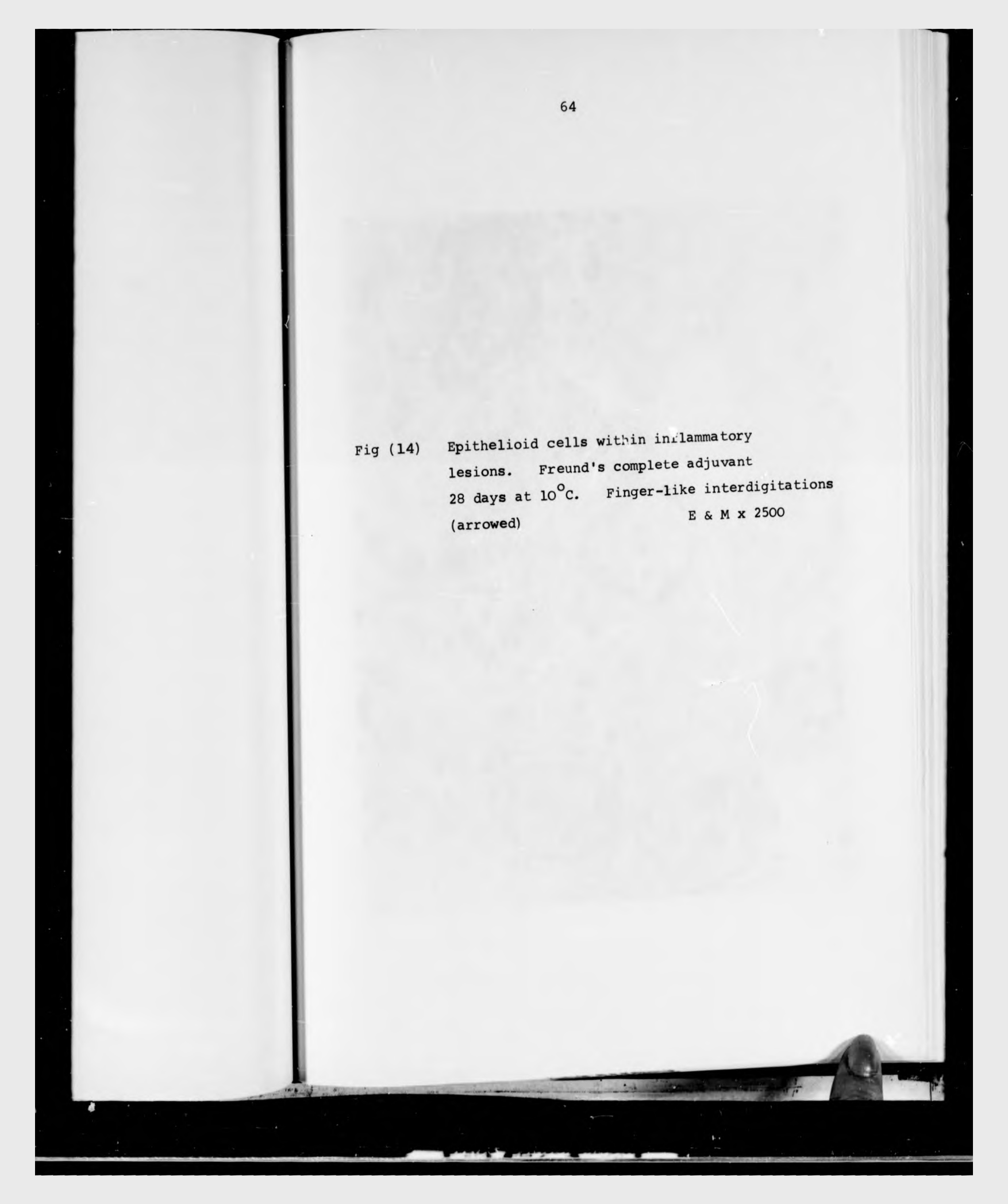
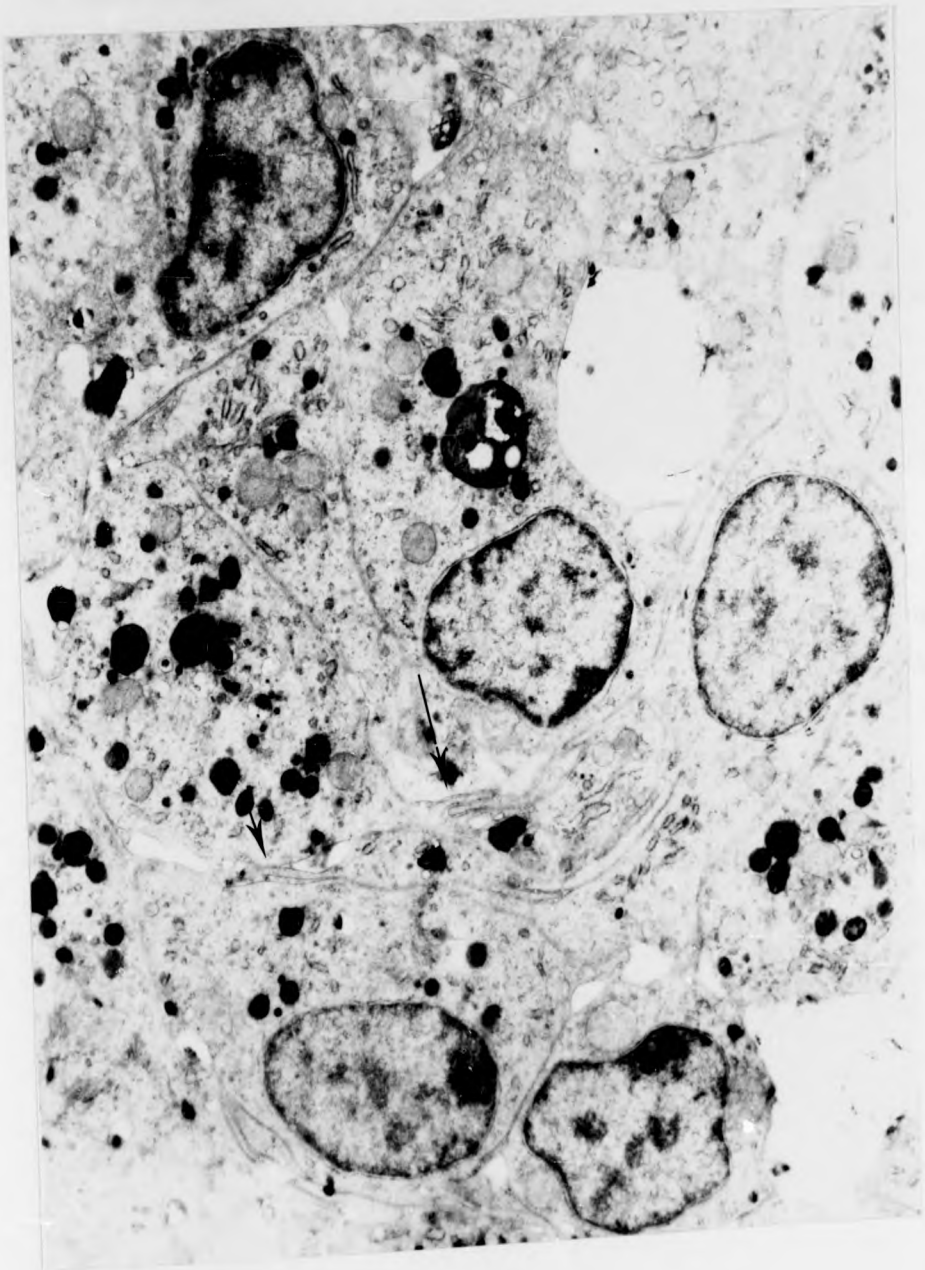


Fig (14) Epithelioid cells within inflammatory lesions. Freund's complete adjuvant 28 days at 10°C. Finger-like interdigitations (arrowed) E & M x 2500



c) Giant cells:-

The multinucleated giant cell had the same basic components as the adjacent epithelioid cells shown in figures (15, 16). However, they were so large (18 μm to 60 μm) that it was almost impossible to secure a complete cross section of a giant cell in any one field of view. The nuclear chromatin was scattered around the periphery of the nucleus in a similar fashion in each individual nucleus. The mitochondria were located centrally, within the ring of nuclei and were interspersed with darkly staining lysosomes of varying sizes from (0.2 μm to 0.7 μm). There was some evidence of focal aggregations of rough surfaced endoplasmic reticulum around the periphery of the cells, but it was not possible to determine whether the focal distribution was associated with derivation from any individual contributing macrophages which had themselves been rich in such organelles. The cytoplasmic membrane was regular and did not show interdigitation with other cells, as in the case of the epithelioid cells, or pseudopodia as in the macrophages. The individual Golgi components were well developed and situated close to relevant nuclei.

d) Lymphocytes:-

The nucleus of the lymphocyte dominated the cell, being surrounded by only a thin rim of cytoplasm. Occasional mitochondria were present within their cytoplasm but few other organelles were evident. Their nuclear chromatin was dense but dispersed marginally (Fig 17).

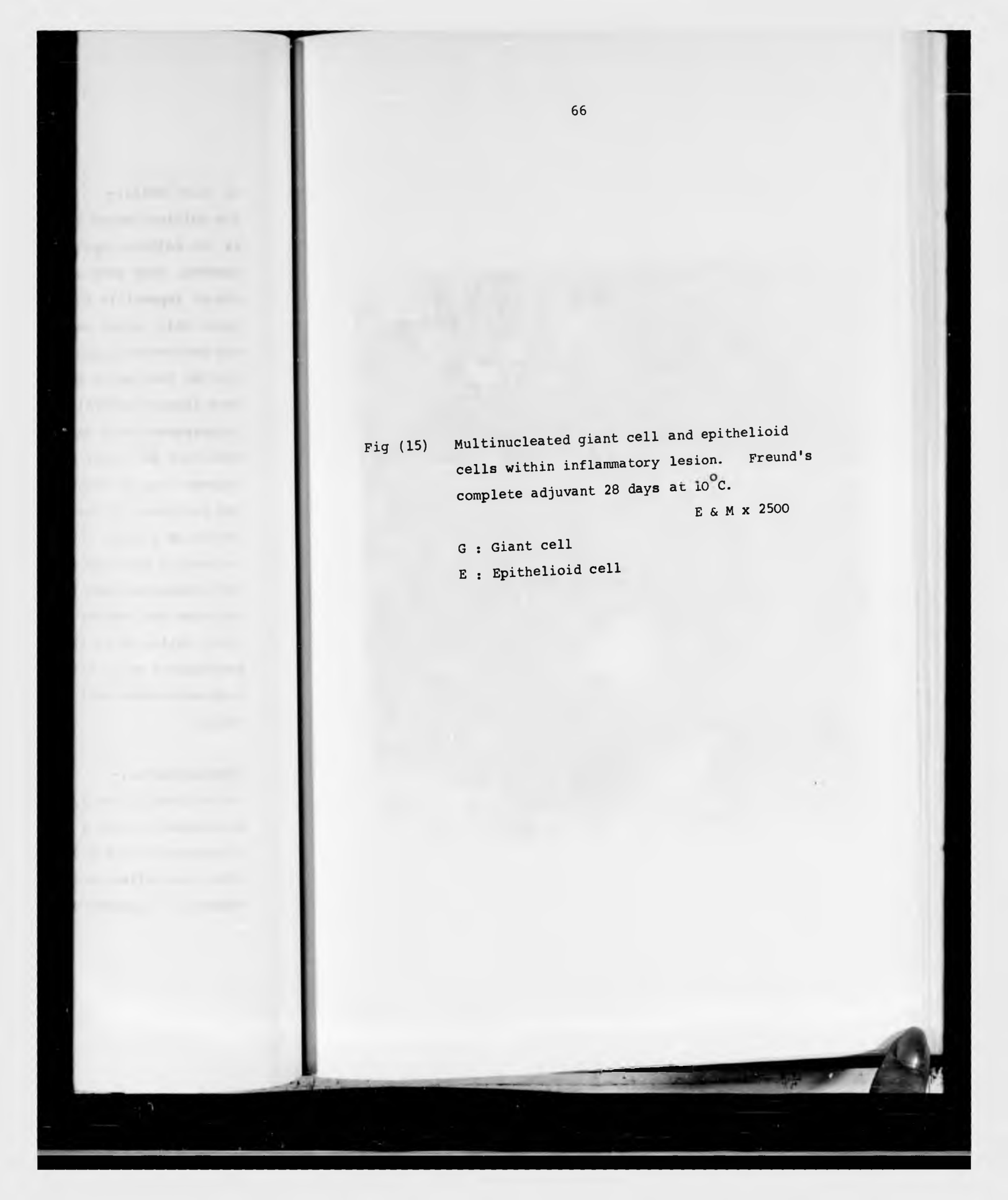


Fig (15) Multinucleated giant cell and epithelioid cells within inflammatory lesion. Freund's complete adjuvant 28 days at 10°C.

