LNPF has not yet been shown to possess any pharmacological activity other than an ability to increase vascular permeability. Thus, it fails to stimulate guinea-pig ileum or rat uterus, or to affect blood pressure in the rat, cat, dog or guinea-pig. Owing to its inert pharmacological behaviour it has been possible to differentiate LNPF from a variety of other permeability factors including histamine, 5-HT, bradykinin, kininogenase, substance P and globulin PF.

LNPF has been shown to rise in concentration at the site of a developing tuberculin reaction and to fall in concentration as the inflammation wanes. A similar rise and fall in concentration of LNPF has been noted in cutaneous hypersensitivity reactions in guinea-pigs (dinitrochlorobenzene) and rats (pertussis), and in experimental allergic thyroiditis. In addition, these reactions have been markedly reduced by treatment with antibody sera prepared against LNPF. Recently considerable interest has been shown in the ability of antilymphocyte serum to suppress delayed hypersensitivity reactions and to prolong homograft life (Levey & Medawar 1966). Antilymphocyte serum is believed to supress the delayed hypersensitivity reaction by 'blindfolding' lymphocytes and thus rendering them incapable of recognizing antigen. It seems possible that antilymphocyte serum owes part of its efficacy to an ability to suppress LNPF released at the challenged site. It has been shown that antilymphocyte serum has a more striking action on the lymph node than anti-LNPF in that it suppresses the numbers of both large pyroninophilic cells (immunoblasts) and small lymphocytes, whereas anti-LNPF has no apparent effect on the numbers of small lymphocytes (Turk & Willoughby 1967).

The problem of the mediation of the inflammation of the delayed hypersensitivity reactions is of profound clinical importance. Should the role of the lymph node permeability factor be confirmed and means developed to bring about its suppression, this could lead to a therapeutic agent with a wide spectrum of activity.

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The Fibroblast

The conventional description of the fibroblast is a morphological one. It is described as an elongated cell with long branching processes. Often the word stellate is used, but it is not very appropriate as the processes predominantly project from either end of the cell. The nature of the cell is also inferred from its surroundings; that is, the assumption is made that an elongated cell with processes lying amongst collagen fibres has formed the fibres and therefore is a fibroblast. In some circumstances this may well be a misleading assumption as the form of a macrophage may be constrained into an elongated shape if it is resting amongst collagen fibres. This fallacy is clearly a potential pitfall in experiments which claim to demonstrate the derivation of the fibroblast from macrophages.

The form of fibroblasts varies with their activity. By light microscopy the inactive cell, the

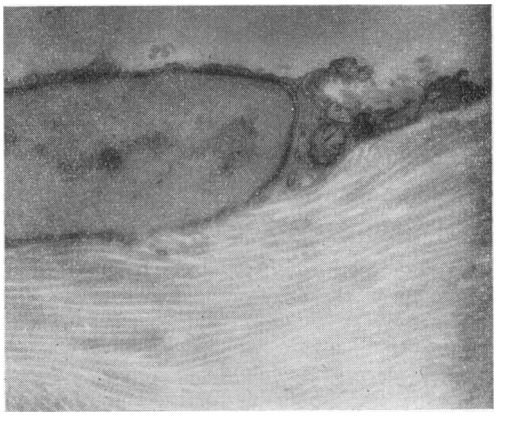

Fig 1 *Above inactive fibroblast. Part of its nucleus can be seen with surrounding cytoplasm containing mitochondria. Osmic-thiocarbohydrazide-osmic. Epon embedded.* × *20,000*

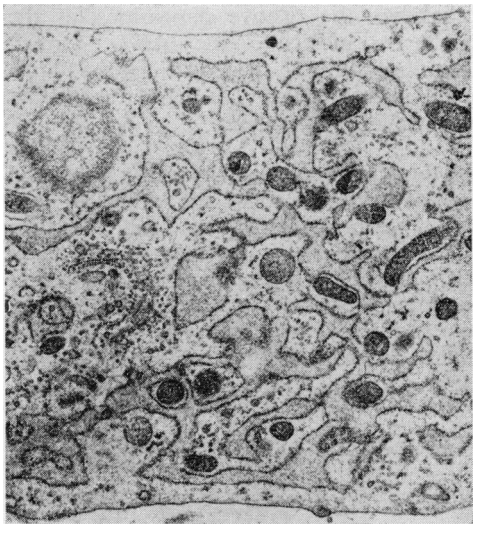

Fig 2 *Part of the cytoplasm of an active fibroblast showing a large amount of prominent endoplasmic reticulum with somewhat dilated cisternæ. Osmic fixed. Epon embedded, lead stained. Approx.* × *10,000*

fibrocyte, is a rather thin, elongated cell with thin processes, only a small amount of cytoplasm and a long thin nucleus. The active cell, the fibroblast, is larger with a plumper oval nucleus and a greater amount of basophilic cytoplasm.

The findings by light microscopy are confirmed and extended by electron microscopy. The inactive cell, as in the Achilles tendon of the rat (Fig 1), has a lesser amount of cytoplasm with fewer mitochondria and a small amount of endoplasmic reticulum. The actively synthesizing fibroblast is almost filled by endoplasmic reticulum. Mitochondria and the Golgi apparatus are prominent (Fig 2).

These morphological findings are fairly characteristic but the specific property of the cell is its ability to synthesize collagen fibres. Fortunately this can be demonstrated very precisely by performing autoradiographs of the cells after the injection of H^3-proline. It can be done using either light or electron microscopy. Ross & Benditt (1962) have followed this process in healing wounds in normal and scorbutic guinea-pigs at intervals of one and four hours, and one, three and seven days. They made autoradiographs of the wounds by embedding the tissue in epoxy resin, cutting 1μ sections and coating them with nuclear track emulsion. By doing grain counts of unit areas over cells and over interstitial tissue they showed that a maximum concentration of H^3-proline was attained over the cells in four hours and then the proline, presumably now included in collagen molecules, moved into the interstitial tissue.

More detailed analysis can be obtained using autoradiography with the electron microscope (Ross 1965). By this technique silver grains indicating the presence of H^3-proline can be seen fifteen minutes after administration primarily over the endoplasmic reticulum, after thirty minutes over the Golgi complex, and then in the peripheral cytoplasm and eventually over the extracellular collagen.

This analysis confirms what has been universally accepted, that the fibroblast synthesizes collagen. However, the old controversy remains as to where exactly the fibrils are synthesized. The alternatives are that the actual collagen fibrils are synthesized within the cell and somehow passed out through the cell membrane into the interstitium, or that the fibrils are assembled extracellularly from some soluble precursor.

The weight of evidence seems to me to be in favour of the view that the fibrils are formed extracellularly.

There has been no convincing evidence of collagen fibrils within the cytoplasm of fibroblasts. Several workers have published electronmicrographs which they claim demonstrate fibrils passing through the cell membrane of fibroblasts into the extracellular space. The cell membrane in these pictures is usually indistinct and often described as 'disintegrating'. Goldberg & Green (1964) have convincingly demonstrated that such appearances are due simply to oblique sectioning of intact cell membrane.

The idea of the extracellular assembly of collagen fibrils from a soluble precursor, presumably the tropocollagen molecule, is supported by two pieces of evidence: (1) It is possible to produce, *in vitro*, from solutions of tropocollagen, collagen fibrils that are indistinguishable from those formed in the tissues. (2) The first collagen fibrils formed are only about 100 Å in diameter. They increase in diameter in the extracellular tissue to a diameter of about 800 Å presumably by the accretion of tropocollagen molecules.

Solutions of tropocollagen molecules can be readily obtained by dissolving rat tail tendon in dilute acetic acid. Fibrils with transverse bandings of different periodicities can be obtained by treating the solution in different ways. Fibrils with the normal 640 Å banding can be produced simply by increasing the concentration of sodium chloride to 1%.