E & M x 2500

G : Giant cell

E : Epithelioid cell

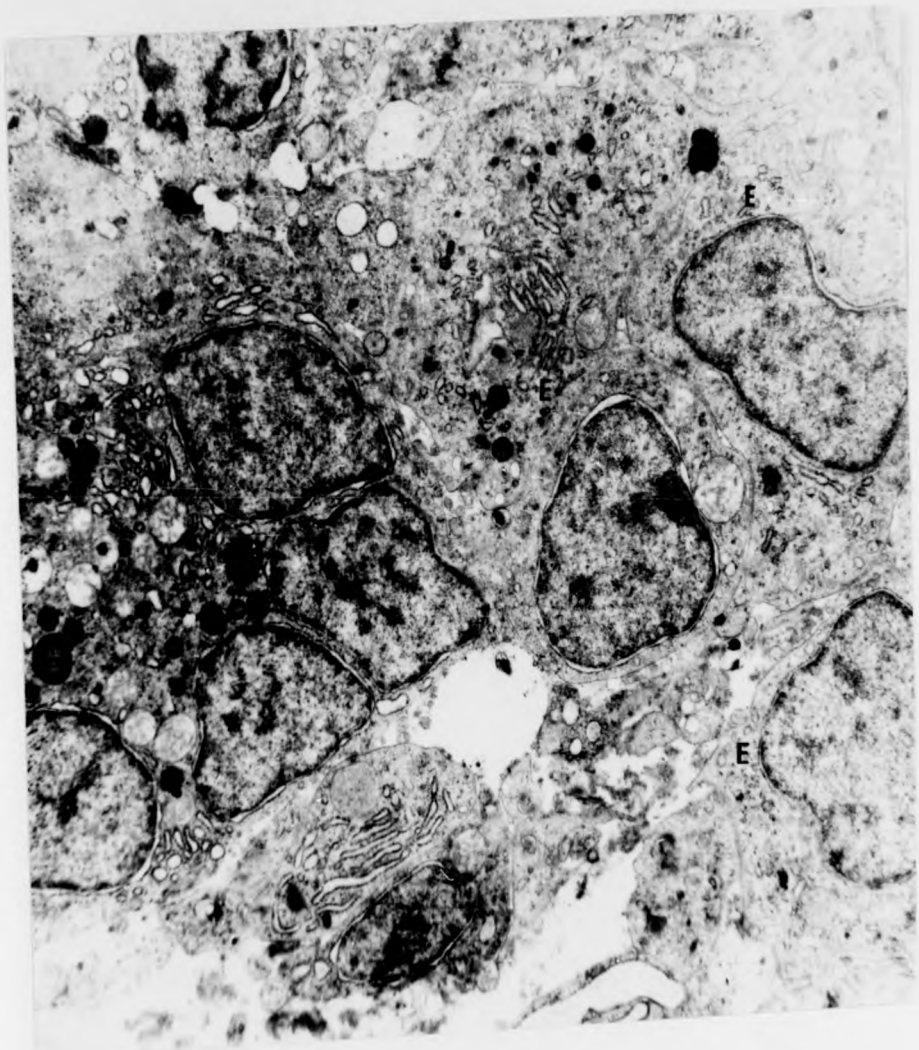

E
E
E

Fig (16) The giant cell E & M x 4000

N : Nucleus

L : Lysosome

M : Mitochondria

G : Golgi apparatus

R : Rough endoplasmic reticulum

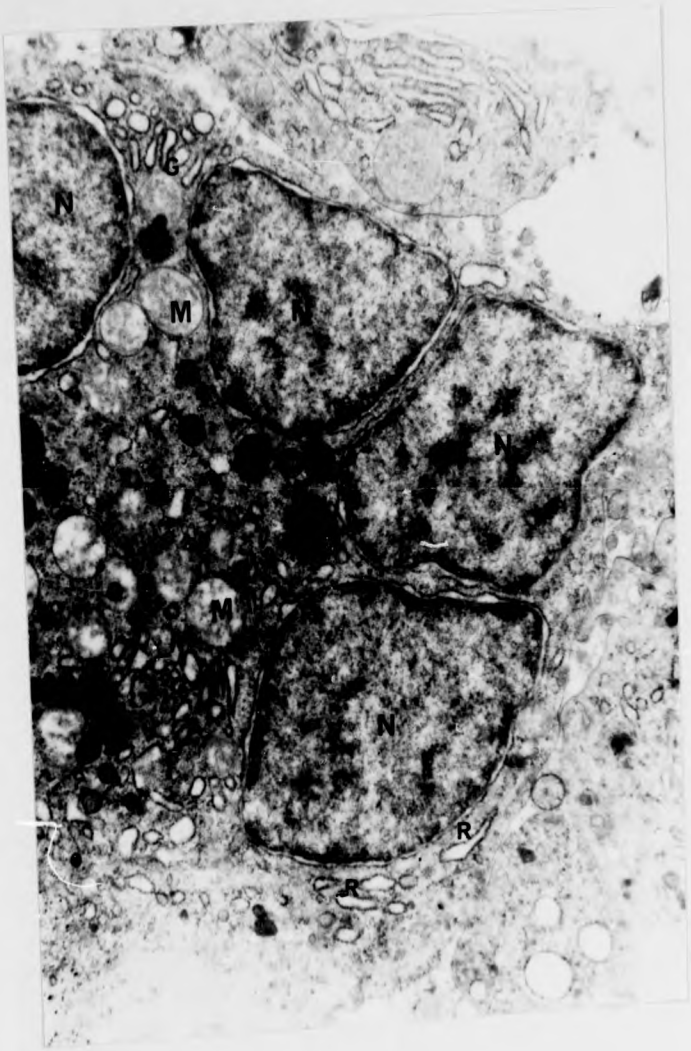
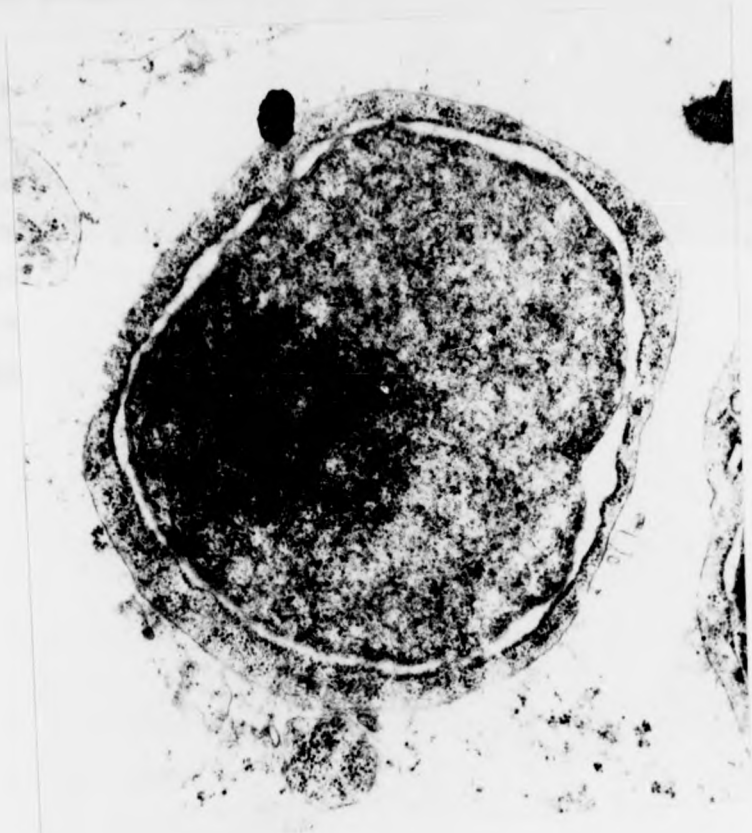

N
M
N
N
M
N
R
R

Fig (17) A lymphocyte, within an inflammatory lesion. Freund's complete adjuvant 21 days at 10°C.

E & M x 6000



e) Plasma cells:-

The main feature of the plasma cells was the very extensive endoplasmic reticulum which occupies the cytoplasm. The endoplasmic reticulum was dilated into large cisternae in some of the more mature plasma cells. Mitochondria were present between endoplasmic reticulum stacks (Figs 18, 19).

f) Fibroblasts:-

The ultrastructure of fibroblasts varied depending on the degree of fibroblast activity of the area examined. The active cells had scattered nuclear chromatin and extensive endoplasmic reticulum with dilated cisternae whereas in the less active cells the chromatin was more dense and endoplasmic reticulum much less well developed (Fig 20).

Fig (18) A plasma cell within an inflammatory lesion. Freund's complete adjuvant 21 days at 10°C.

E & m x 6000

M : Mitochondria

N : Nucleus

R : Rough endoplasmic reticulum

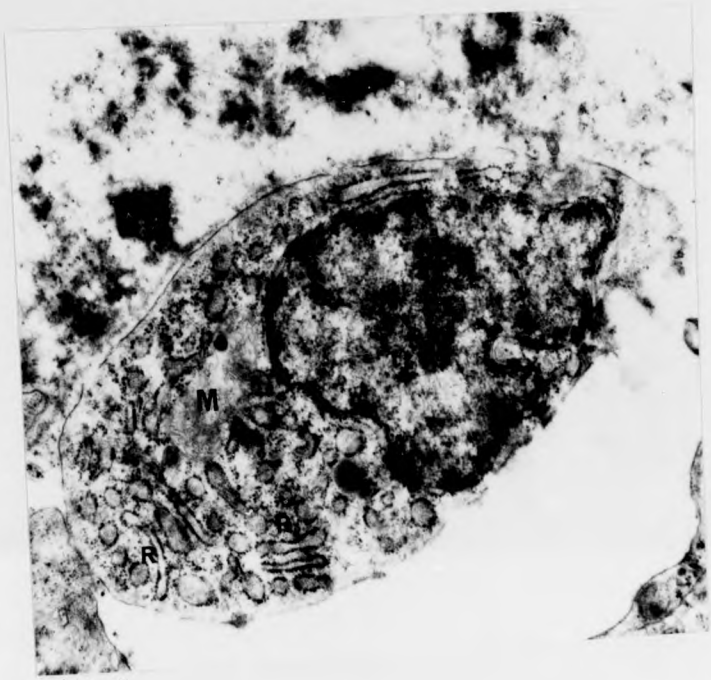
Fig (19) A plasma cell within an inflammatory lesion. Freund's complete adjuvant 21 days at 10°C.

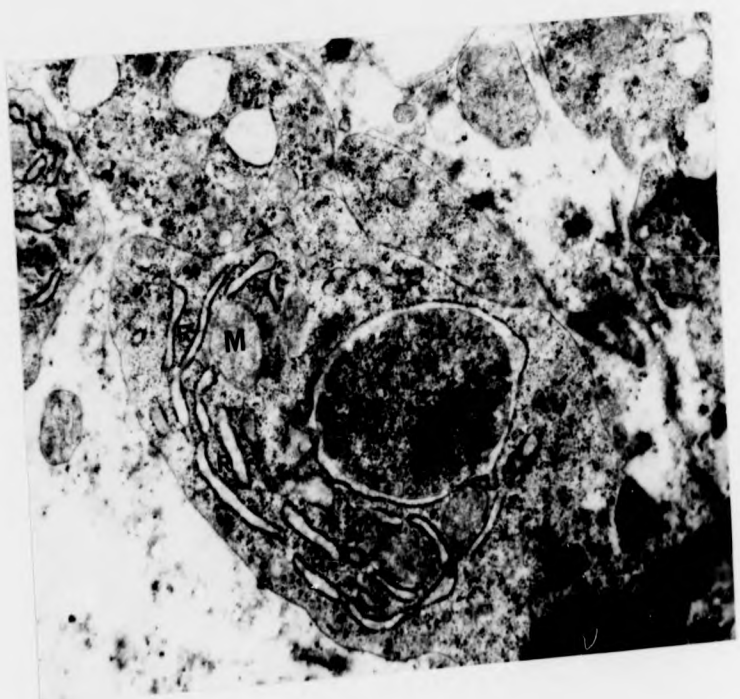
E & M x 6000

M : Mitochondria

R : Rough endoplasmic reticulum

N : Nucleus


M
R


M

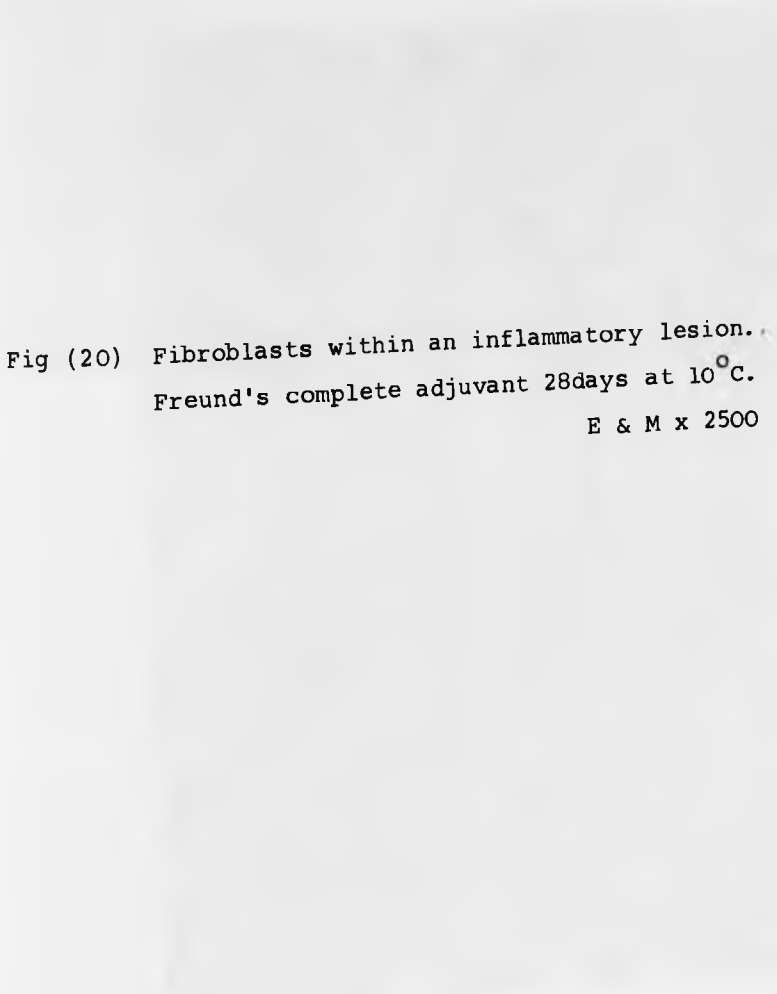


Fig (20) Fibroblasts within an inflammatory lesion. Freund's complete adjuvant 28days at 10°C. E & M x 2500

Experiment 2.

A comparison of the effect of temperature on the development of multinucleated giant cells in response to inoculation of Freund's complete adjuvant.

Since inhibition of the rate of inflammatory response development due to reduction of temperature has been reported by several workers in teleost fish (Janssen and Waaler 1967; Finn and Nielson 1971; McQueen et al 1973; Roberts et al 1973; Anderson and Roberts 1975), the persent experiment was devised to define the effect of lowering of environmental temperature on giant cell development. The study was basically similar to the previous experiment but the environmental temperature was reduced from 10°C to 5°C.

Materials and Methods:-

Nine 2+ plaice were used. They were 18-20 cm in length and were acclimitized for two weeks in a cold room at 5°C. before intramuscular injection of 0.1 ml Freund's complete adjuvant which was diluted with physiological saline as described in general materials and methods. Fish were sacrificed at 3, 8, 16, 21, 28, 35, 42, 49 and 56 days after injection and samples were excised from the injection sites. The histopathological sections from these samples were stained by the following staining methods: Haematoxylin and eosin, Ziehl-Nielson, Masson's trichrone, Unna-Pappenheim.

Results:-

Three days after inoculation sections showed that there was a sparse inflammatory response comprising mainly lymphocytes and a few neutrophil around the dilated vessels. A few adjuvant lacunae were present at the reaction site. However, eight days after inoculation muscle fibre necrosis had developed with myophagia, at the injection site. The inflammatory infiltate comprised predominantly macrophages, which had invaded the degenerated muscle fibres to produce the myophagia.

The most striking feature of the inflammatory reaction in the sampling at the 16th, 21st, 28th, 35th and 42nd days was that while active myophagia was taking place on the many degenerative muscle fibres, at the same time epithelioid cells were diffusely aggregated among the myophagic necrotic muscle fibres (Fig 21). Epithelioid cells also surrounded some of the adjuvant lacune. The necrotic muscle fibres which showed active myohpagia were invaded also by macrophages and neutrophils. The neutrophils commonly possessed a kidney shaped rather than a lobulated nucleus. The inflammatory infiltrate occasionally contained plasma cells, although, at high temperature they had been considerably greater in number. The inflammatory reaction at low temperature at these stages was found to be quite different in many respects from the inflammatory at high temperature (10^{o}C.). At the high temperature, myophagia was not seen at these stages, since necrotic muscle fibres had already been completely cleared by macrophage activity and replaced by a mixture of macrophages, epithelioid cells, lymphocytes, plasma cells, fibroblasts and multinucleated giant cells.

By the 42nd day at 10°C, the resolution of the granuloma had started with development of fibrous tissue and regeneration of the muscle fibres at the periphery of the lesion but at low temperature this was not so and although occasionally, phagocytosis of acid-alcohol fast bacilli by macrophages was seen, no evidence of giant cells was seen at any stage of this experiment.

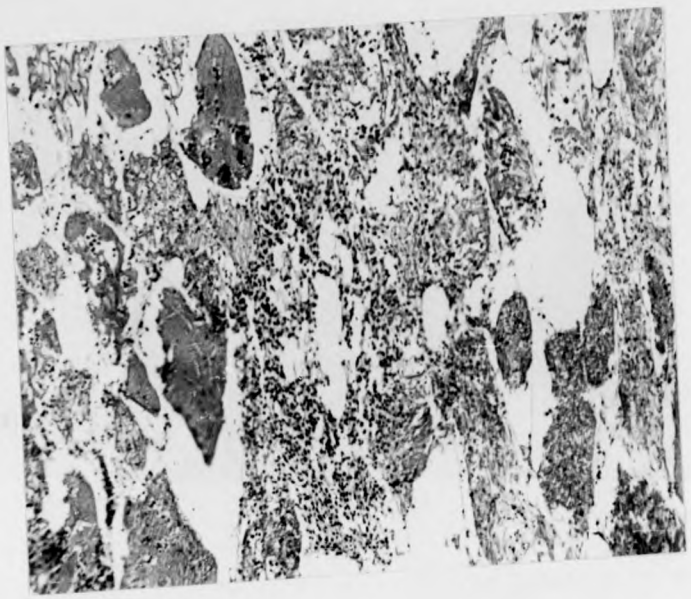
Between the 49th - 56th days, clearance of the necrotic muscle fibres was considerable and they were replaced by the inflammatory cells, which were predominatantly epithelioid cells, macrophages, lymphocytes, plasma cells and fibroblasts (Fig 22, 23). However, there were still a few muscle fibres showing myophagia at the periphery of the lesion. Epithelioid cells, lymphocytes and plasma cells were arranged in an almost folicular fashion usually around the adjuvant lacunae, but no multinucleated giant cells were seen among the inflammatory cells. There was no development of fibrous tissue or regeneration of muscle fibres or degenerative changes within vessels, in sharp contrast to the situation at 10°C after this time.

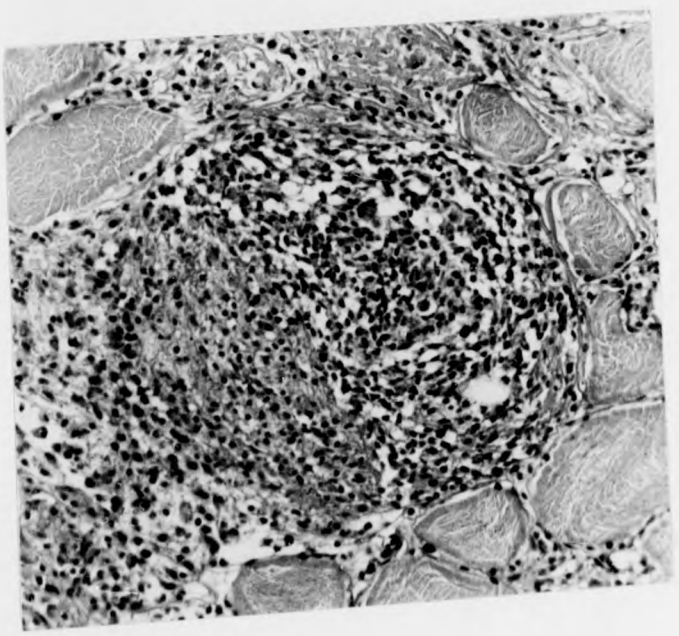
Fig (21) Low power micrograph of inflammatory area following injection of Freund's complete adjuvant 21 days at $5^{o}C$. Myopagia is still containing at the same time as epithelioid cells are present

H & E x 200

Fig (22) Freund's complete adjuvant at $5^{o}C$ after 49 days. The myophagia is now complete and the tissue replaced by epithelioid cells and macrophages

H & E x 500





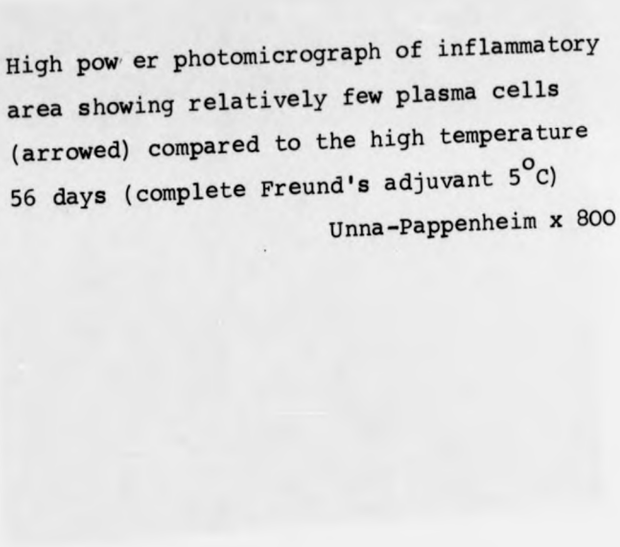


Fig (23) High pow er photomicrograph of inflammatory area showing relatively few plasma cells (arrowed) compared to the high temperature 56 days (complete Freund's adjuvant 5°C)

Unna-Pappenheim x 800

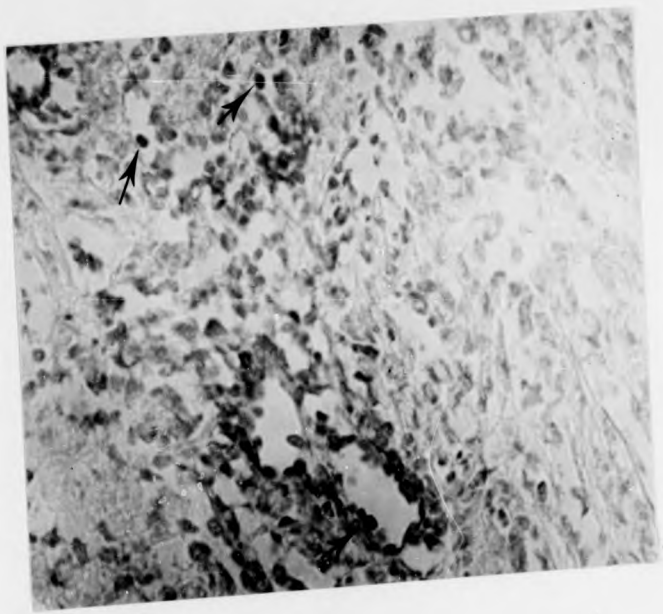


Fig 24 Duration of the inflammatory cell response within the complete Freund's adjuvant granuloma at 5°C. Commencement and termination of sampling days are shown by arrows.

□ Neutrophils

△ Macrophages

○ Epithelioid cells

● Lymphocytes

▲ Plasma cells

■ Fibroblasts

TIME (10 days intervals)

Experiment 3.

The development of multinucleated giant cells in response to inoculation of incomplete Freund's adjuvant at 10°C.

Development of giant cells in the granulomatous lesions of mammals in response to incomplete Freund's adjuvant has been reported by several workers (Freund 1965; Spector and Willoughby 1968). The purpose of this study was to determine whether the incomplete adjuvant could provoke giant cell production in the plaice as had been the case with the complete, Mycobacteria containing adjuvant. This study was carried out at light microscope level only.

Material and Methods:-

Prior to the main experiment, a pilot experiment was carried out at 10°C: for this six small plaice were used. They were 7-8 cm in size and were inoculated intramuscularly with a 0.05 ml insult of incomplete Freund's adjuvant which was diluted with an equal volume of sterile physiologic saline before injection. The purpose of the pilot experiment was to determine suitability of the chosen dose, and to determine the most suitable sampling days. The fish were sacrificed at 3, 8, 10, 16, 18 and 21 days after inoculation. Examinations of histological sections from the various samples showed that the dose used produced adequate inflammatory responses but since no multinucleated giant cells were observed a longer term sampling period was deemed necessary to be certain that they did not occur at a later stage.

In the main experiment, eight 1^{+} plaice were used. They were 10-12 cm in length. The fish were inoculated with the increased volume of insult of 0.1 ml incomplete adjuvant which was equally diluted with the sterile physiological saline before injection. They were sacrificed at 3, 8, 16, 21, 28, 35, 42, 56 days after inoculation. Tissue samples were taken from the injection site, and quickly fixed in 10% formal saline. Sections from this sample were stained with haemotoxylin and eosin, Masson's trichrome and Unna-Pappenheim's method.

Microscopy:-

Inoculation of incomplete Freund's adjuvant intramuscularly into the fish provoked an inflammatory reaction in the area of the injection site after three days. The lesion consisted of neutrophils, Lymphocytes and, predominantly, macrophages. Among the degenerated muscle fibres, there were few adjuvant lacunae. By the eighth day, the inflammatory cells were increased in number and in addition, occasional plasma cells were seen.

By the 16th and 21st days after inoculation, the inflammatory reaction predominantly comprised epithelioid cells rather than macrophages and there were slightly increased numbers of plasma cells and lymphocytes. In addition to these cells, there was a surrounding network of a delicate tracery of collagen fibres.

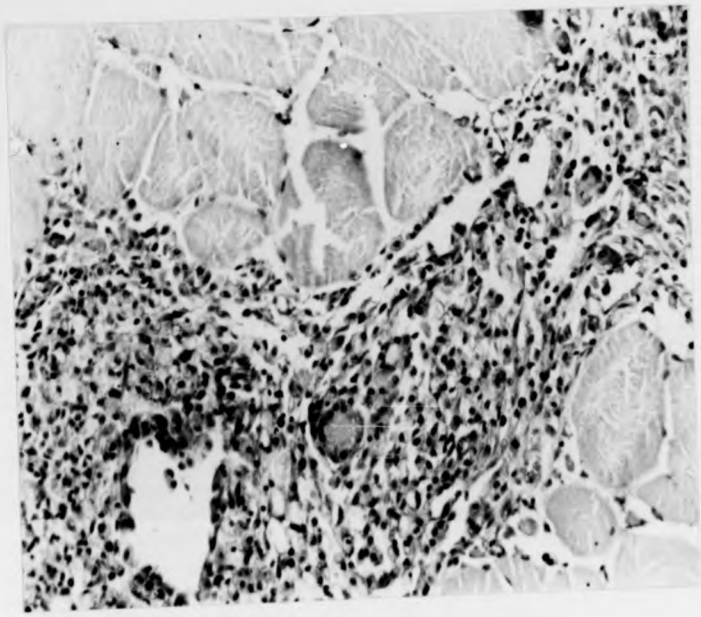
Epithelioid cells surrounded the adjuvant lacunae in sheets with some plasma cells and lymphocytes intermixed but no multinucleated giant cells were seen among them, although, at these stages multinucleated giant cells had been seen among the inflammatory cells in response to complete Freund's adjuvant at 10°C. However, giant cells did appear in the sections taken at 28 days and were present up to the last sampling date at 56 days. Multinucleated giant cells both Langhan$ and foreign body type were seen among the inflammatory cells. (Figs 25, 26). The epithelioid cell was the predominant cell of the inflammatory response rather than macrophages and surrounded the adjuvant lacunae in dense sheets intermingled with macrophages and plasma cells (Fig 27). By the 42nd and 56th days, the sheets contained discrete follicular epithelioid cells granulomas. Langhans type giant cells were circular to oval in shape and their size varied from 17 micron to 35 micron. Their nuclei had the typical horse shoe configuration of their type (Fig 28) the number of nuclei varying from 4 to 14. Foreign body type multinucleated giant cells were typically irregular or oval in shape (Fig 29) and their size varied from 27 micron to 60 micron in diameter. They contained varying numbers of nuclei from 15 to 19, their nuclei being scattered randomly within the cytoplasm. Both the Langhans and foreign body type giant cells contained a variety of vacuoles within their cytoplasm.

Fig (25) The inflammatory area containing dense sheets of epithelioid cells, intermingled some macrophages, lymphocytes, few plasma cells, and in the middle of the lesion Langhans type giant cell which contain small vacuoles (Freund's incomplete adjuvant 28 days 10°C)

H & E x 500

Fig (26) The inflammatory area containing foreign body type giant cell at the edge of an adjuvant lacuna (incomplete adjuvant 28 days 10°C)

H & E x 800



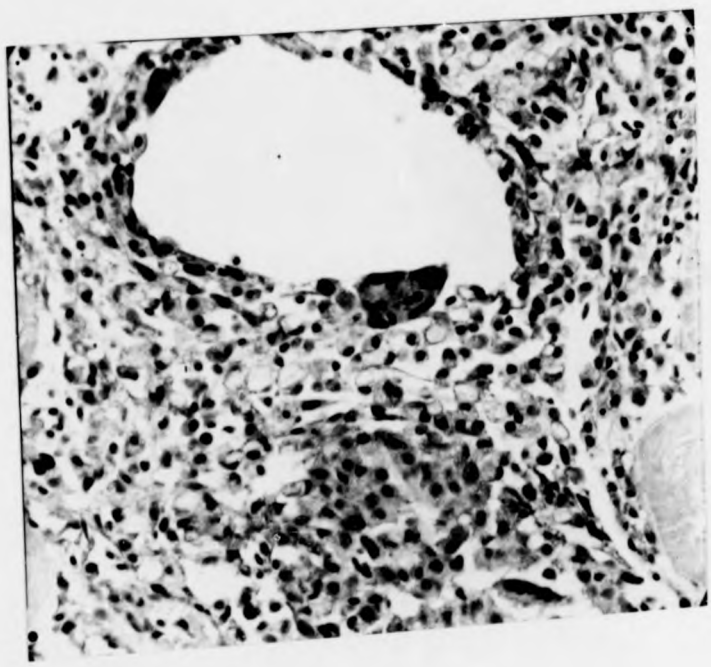
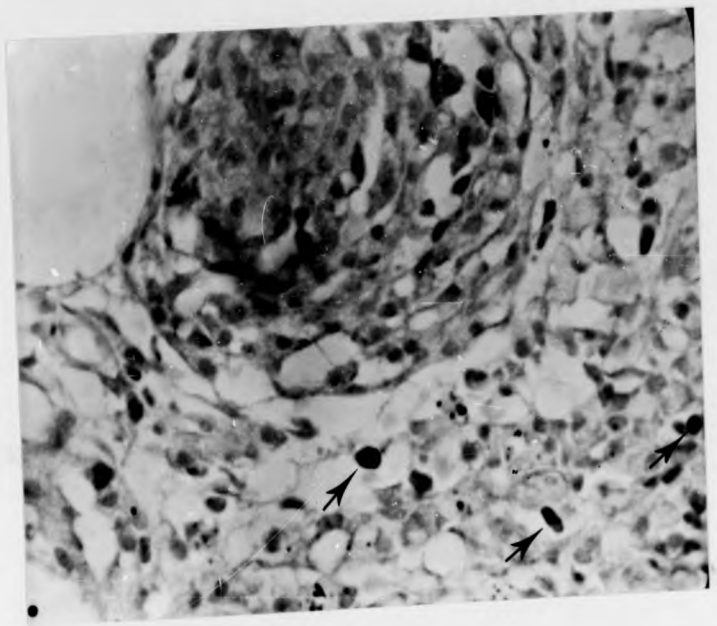


Fig (27) Plasma cells (arrowed) within a stroma of epithelioid cells in incomplete adjuvant granuloma 42 days at 10^{o}C.

Unna-Pappenheim x 800

Fig (28) The inflammatory area containing a Langhans type giant cell. Its nuclei peripherally placed in a horse shoe configuration (incomplete adjuvant 42 days)

H & E x 800



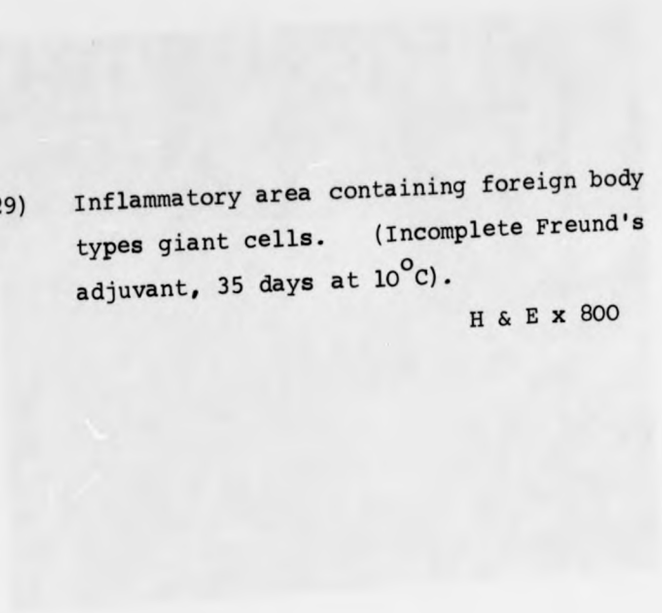


Fig (29) Inflammatory area containing foreign body types giant cells. (Incomplete Freund's adjuvant, 35 days at 10°C).

H & E x 800

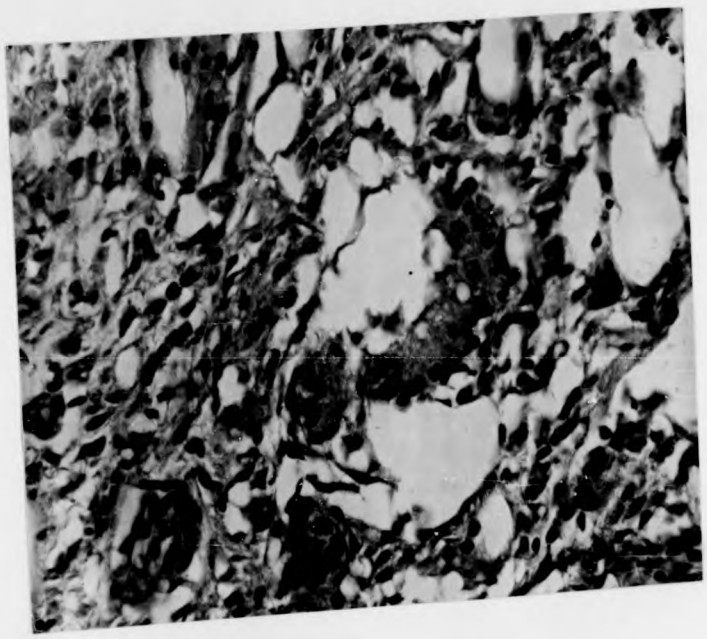


Fig (30) Duration of the inflammatory cell response within incomplete Freund's adjuvant granulomata at 10°C. Commencement and termination of sampling days are shwon by arrows.