Fig 3 *Reconstituted collagen fibril negatively stained with potassium phosphotungstate.* × *150,000*

Fig 3 shows such a reconstituted fibril negatively stained with potassium phosphotungstate (Millington 1966). The 640 Å banding is seen to consist of a light band and a slightly wider darker band. To account for this transverse banding, Grant *et al.* (1965) suggest that the tropocollagen molecules, that are 2,800 Å long and 15 Å diameter, have along their length 9 zones: 5 are bonding zones, each about 265 Å long, separated by 4 non-bonding zones each about 375 Å long. The molecules aggregate laterally with bonding and non-bonding zones in register. The light bonding zone and the dark non-bonding zone then form together the 640 Å repeat (265+375).

Several workers have shown that collagen fibrils increase in size extracellularly from about 100Å diameter to about 800 Å. At any given moment in their development all the fibrils of any particular group are all about the same diameter, so that the mechanism whereby tropocollagen molecules are added to the outer surface of the fibrils must be a precisely controlled one that for some reason stops when the fibrils are about 800 Å in diameter.

Some years ago (Brewer 1959) it was shown, from observations on the birefringence of newly formed collagen, that there was a transversely arranged molecular component in newly formed collagen that disappeared as the collagen matured. This component could be removed by extraction with 0·2 M NaCl, a procedure which extracts neutral salt-soluble collagen. It was suggested at the time that this form of collagen might be arranged at right angles to the collagen fibrils and so account for the birefringence. However, when one considers the relative dimensions of the tropocollagen molecule, the newly formed collagen fibrils and the space between the fibrils, as shown diagrammatically in Fig 4, it is difficult to see how this could happen.

Even though the collagen fibrils may be formed extracellularly the fibroblast still influences their formation, particularly their orientation as they tend to be arranged parallel to the long axis of the cell. Quite how this is achieved is not clear but Porter & Pappas (1959) suggest that the initial very thin collagen fibrils are formed actually on the outer surface of the cell membrane. Such an association could influence the orientation of the fibrils.

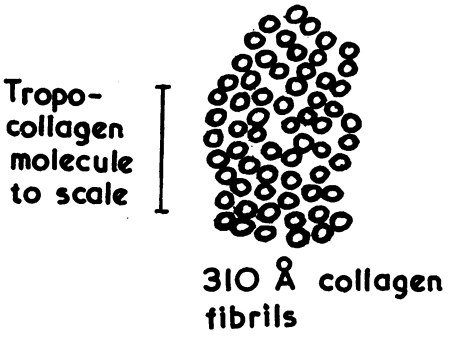

Fig 4 *The relative dimensions of young collagen fibrils and the tropocollagen molecule are illustrated. The circles are tracings of the outlines of young collagen fibrils cut transversely showing how closely they are packed*

In addition to forming collagen the fibroblast forms mucopolysaccharide. This formation goes on at the same time as the formation of collagen. The mucopolysaccharide can be demonstrated in electron microscope preparations by Hale's colloidal iron technique (Curran *et al.* 1966).

The mucopolysaccharide passes from the cell into the interstitial tissue where it lies about the collagen fibrils.

In vitro a variety of polysaccharides are able to precipitate fibrils from solutions of collagen. The fibrils so formed, however, show transverse bandings that differ markedly from the normal 640 Å. There is no evidence as to whether or not they play any part in the formation of fibrils in life. The amount of mucopolysaccharide is greater in newly formed fibrous tissue; it becomes less in amount as the fibrous tissue matures. There is some evidence that in mature collagen the mucopolysaccharide stabilizes the collagen (Jackson 1953).

Cells other than the fibroblast have the ability to form collagen. The two obvious examples are the osteoblast and the chondrocyte. They probably also secrete different mucopolysaccharides in different proportions so that they each produce their characteristic interstitial tissue.