□ Neutrophils
△ Macrophages
○ Epithelioid cells
◇ Giant cells
● Lymphocytes
▲ Plasma cells
■ Fibroblasts

TIME(10 days intervals)

Experiment 4.

The development of the multinucleated giant cells and granulomata in response to inoculation of piscine Mycobacteria at 10°C.

Mycobacterium tuberculosis has long been known as a bacterial stimulus to the production of multinucleated giant cells in granulomatous inflammatory reactions of mammals. This has already been discussed in detail in the general review of the literature. Since, in fish pathology there is argument as to the existence of the multinucleated giant cells in tuberculous lesions (vide supra), it was considered essential to study the pathogenesis of fish Mycobacteria induced granulomata to confirm or refuse these findings. This study was carried out at light microscope level and was coupled with a study of the immunological components of the response.

a) Light Microscopy:-

Materials and Methods:

The following pilot experiment to choose the ideal dose level of irritant which would provoke an adequate cellular inflammatory response at the injection site was performed in five 1^{+} fish. Each fish was inoculated intramuscularly on the aboral site with 0.1 ml of a heavy culture suspension of piscine Mycobacteria, isolated from halibut (Hendrie per.comm.). Fish were sacrificed one fish at a time at 16, 28, 35, 50 and 60 days after inoculation. Histopathological examinations of the sections which were obtained from different sampling days showed that adequate collections of epithelioid cells resembling small tubercles but without caseation or giant cells were produced but that the level of inoculation was inadequate for a good response.

In the main experiment seven fish similar to those used above were used. They were infected with an increased volume (0.3ml) of heavy culture of physiological saline suspension of mycobacteria. The culture was inoculated three times at three different points which were adjacent to each other. They were sacrificed at 12, 18, 24, 27, 35, 40 and 56 days after inoculation.

Microscopy:

The injection of the mycobacterium induced degeneration of muscle fibres over a large area by 12 days after inoculation. The necrosis of muscle fibres was characterized by break down of their sarcoplasm with invasion by macrophages. The inflammatory exudate comprised predominantly macrophages, a few polymorphonuclear leucocytes (neutrophils), lymphocytes and plasma cells, while extracellular deposits of acid-alcohol fast bacilli were seen they were also observed within the cytoplasm of the many macrophages.

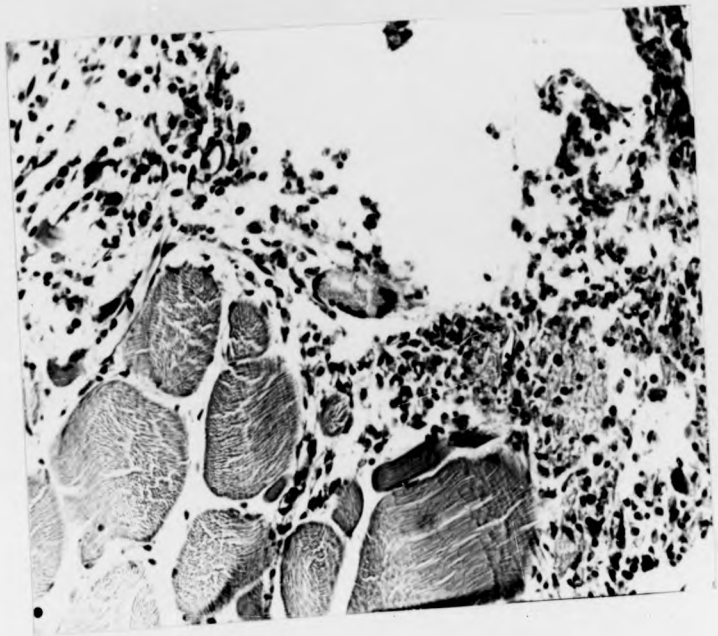
Myophagia was complete by the 18th day and many macrophages aggregated within the inflammatory area. The epithelioid cells were first seen at this stage, in small collections within the inflammatory area. Among the inflammatory cells occasional Langhans type multinucleated giant cells, some lymphocytes and fibroblasts and an increased number of plasma cells were seen. The giant cells were typically circular to oval in shape and their multiple nuclei were distributed at the periphery of the cells, again large vacuoles were seen within the cytoplasm. Many of the macrophages were laden with large numbers of acid-alcohol fast bacilli.

On the subsequent sampling stages (24th-27th days), the inflammatory reaction comprised an increased number of multinucleated giant cells among the other inflammatory cells, the plasma cells, lymphocytes, fibroblasts and small collections of epithelioid cells. However, fully formed epithelioid granulomata were not yet present. At these stages Langhans type giant cells were very frequent among the inflammatory cells (Fig 31). They were circular or circular to oval in shape and their size measured between 23-55μmand their darkly stained nuclei varied between 7-23. Their cytoplasm contained small or large vacuoles and Ziehl-Nielson stained sections indicated the presence of large numbers of acid-alcohol fast bacilli within their cytoplasm (Fig 32). The fibroblasts were especially prominent in the area of muscle damage.

The most striking feature of the 35th-40th - 56th days was the development of the many, discrete, whorling patterned epithelioid cell granulomata (tubercles). Some of these had central caseation in which acid-alcohol fast bacilli abounded (Fig 33). The numbers of multinucleated giant cells had decreased at this stage, as the epithelioid cell granuloma developed, although they were seen occasionally among the epithlioid cells. The tubercles contained predominantly epithelioid cells, although very occasionally, Langhans type giant cells, (Figs 34, 35) many plasma cells (Fig 36) and some collections of lymphocytes (Fig 37) and around the margin of the tubercles fibroblasts were seen.

Fig (31) The inflammatory area of the piscine Mycobacterial lesion showing Langhans type giant cell with peripherally placed nuclei. 24 days at 10°C
H & E x 500

Fig (32) Langhans type giant cells and some macrophages containing piscine Mycobacterium within their cytoplasm (24 days at 10°C).
Ziehl-Nielson-Tartarzine x 800



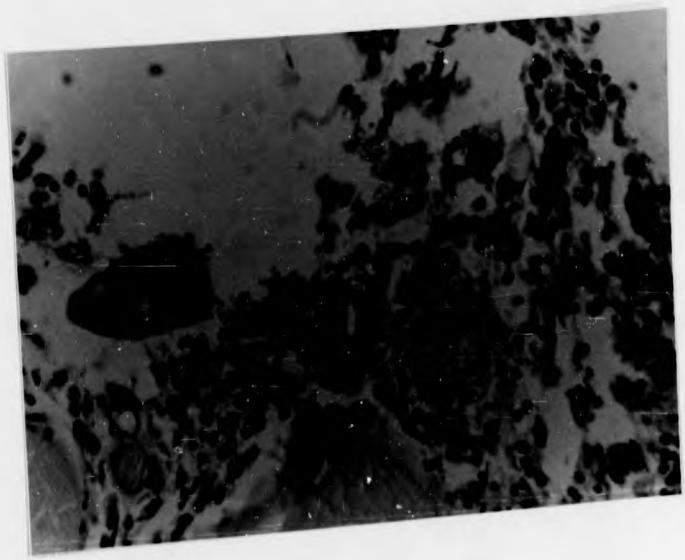
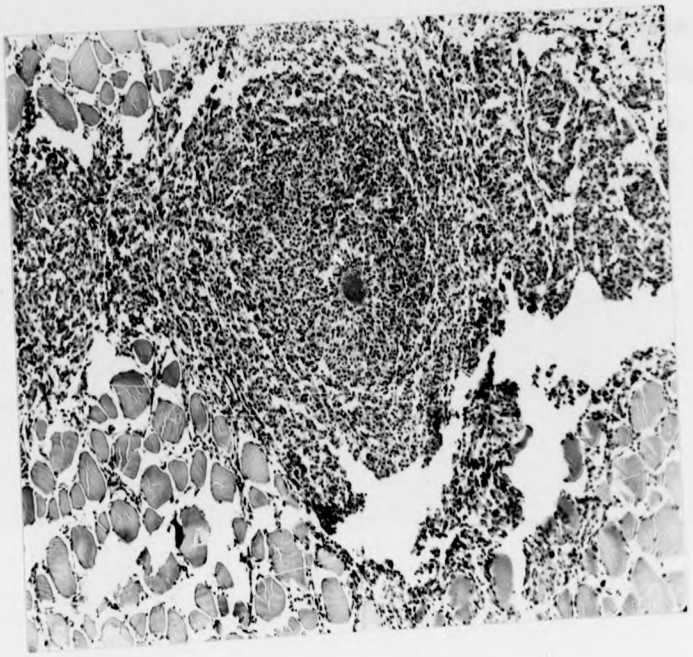


Fig (33) Tubercle development in fish injected with piscine Mycobacteria. The tubercle contains a caseous necrotic area with abundant bacteria in the centre. 35 days post injection at 10°C.

H & E x 200

Fig (34) A Langhans type giant cell among epithelioid cells in piscine Mycobacterial lesion after 35 days at 10°C.

H & E x 800



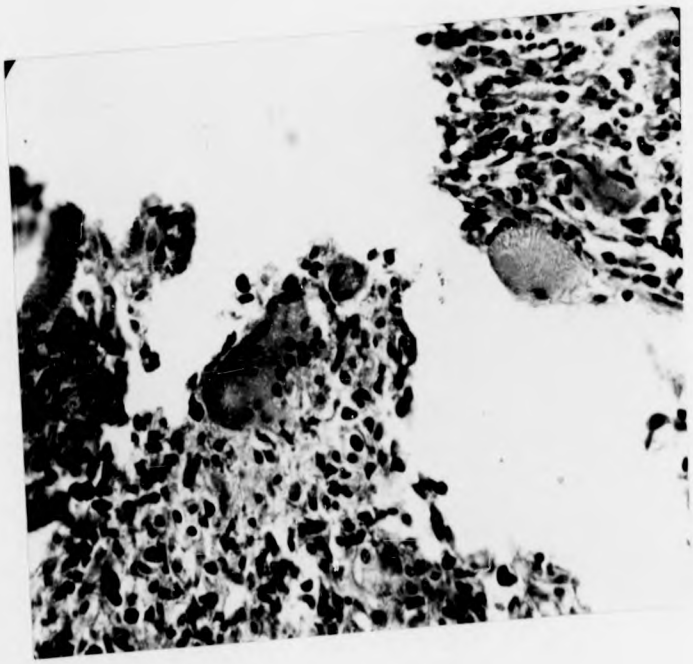
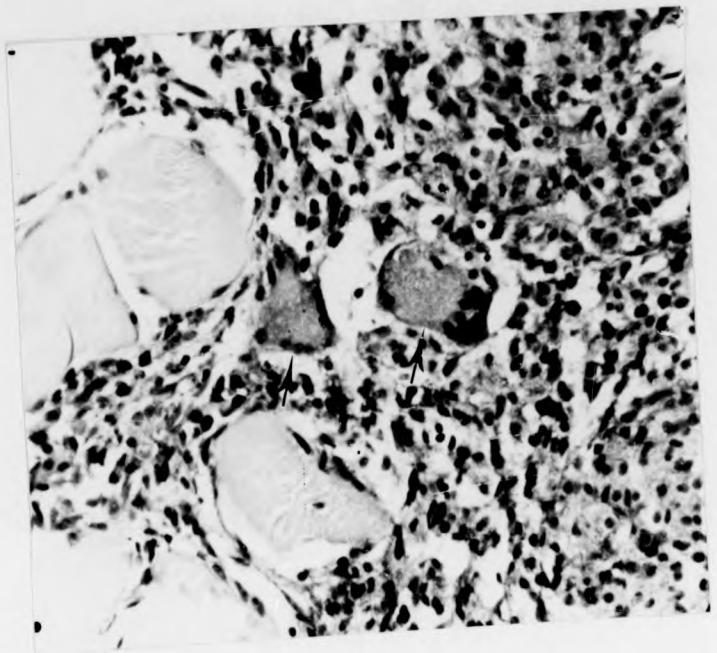
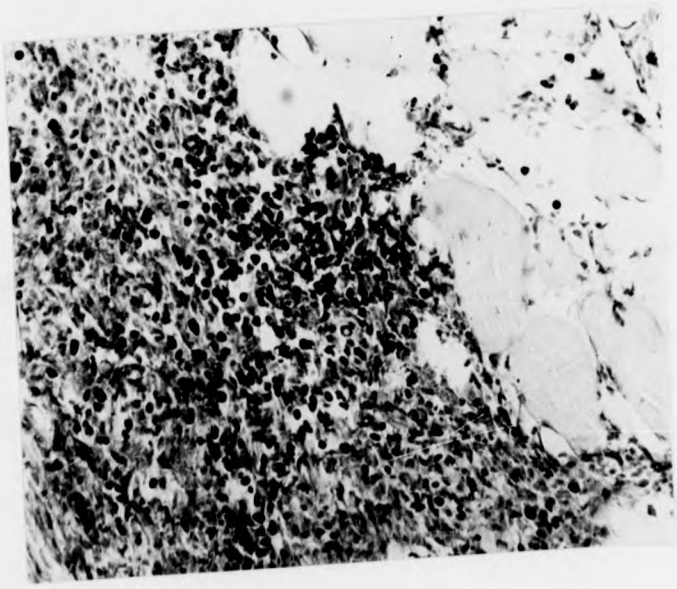


Fig (35) Two Langhans type giant cells among epithelioid cells. One of containing a few bacilli within its cytoplasm, the cells (arrowed). (35 days, 10°C)
Ziehl-Nielson-Tartarzine x 800

Fig (36) Abundant plasma cells among the epithelioid cells of piscine Mycobacterial lesion after 35 days at 10°C
Unna-Pappenheim x 500





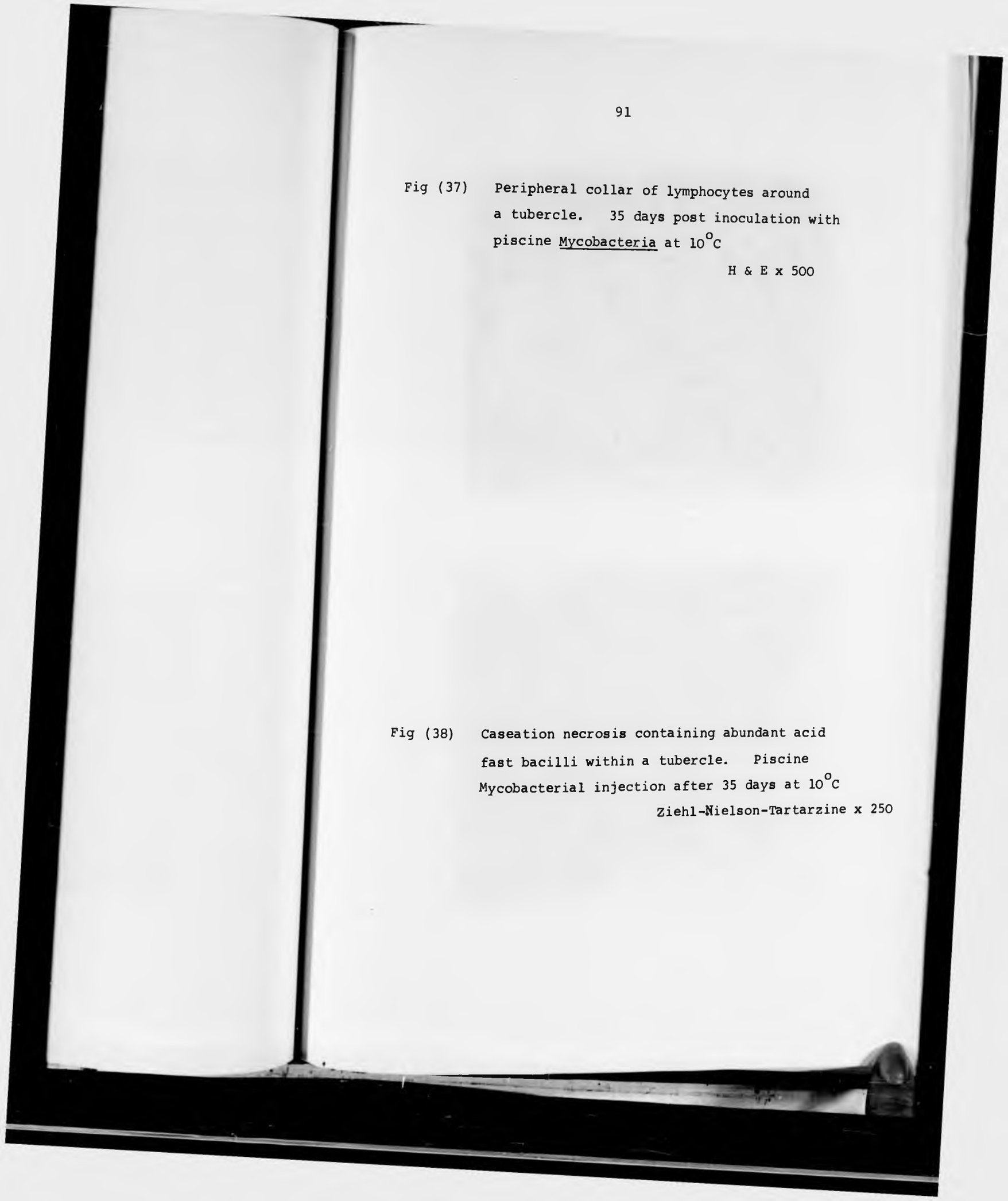
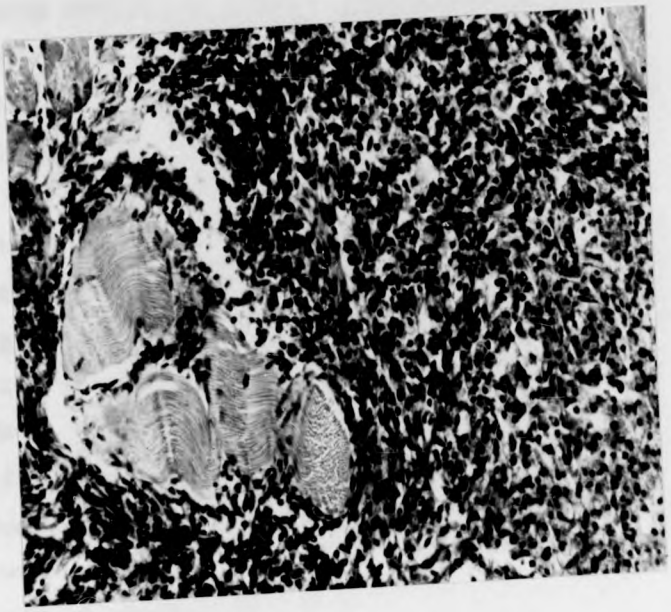


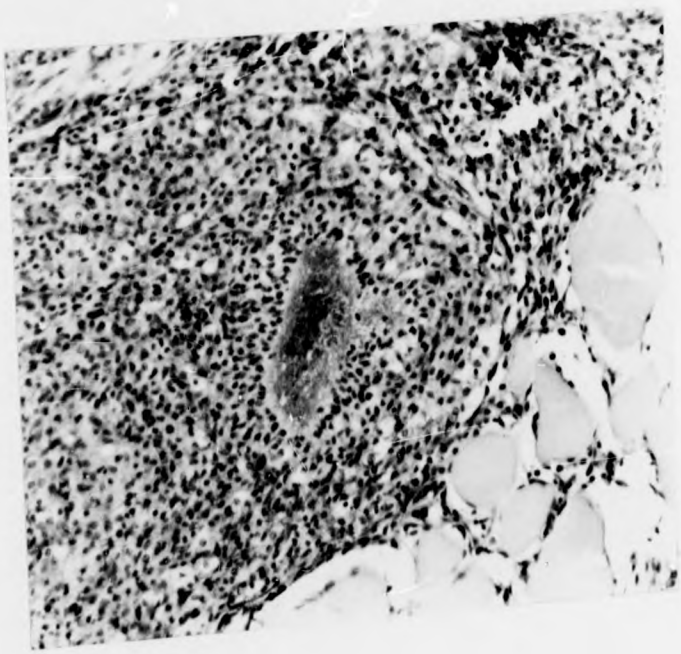
Fig (37) Peripheral collar of lymphocytes around a tubercle. 35 days post inoculation with piscine Mycobacteria at 10°C

H & E x 500

Fig (38) Caseation necrosis containing abundant acid fast bacilli within a tubercle. Piscine Mycobacterial injection after 35 days at 10°C

Ziehl-Nielson-Tartarzine x 250



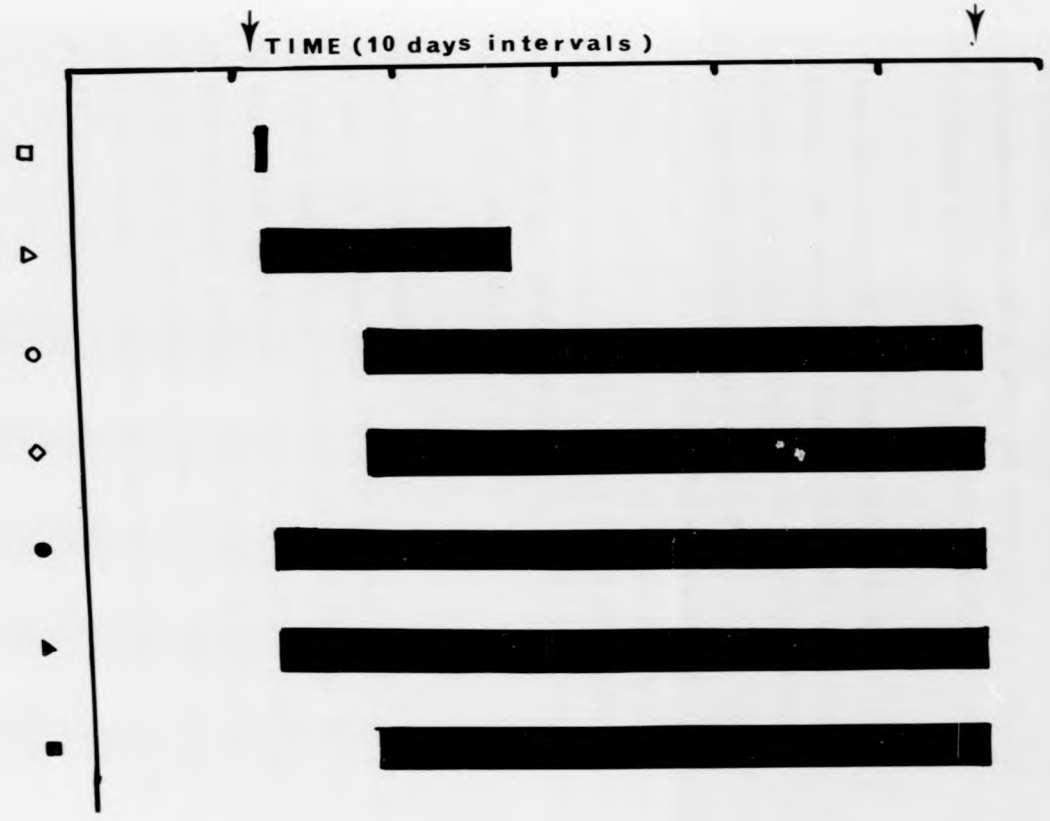


Tuberculous bacilli at these stages became very infrequent within the cytoplasm of the inflammatory cells (epithelioid cells and giant cells), although they were abundant within the centres of caseous necrosis (Fig 38).

The histological similarity of the tuberculous lesions at these late stages to the granulomas due to talc and beryllium (vide infra) was marked with the development of the complete epithelioid cell response, in a whorling pattern, each whorl representing a discrete granuloma or tubercle. However, this differed considerably from the findings with both complete and incomplete Freund's adjuvant granuloma at 10°C, where there was also an extensive population of macrophages among the whorls of epithelioid cells.

Fig (39) Duration of the inflammatory cell response within piscine Mycobacterium granulomata at 10°C. Commencement and termination of sampling days are shown by arrows.

- □ Neutrophils
- △ Macrophages
- ○ Epithelioid cells
- ◇ Giant cells
- ● Lymphocytes
- ▲ Plasma cells
- ■ Fibroblasts


TIME (10 days intervals)

b) Immunology of the response to piscine Mycobacterium:-
It has been postulated that the necrotising changes associated with granulomata and the development of epithelioid & giant cells may be related to a hypersensitivity reaction between antigen of the irritant causing implicated in various granulomatous diseases such as tuberculosis, leprosy and berylioisis although it is as yet unconfirmed (Crowle 1962).

During the study of the basic granuloma and giant cell response, the extensive observations on the presence of lymphocytes and other immunocytes suggested that an investigation of a possible delayed type hypersensitivity response should be carried out, on the fish infacted with piscine Mycobacteria and held at 10°C, by means of the Migration Inhibition Test (George and Vaughan 1962), which provides evidence of the presence of any sensitized, antibody bearing lymphocytes within the circulation.

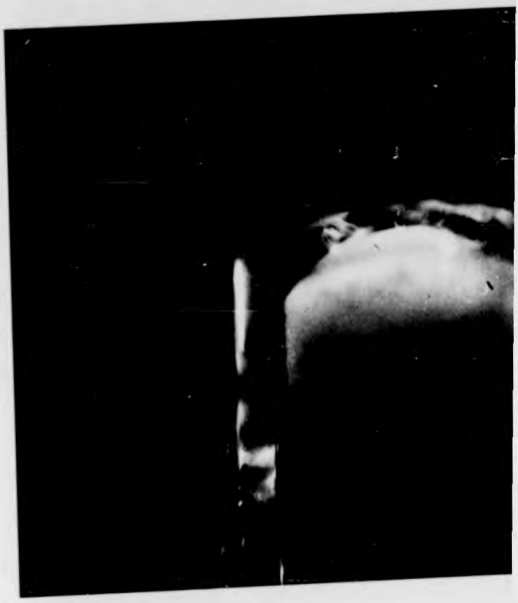
Materials and Methods:
One year old plaice were utilized, blood being sampled from the renal portal vein (Wardle 1971) of fish at 12, 16, 30 and 32 days after previous injection of piscine Mycobacteria in physiological saline. The migration inhibition test was carried out as described in the general material and methods section, using the method of George and Vaughan (1962) modified by Timur M (1975 pers.comm.).

Results:

There was no evidence of inhibition of migration of macrophages from the buffy coat of the blood samples at 12 and 16 days after injection of Mycobacteria. In all of these cases the white caps of cells extended out into the medium, developing a fan-like head on the capillary tube in both test and control tubes. However, the cap of the buffy coat on the samples of the blood from fish injected 30 and 32 days previously completely failed to migrate from the tube (Fig 40), although the cells of control tubes, where no antigen had been added to the medium, migrated out into the medium without inhibition (Fig 41).

Fig (40) Migration of Leucocytes inhibited from test tube to medium in which antigen added

Fig 41 Migration of Leucocytes from control tube to medium induced a fan-like head on the capillary tube, where no antigen had been added to the medium or where a negative result occurred.



Experiment 5.

The development of the giant cells and granulomata in response to inoculation of talc at 10°C.

Talc (magnesium silicate) is known as a cause of granuloma formation and giant cell production in mammals (vide supra) The purpose of the present study was to define in general the chronic inflammatory response, and specifically to investigate the multinucleated giant cell response in plaice after injection of the talc at 10°C. This study was carried out at the light microscope level only.

Materials and Methods:-

A pilot experiment was carried out with six fish to determine the optimum dose rate and sacrifice time. These fish were inoculated with 0.1 ml of one per cent talc suspension intramuscularly on the aboral side of the lateral line. They were sacrificed one fish at a time at 7, 21, 28, 35, 42 and 56 days after previous inoculation. The histopathologic picture of the inflammatory lesions of different sampling days simulated tuberculous lesions and comprised epithelioid cells and some multinucleated giant cells which were bearing talc particles. However, these lesions were very small and interpretation was difficult. For this reason the main experiment was carried out with an increased volume of irritant at an increased dose level for each fish in order to achieve a greater cellular reaction.

In the main experiment eight fish were utilized. They were 14-15 cm in length and each was inoculated with 0.15 ml 3 per cent talc suspension in physiological saline, intramuscularly. The inoculation was carried out three times into three adjacent sites. The injection site was marked by fin clipping and fish were sacrificed at 8, 16, 21, 28, 35, 42, 49 and 60 days after inoculation. Histological samples were obtained and fixed as described before in the general part of the materials and methods. The histological sections were stained by haemotoxylin and eosin, Masson's trichrome and Unna-Pappenheim's staining methods and in addition use was made of the bi-refringent properties of talc to examine the specimens in polarized light in a Leitz Polarizing microscope.

Microscopy:-

The inflammatory infiltrate after the eight day was composed of macrophages, lymphocytes and scattered plasma cells. In addition epithelioid cells, which had already been formed atthis very early stage, were lying among the talc deposites. These talc particles were seen as refractile crystals within the tissue. By the 16th-21st and 28th days, the lesions simulated tuberculous lesions with the difference that the talc perticles were very obvious lying free or within the cells. By the 16th day, some macrophages were seen intermingled with epithelioid cells but in subsequent stages they had disappeared. The inflammatory response also contained plasma cells (Fig 42), fibroblasts and multinucleated giant cells. Many multinucleated giant cells containing large talc particles were scattered among the epithelioid cells. These

giant cells varied in size, shape, nuclear content and nuclear configuration. Some resembled classical foreign body type giant cells, being circular to oval in shape and containing randomly scattered nuclei around the phagocytosed large refractile particles, other resembled Langhans type giant cells being circular oval or flask shaped and contained multiple nuclei at the perihpery of the cell, and also they contained large talc particles within their cytoplasm. However, another type of giant cell oval or oval to circular in shape, with a nuclear configuration which was a mixture of the Langhans type and foreign body type was also seen. The phagocytosed talc particles occupied most of the cell cytoplasm of these intermediate type multinucleated giant cells, as refractile particles, as seen in the Langhans type and foreign body type giant cells, while some of its nuclei were distributed in a line and the rest scattered randomly within the rim of cytoplasm. The multinucleated giant cells varied in size from 18µm to 70µm and in nuclei number from 3-20.

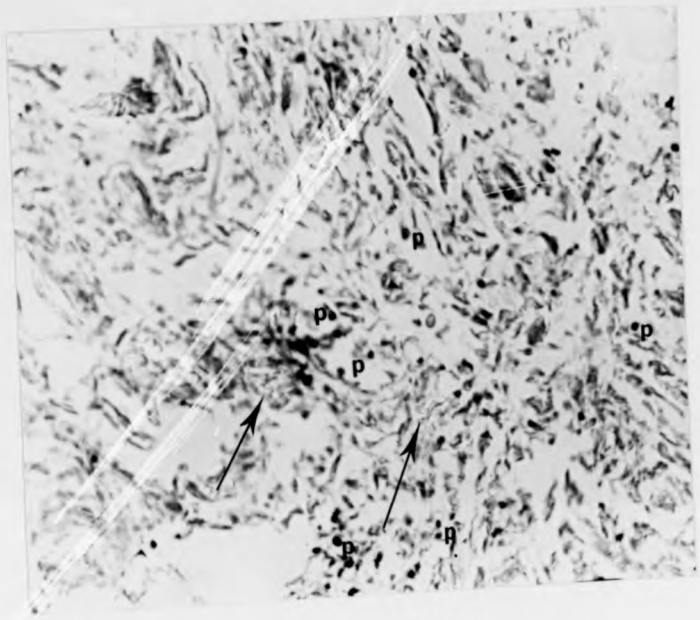
Tuberculoid lesions containing talc continued to be seen on the 35th, 42nd and 49th days after inoculation. They were composed of epithelioid cells, many multinucleated giant cells, scattered plasma cells and fibroblasts at the periphery of the lesions. Occasionally some regenerating muscle fibres were seen. By the 60th day, some of the follicular granulomata were entirely composed of multinucleated giant cells in which talc occupied most of the cell with very little of the cytoplasm remaining (Figs 43, 44, 45) but the others were mainly composed of epithelioid cells and some rather small refractile talc particles bearing multinucleated

Fig (42) Inflammatory area containing talc granules (arrowed) and containing several plasma cells (p). Talc granuloma 28 days 10°C

Unna-Pappenheim X 500

Fig (43) Late stage (60 days) inflammatory area containing many foreign body type giant cells among the epithelioid cells. Refractile talc particles occupied most of the cytoplasm of the giant cells

H & E x 500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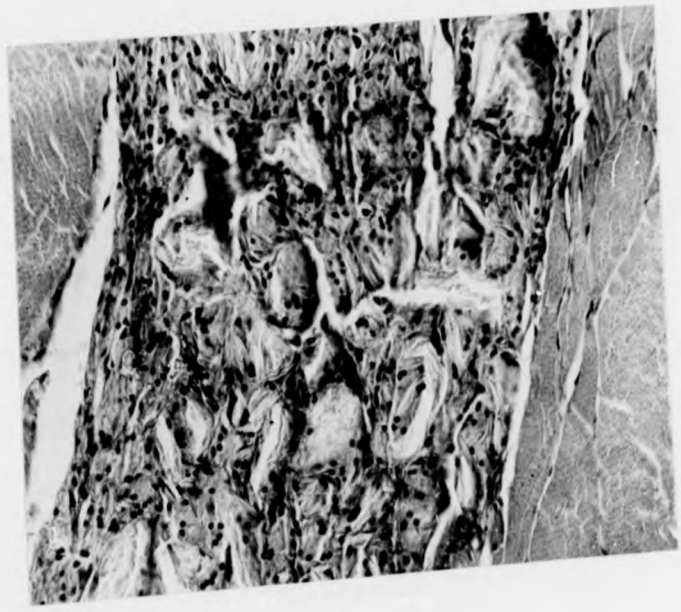
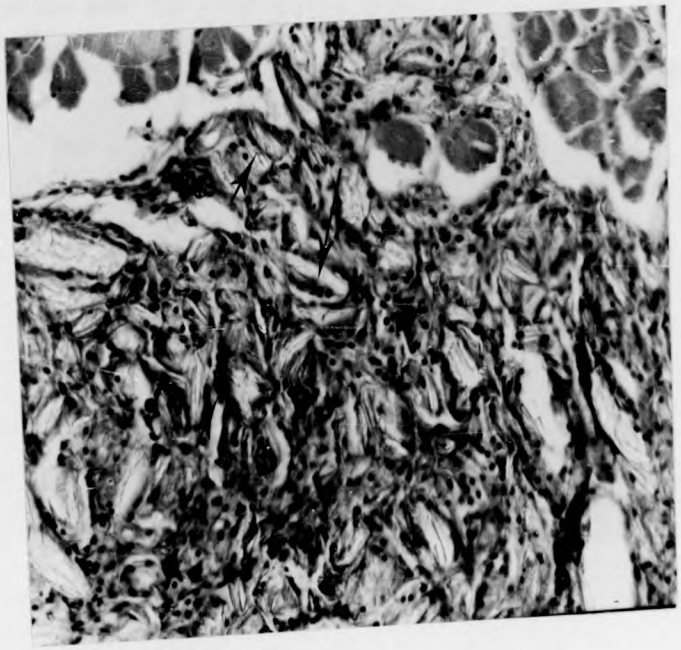


Fig (44) Late stage response to talc (60 days) and Langhans type giant cells containing large particles of talc within their cytoplasm, and their multiple nuclei placed at the periphery of the cytoplasm of the cells (arrowed).

H & E x 500

Fig (45) Intermediate type giant cell in late stage (60 days) granuloma showing the nuclei (arrowed) at the periphery of the cytoplasm and also scattered in the middle of the cytoplasm

H & E x 500



giant cells and few plasma cells (Fig 46).

Polarized light microscopy was very useful at all stages of the study of the talc granuloma, as it allowed very small amounts of talc to be readily observed, either in cells or lying free.

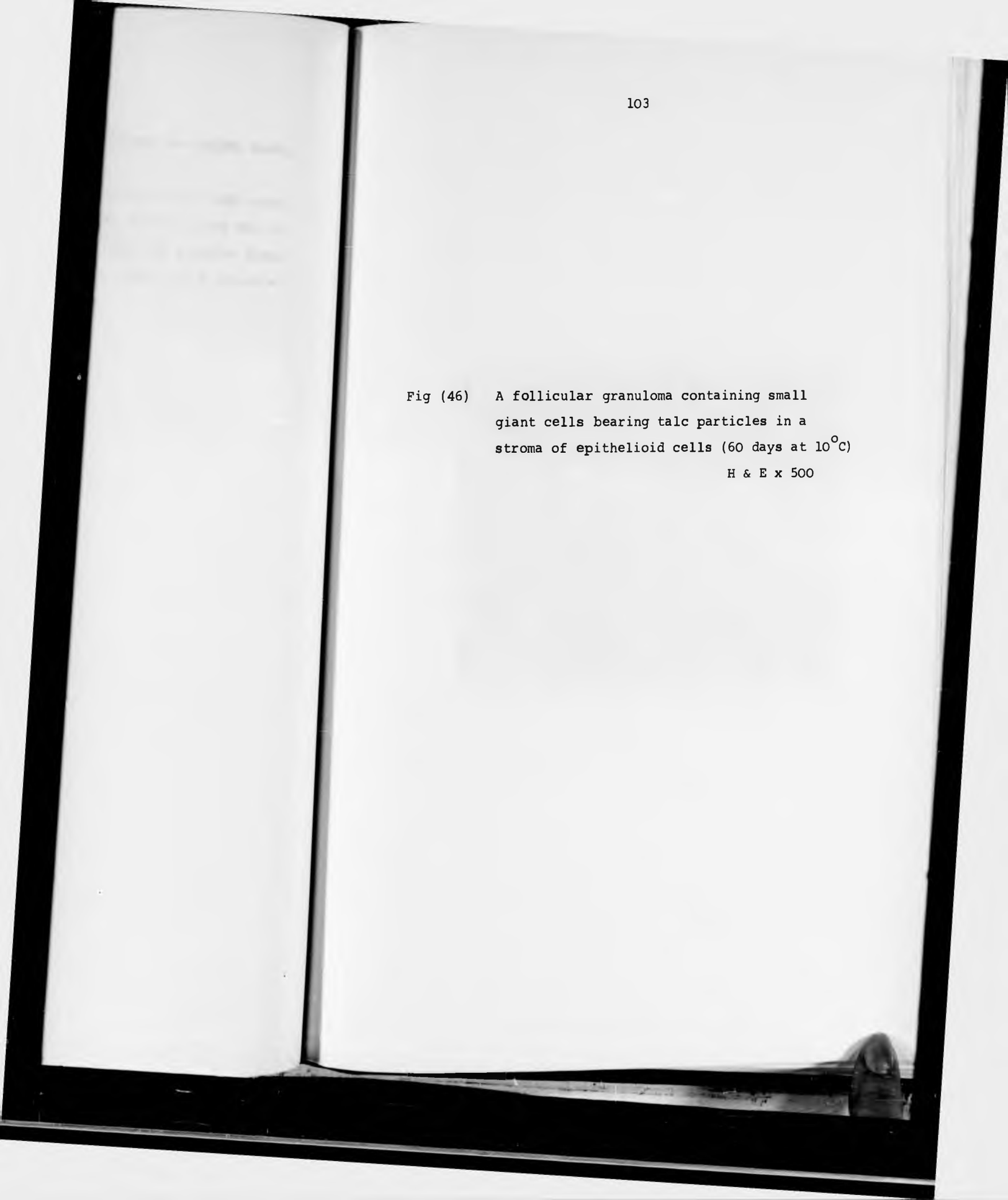


Fig (46) A follicular granuloma containing small giant cells bearing talc particles in a stroma of epithelioid cells (60 days at 10°C)
H & E x 500

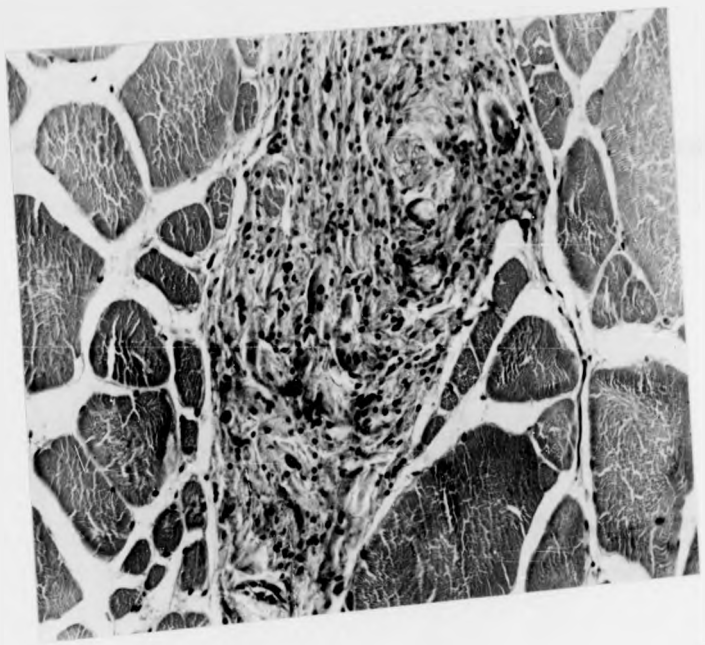
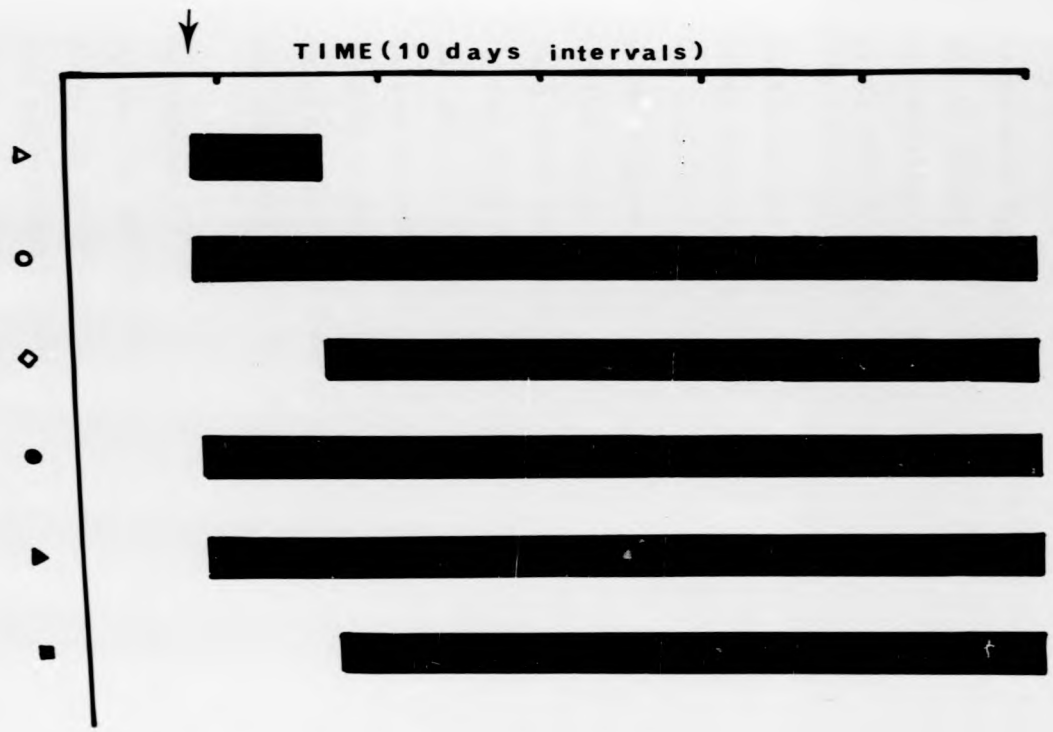


Fig (47) Duration of the inflammatory cell response within talc granulomata at 10°C. Commencement and termination of sampling days are shown by arrows.

△ Macrophages
○ Epithelioid cells
◇ Giant cells
● Lymphocytes
▲ Plasma cells
■ Fibroblasts


TIME(10 days intervals)

Experiment 6.

The development of multinucleated giant cells and granulomata in response to inoculation of beryllium oxide at 10°C.

Beryllium is well recognised as a cause of granulomatous lesions resembling miliary tuberculosis and characterised histologically by the presence of multinucleated giant cells in mammals (vide supra). The purpose of the present study was to define the generalized chronic inflammatory response to beryllium with special reference to the multinucleated giant cell response in plaice.

Materials and Methods:

Seven fish were used and they were 1^{+} year old. Each fish was inoculated intramuscularly with 0.15 ml of 3% beryllium oxide suspension in physiological saline at the aboral side of the lateral line. The irritant was inoculated three times into three adjacent points and the injection site was marked by fin clipping. The fish were sacrificed at 7, 12, 16, 21, 28, 35, and 42 days after inoculation the lesion bearing tissue excised, and fixed and stained as described in the general materials and methods section. Microscopic examinations were made using standard light microscopy and polarized light microscopy.

Microscopy:

By the 7th day, muscle necrosis was apparent at the injection site, the sarcoplasm of the muscle fibres being broken down and phagocytosed by macrophages. Numerous small deposits of beryllium were seen as refractile green to yellow coloured particles and these particles were surrounded by macrophages.

Many of the macrophages were laden with beryllium so that it appeared as a refractile yellow to green colour and obscured all except the eccentric strongly basophilic nucleus.

On the 12th day, aggregation of the numerous macrophages within the inflammatory area was well seen, and many of them were loaded with beryllium oxide and the inflammatory response also comprised plasma cells, lymphocytes, and fibroblasts. By the 16th day, some of the inflammatory response cells were recognisable as epithelioid cells. On the subsequent stage at 21 days, the inflammatory infiltrate also contained multinucleated giant cells. The cytoplasm of the cells were occupied by a large amount of beryllium and their nuclei were lined up at the periphery in a horse-shoe configuration, so-called Langhans type giant cells (Fig 48). Their size varied between about 17-30μm and their nuclei numbered between 5 & 12. At this stage plasma cells were also increased in number (Fig 49).

The epithelioid cell granulomata were developed by the 28th days, and a similar histopathological picture was seen at subsequent stages at the 35th and 42nd days (Fig 50). The epithelioid cells were arranged in a whorling pattern and simulated tuberculous lesions (Figs 51, 52). At these stages, the epithelioid cell granuloma comprised Langhans type multinucleated giant cells which were bearing beryllium oxide (Figs 53, 54) as were most of the epithelioid cells and there were scanty plasma cells, some lymphocytes and fibroblasts.

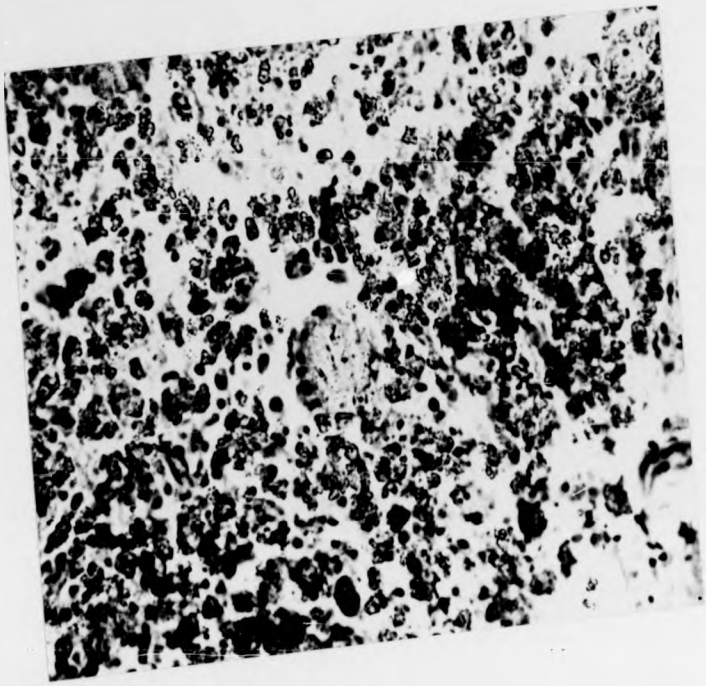
In the examinations of the histological sections by polarized

Fig (48) Inflammatory area containing macrophages loaded with beryllium oxide, several plasma cells, and a Langhans type giant cell. Giant cell containing refractile beryllium within its cytoplasm (21 days, 10°C)

H & E x 800

Fig (49) Plasma cells within beryllium granuloma after 21 days at 10°C

Unna-Pappenheim x 800



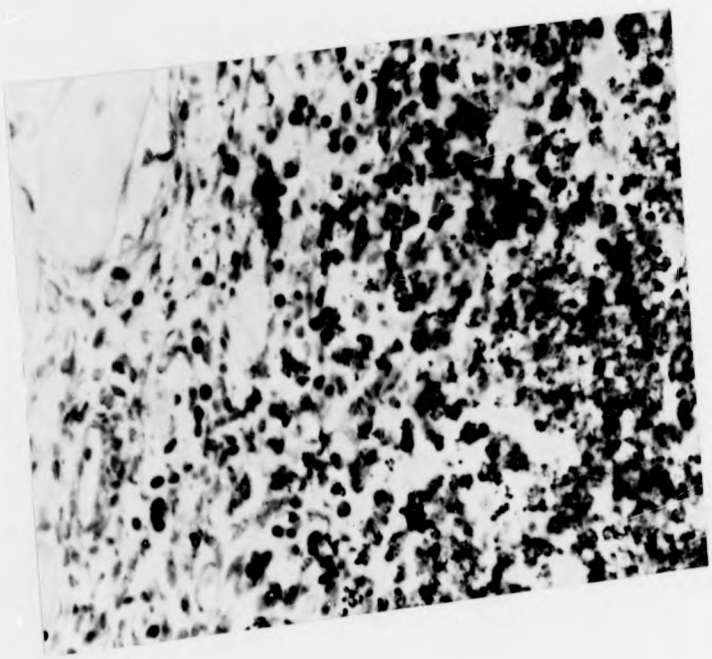
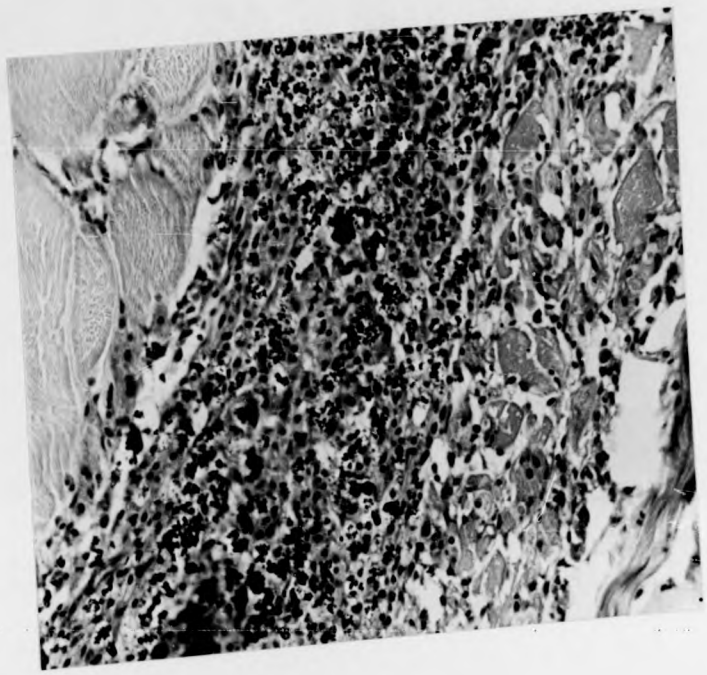


Fig (50) Epithelioid cells containing beryllium particles after 42 days

H & E x 500

Fig (51) Tubercle like structure containing refractile beryllium oxide granules (42 days at 10°C)

H & E x 800



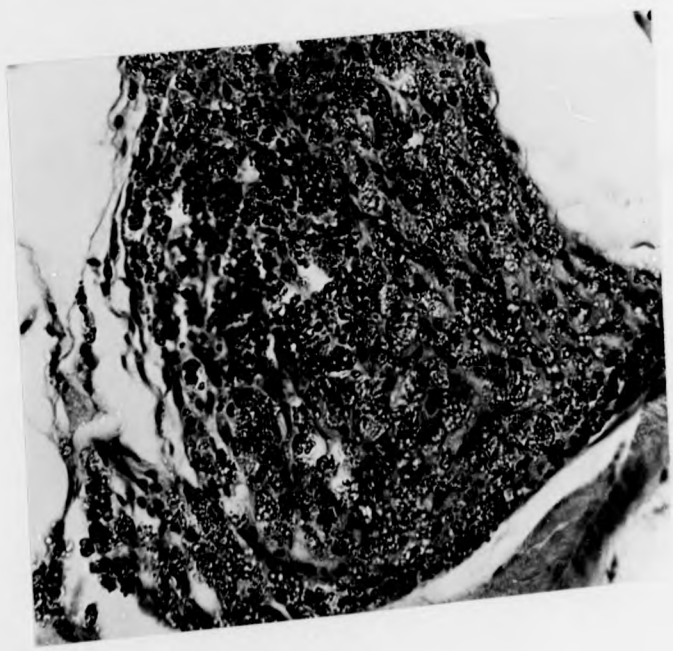
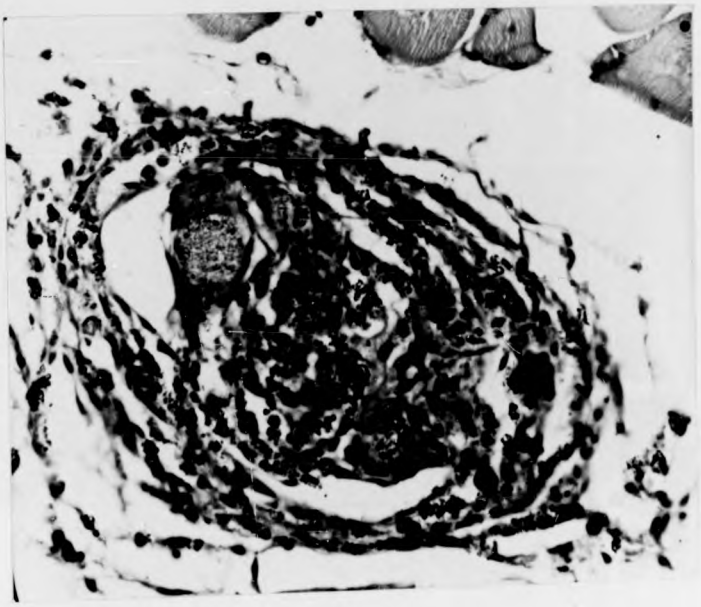


Fig (52) A small tubercle with a whorling pattern containing beryllium oxide within its epithelioid cells (42 days, 10°C)

H & E x 500

Fig (53) A Langhans type giant cell (arrowed) bearing beryllium particles. The other cells in the stroma are epithelioid cells (42 days, 10°C)

H & E x 800



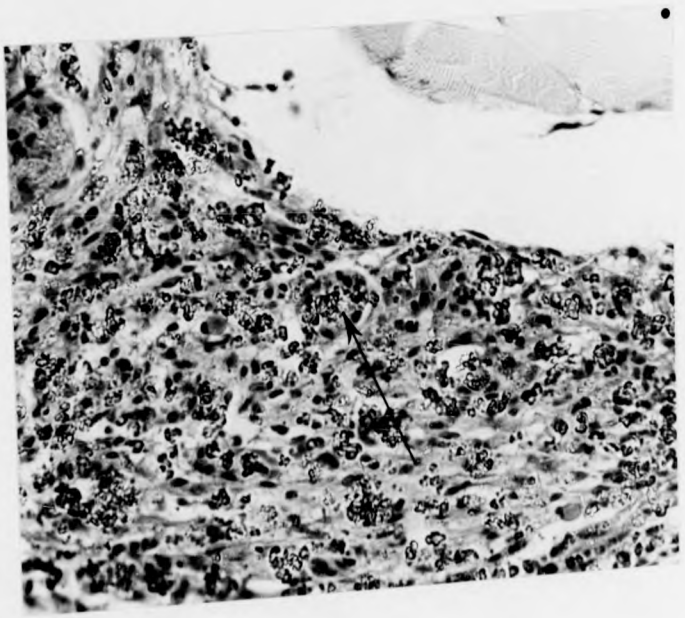
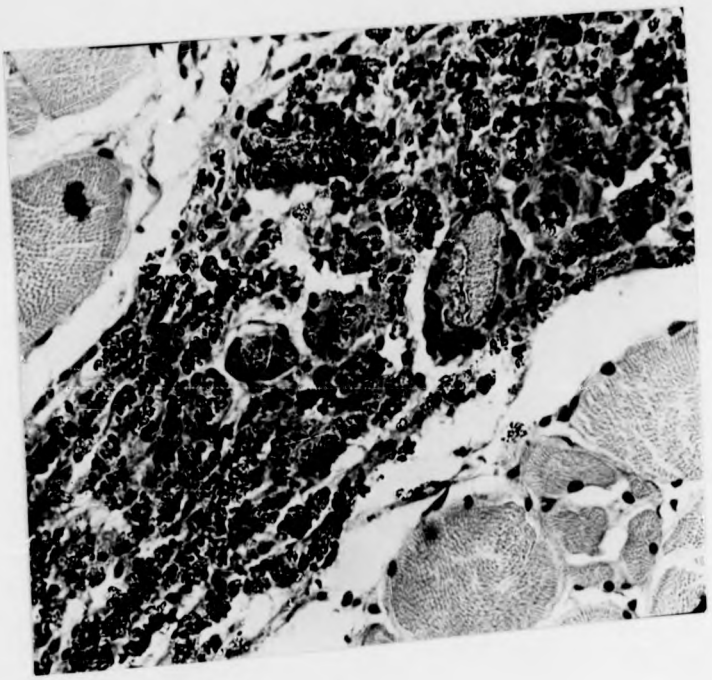


Fig (54) Langhans type giant cells among the inflammatory cells, containing beryllium oxide within their cytoplasm (42 days, 10°C)
H & E x 800



light microscopy, the beryllium oxide showed clear birefringe properties on direct examination and this allowed simple and rapid recognition of the beryllium either in the cells or lying freely within the inflammatory area.

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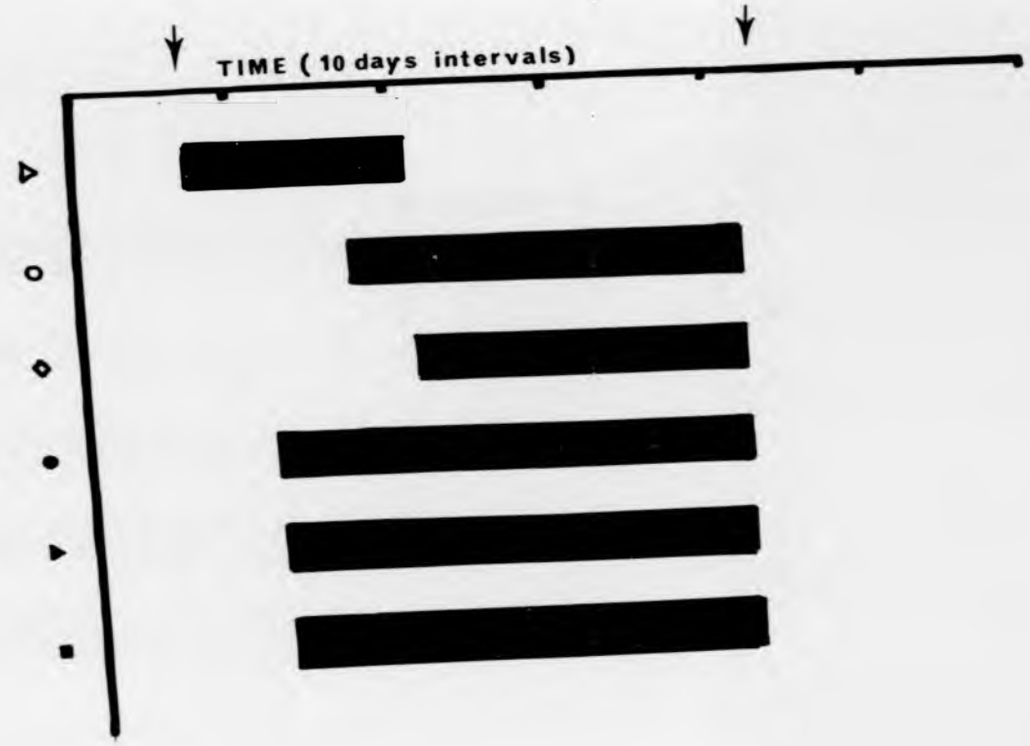
Fig (55) Duration of the inflammatory cell response within beryllium oxide granulomata at 10°C. Commencement and termination of sampling days are shown by arrows.

△ Macrophages
○ Epithelioid cells
◇ Giant cells
● Lymphocytes
▲ Plasma cells
■ Fibroblasts


TIME (10 days intervals)

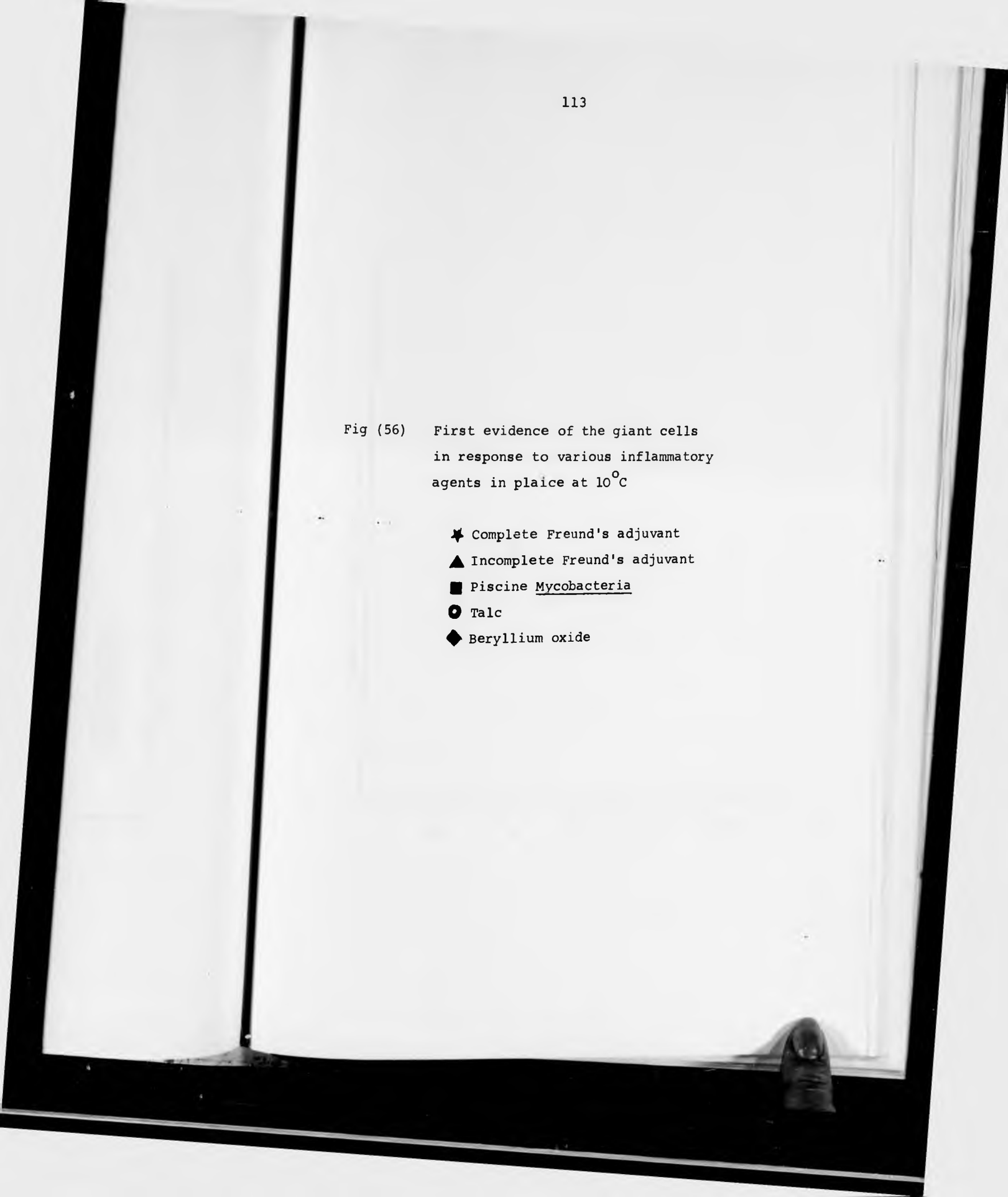


Fig (56) First evidence of the giant cells in response to various inflammatory agents in plaice at 10°C

- ✱ Complete Freund's adjuvant
- ▲ Incomplete Freund's adjuvant
- ■ Piscine Mycobacteria
- ● Talc
- ◆ Beryllium oxide

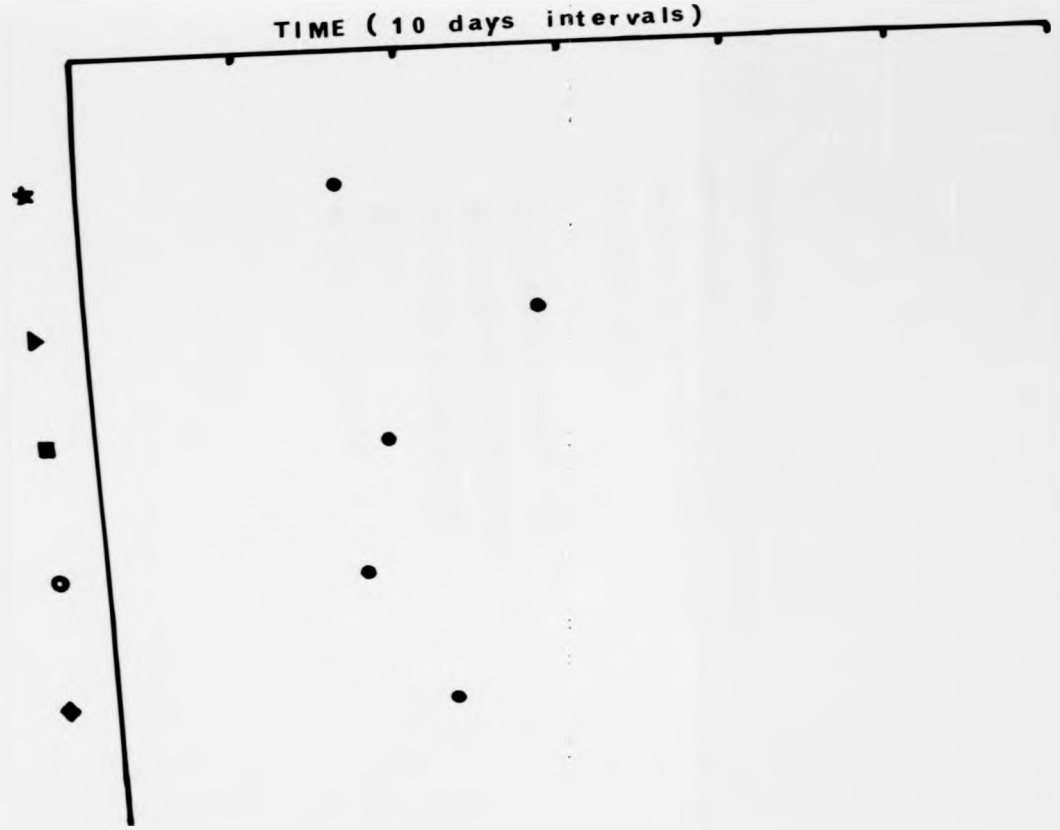

TIME (10 days intervals)

Fig (57) The time of the maximal [illegible] occurrance of the giant cells in response to various inflammatory agents at 10°C.

- ✱ Complete Freund's adjuvant
- ▲ Incomplete Freund's adjuvant
- ■ Piscine Mycobacteria
- ● Talc
- ◆ Beryllium oxide

TIME (10 days intervals)

SECTION IV

DISCUSSION

The chronic inflammatory response of the plaice, as manifested by the results of the main experiment of this study, (the sequential pathogenesis of the response to Freund's complete adjuvant at 10°C), shows many features of similarity to the chronic inflammatory response of higher vertebrates, not least in its development of giant cells. The cells found in the different stages of the response were similar to those which occur in higher animals, comprising macrophages, polymorphonuclear leucocytes, epithelioid cells, lymphocytes, plasma cells and fibroblasts as well as the giant cells which were the main component of the present study.

Probably the most similar study to the present one was that reported by Steiner et al (1960), on the response in the rabbit to inoculation with complete Freund's adjuvant. In that study the adjuvant induced sarcoid like non-caseating granulomata consisting of, as in the present study, epithelioid cells intermingled with macrophages, lymphocytes, plasma cells and their precursors, fibroblasts and occasional giant cells. They found giant cells of both Langhans and foreign body type, with the foreign body giant cells usually being found adjacent to the actual oil lacuna. Spector and Lykke (1966) confirmed this occurrance of Langhans type giant cells in their study on Freund's adjuvant granulomata in the rat.

Complete Freund's adjuvant granolomata was first studied in fish by Finn and Nielson (1971) who noted the presence of unspecified giant cells but did not even mention the presence of epithelioid cells or plasma cells. Their findings, as far as they went, agreed with the results of the present study but really did not continue for a sufficiently long period for serious observations of the chronic lesion.

The morphology of the both types of plaice giant cell in the complete Freund's adjuvant granuloma was very characteristic and typical of the published descriptions in homeotherms, the Langhans cells being round or round to oval, with the distinctive horse-shoe of subperipheral nuclei, and the foreign body type irregular or elongated with nuclei arranged haphazardly throughout their cytoplasm. Acid-alcohol fast bacteria were never seen in the cytoplasm of the giant cells although they were often found within either epithelioid cells of macrophages and it is tempting to conclude that the process of development into giant cells, whether by nuclear division or fusion of either macrophages or epithelioid cells (and no firm evidence was obtained to favour one or other method) allowed the destruction of ingested Mycobacteria. The piscine tuberculous lesions (vide infra) did not produce foreign body giant cells but since both types were produced in both complete and incomplete adjuvant studies, it seems likely that the foreign body cells occurring in the complete adjuvant experiment were in response to the mineral oil rather than the Mycobacteria.

The role of the focal aggregations of lymphocytes, which were very conspicuous after the 28th day, was particularly interesting. Such accumulations are also a feature of the mammalian granuloma around this time (Spector and Lykke 1968) and it is likely that they are responsible for the development of the cell mediated immunity, which is discussed below with special reference to the piscine Mycobacteria.

The ultrastructure of the cells involved in the adjuvant granuloma reinforced the impression of general harmony with the features of inflammatory cells of higher animals. Timur M (1975) has described the ultrastructure of the inflammatory cells of the plaice carrageenin granuloma and noted in the macrophages of his study that lysosomes were absent. Apparently this is also a feature of mammalian macrophages in carrageenin granuloma but in the present study the macrophages and epithelioid cells had similar lysosomal complements to those of higher animals, with greater numbers occurring in the macrophages. The feature of the giant cell which posed most technical difficulty in the ultrastructural study was their size, since the field of vision even at low power, with an electron microscope, is limited by the size of the supporting grid and consequently it was virtually impossible to obtain a complete section of such a cell. Generally the organelles of the giant cells resembled epithelioid cells and once again emphasized their similarity to the equivalent homeotherm cell.

The inflammatory cells, apart from epithelioid cells, giant cells, plasma cells and fibroblasts are also seen in circulating blood and there was no significant different in their ultrastructure from that observed in the circulating blood of plaice by Ferguson (1975). The plasma cell has not, to the authors knowledge, been previously described ultrastructuraly in a teleost but its features, both tinctorially, and ultrastructurally, were characteristic of that cell in higher animals (Movat and Fernando 1962). The

main feature of the plasma cell is the grotesquely developed endoplasmic reticulum. The Unna-Pappenheim staining method (Methyl-green pyronin) is a staining method which is particularly inconsistent in his results but when it was successfully performed the teleost plasma cells, and their precursors the large pyroninophils, were very distinctive.

Although at 10°C the Freund's complete adjuvant granuloma, and its giant cells, was very similar in the plaice to that of the higher animals, the granuloma produced by use of Freund's incomplete adjuvant, that is the mineral oil and water suspension without its complement of Mycobacteria, was somewhat different. Again both Langhans and foreign body type giant cells occurred, with the foreign body giant cells similarly localized around the lacunae, but the significant feature of the differences were the absence of the focal accumulations of lymphocytes around the lesion which had been a feature of the lesion containing Mycobacteria and in contrast, the presence of fewer plasma cells. Presence of more plasma cells and accumulations of lymphocytes would therefore as expected appear to be related to the presence of Mycobacteria.

One feature of the poikilotherm inflammatory response which is not mirrored in the homeotherms is its modification by temperature (Finn and Nielson 1971; Roberts et al 1973). In the present study the general findings in terms of temperature modification was not dissimilar to that of other workers (Finn and Nielson 1971 in trout; McQueen et al 1973 in plaice; Roberts et al 1973 in Atlantic salmon; Anderson and Roberts 1975 in Atlantic salmon and Tanichthyes albonubes) but the

finding of special significance to the present study was the discovery that giant cells were never seen in the low temperature experiment. All the other cellular responses were seen and no real explanation can be given for this finding. Macrophages and epithelioid cells were evident in significant numbers and contained ingested mycobacteria so that presumably the blockage in their transformation to giant cells was directly related to temperature. It is possible that the development was merely retarded and that had the experiment been continued beyond the period of 56 days, giant cells might have been produced, although the other features, including fibrosis and the appearance of plasma cells, albeit in small numbers, many of which occurred after the development of giant cells at 10^{o}C, had developed before termination of the experiment and only myofibriller regeneration, which was a very long term developed even at 10^{o}C, was similarly lacking at 5^{o}C. There is, however, some support for a retardation rather than prevention by temperature, since Timur M (1975) in his study of the carrageenin granuloma of plaice found that very occasional Langhans type giant cells could be seen in the carrageenin granuloma after 85 days at 5^{o}C.

Although the Freund's adjuvant granuloma provides a very useful model which is directly comparable with studies in homeotherms, the major stimulus to the commencement of the present study was the dichotomy in the literature between those who considered that giant cells could occur in fish

(Betegh in 1922 (reviewed by Alexander 1931); Aronson (1926)) and Sutherland (1922); Nigrelli and Vogel (1963), who could not find giant cell in the tuberculous lesions which they studied. Consequently the results of the experimental inoculation of the Mycobacteria of piscine origin were considered particularly significant. There is no doubt from the present study that Mycobacteria of piscine origin do invoke the production of giant cells although only of the Langhans type and the tubercle produced goes through a similar pathogenesis to that of the mammalian tubercle. The giant cells were only numerous over the period of the 24th to 27th days at 10°C. Once the granuloma was fully mature and the macrophages given way to epithelioid cells they decreased in number so that it is possible that the material examined by Sutherland (1922) and Nigrelli and Vogel (1963) was from very late stages of the condition and hence beyond the stage when giant cells were prevelant.

The macrophage migration-inhibition experiment, which gave clear evidence of the presence of a cell mediated immune response to Mycobacteria after 30 days, provides biological confirmation of the suspected function of the lymphoid infiltrate which was very conspicuous at this stage. It is presumed that the sensitized lymphocytes would also release other lymphokine factors in addition to the macrophage inhibition factor. These could be responsible for the other features of the pathogensis of the tubercle such as the caseous necrosis as is suspected in homeotherms (McCall 1971).

Although the study of the giant cell response to tuberculous microorganisms was the prime interest of the study, foreign body granulomata are also found in fish, indeed Wolke and Trainer (1971) described both foreign body and Langhans type giant cells developing in the white sucker in response

to silicaceous diatom spines, which provides a natural situation directly anologous to the experimental talc (silica) granuloma described above. The giant cells of both talc (magnesium silicate) and beryllium oxide granulomata were of varying morphology. Both Langhans and foreign body type cells were seen, in addition the elongated cell, which, at least in the talcum powder granuloma, had a nuclear configuration bearing features of similarity to both the Langhans and the foreign body type cells in that some of the nuclei were placed in a line at the periphery of the cell while the rest were distributed randomly around the phagocytosed talc particle i.e. the so called intermediate type giant cell. In the beryllium oxide granuloma only the Langhans type giant cells were seen and they were bearing large beryllium particles within the cytoplasm and the nuclei were placed at the periphery of the cells.

The two types of giant cells, Langhans and foreign body were certainly morphologically distinct but the occurrence of Langhans type giant cells in both infective (tuberculous) and foreign body granulomata, and the presence, especially in the silicaceous granuloma, of the intermediate type of giant cell forcibly illustrates that while the presence of Langhans cells alone indicates the probability of a Mycobacterial lesion there is no absolute relationaship between the cause of other granulomata and the giant cell type which is provoked and it is unfortunate that the derivative term "foreign body type cell" is so suggestive since, certainly in fish, the Langhans type giant cell is frequently seen in foreign body granulomata due to a variety of irritants.

SECTION V

CONCLUSIONS

Conclusions:

1. As in homeotherms, giant cells can be evoked by a variety of agents. In the present study the agents used were complete and incomplete Freund's adjuvant, piscine Mycobacteria, talcum powder, and beryllium oxide 10°C. All produced chronic granulomata containing the classical types of giant cell (Langhans and/or foreign body) such as are found in higher animals.

2. The absence of Mycobacterium butyricum from adjuvant (Freund's incomplete adjuvant) did not affect production of both types of giant cells (Langhans and foreign body) but there were significantly fewer lymphocyte and plasma cells seen at all stages.

3. Lowering of the environmental temperature caused a reduction in the rate of development of the adjuvant granuloma. During the period of study - 56 days, giant cells were not produced, and there was a consierable merging of what were distinct stages at higher temperature such as the persistence of active myophagia at the same time as extensive epithelioid cell development.

4. Mycobacteria isolated from naturally occurring tuberculosis in a marine fish induced Langhans type giant cells after inoculation into plaice at 10°C. Thus the giant cell response is also a possible response to naturally induced tuberculosis in fishes despite reports to the contrary in the literature.

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4. Mycobacteria isolated from naturally occurring tuberculosis in a marine fish induced Langhans type giant cells after inoculation into plaice at 10°C. Thus the giant cell response is also a possible response to naturally induced tuberculosis in fishes despite reports to the contrary in the literature.

5. Talcum powder (magnesium silicate) induced both Langhans and foreign body type giant cells as well as intermediate type giant cells after inoculation into plaice. The giant cells bore large distinctive particles within their cytoplasm.

6. After inoculation of beryllium oxide into plaice, Langhans type giant cells were produced which contained large amounts of phagocytosed beryllium.

7. Ultrastructural examination of the cells involved in the complete adjuvant granuloma showed that the morphology of the various organelles was similar to that of the equivalent homeotherm cell and generally the organelles of plaice giant cells resembled their equivalent components in the epithelioid cells from which they arose.

8. The macrophage migration inhibition test gave evidence for the presence of cell mediated immunity to the piscine Mycobacteria.

SECTION VI

SUMMARY

A sequential histopathology study was carried out in plaice (Pleuronectes platessa) to determine whether the giant cells of the classical chronic granuloma of higher animals could also be induced in a teleost fish. Various irritants commonly associated with giant cells in mammals were used i.e. Freund's complete and incomplete adjuvant; talc (magnesium silicate) and beryllium oxide, and a Mycobacterium isolated from granulomata in a marine fish was also used. Experiments were carried out at 10°C constant environmental temperature except where specific effects of low temperature were being studied, when fish were maintained at 5°C.

Both Langhans type and foreign body type giant cells were present in complete and incomplete Freund's adjuvant, and talc, induced granulomata. In addition in the talc lesions an intermediate form having features of both types, was seen. Piscine Mycobacteria and beryllium oxide, both stimulated the production of only Langhans type giant cells.

Reduction of environmental temperature to 5°C slowed down the rate of development of the granulomata and no giant cells were seen.

Ultrastructural and histochemical studies of the developing granulomata showed that the inflammatory cells involved in the complete adjuvant lesion had close morphological similarities to the equivalent homeotherm cells.

Giant cells were characterised by their large size (16-70μm) and their multiple nuclei, which in Langhans type giant cells were placed at the periphery of the cell, usually in a horse-shoe configuration, and in foreign body type giant cells were scattered randomly throughout the cytoplasm. In the intermediate type giant cells of the talc granuloma some of the nuclei were placed in a line at the periphery of the cell while the rest of nuclei were distributed randomly.

A feature of the development where tubercle bacilli were present (e.g. Complete Freund's adjuvant and piscine Mycobacteria studies) was the presence of an abundant lymphoid infiltrate often arranged as focal lymphoid follicles.
A cell mediated immunity test, the macrophage migration inhibition test, carried out to determine whether such a delayed type hypersensitivity potential was present, indicated that from the 28th day onwards there was an active positive reaction to this test.

SECTION VII

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