Other cells may form collagen under special circumstances. One obvious example is the glomerular epithelial cell which forms collagen when it proliferates to form a crescent. After a little while, the cells within the crescent lose their epithelial shape and come to look like fibroblasts.

There are other situations in which it has been suggested that cells which do not normally form collagen may acquire this ability, e.g. the Kupffer cells in the liver. These thoughts and observations have led to the idea that macrophages can give rise to fibroblasts. This is an old idea but is constantly being revived; however, the experiments claiming to demonstrate this transformation are not generally accepted as being conclusive.

Davis (1963) studied the reaction to asbestos dust in the guinea-pig and rat lung. Initially he found the dust taken up by macrophages in the lung, but later when fibrosis developed the dust was found in fibroblasts. This was interpreted as demonstrating that the macrophages had been converted into fibroblasts. Gillman & Wright (1966) labelled blood monocytes with tritiated thymidine and induced inflammatory lesions in which they later found labelled spindle cells lying amongst collagen. They assumed that these were fibroblasts but the experiment would have been more convincing if it could have been shown that these cells were synthesizing collagen.

In the cellular reaction to intraperitoneal implantations of fragments of egg-white (Curran & Clark 1964) appearances are found which can be interpreted as illustrating macrophage ⟶ fibroblast transformation. However, the fact that fibrosis develops at the site where there were previously macrophages does not prove that the macrophages have been converted into fibroblasts; it may simply be that the macrophages have been replaced by fibroblasts.

Pathologists are all familiar with the appearance of fibrous tissue in scars extending between epithelial cells and also with the appearance of fibroblasts growing into a thrombus as it is organized. It comes as no surprise, therefore, to realize that in tissue culture the fibroblast is a mobile cell although it moves rather slowly. When it moves it has a characteristic shape (Ambrose & Jones 1961). Its locomotory organ is a large fanshaped anterior end which is constantly in motion. There are undulations, 'ruffles', on the under-surface of this anterior end that adhere to the substrate.

The movement of the fibroblasts has been analysed in considerable detail and can be explained in terms of two hypotheses: (1) Contact guidance (Weiss 1958). (2) Contact inhibition (Abercrombie & Heaysman 1954).

'Contact guidance' is a term introduced by Weiss to describe the fact that fibroblasts move with particular ease along fibres such as, for example, the fibrin threads in a plasma clot. In the same way they tend to move along grooves, and in both cases the cells tend to become elongated along the length of the groove or fibre. An interesting associated phenomenon is the fact that the form taken by the fibroblast is determined by the diameter of the fibre; the thicker the fibre the broader is the anterior end of the fibroblast.

'Contact inhibition', described by Abercrombie & Heaysman, is manifested when two fibroblasts meet in tissue culture. When the ruffling membrane of one fibroblast encounters the surface of the other all activity within it ceases and the cell movement stops, apparently inhibited by contact between the two cells.

This phenomenon has important consequences in that fibroblasts cannot move over one another. They cannot readily move into a concentration of other fibroblasts but their movement in areas free of fibroblast is uninhibited so they tend to become evenly distributed.

The active fibroblast is clearly a very busy cell, moving through the tissue actively synthesizing collagen and polysaccharide. The factors which can stimulate the rather insignificant resting fibrocyte to such activity remain a mystery.

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Meeting November 1 1966

A laboratory meeting was held at the Royal Army Medical College, Millbank, London. Professor Francis E Camps gave a lecture entitled **Experiences in the Practice of Forensic Medicine.**

Laboratory demonstrations included cases of interest from the Army Tumour Register, pathology of gunshot wounds, melioidosis, psittacosis, leishmaniasis, helminths and the preservation of frozen blood for transfusion.

Meeting December 6 1966

A laboratory meeting was held at the Middlesex Hospital, London. Demonstrations were given.

Meeting January 31 1967

A laboratory meeting was held at the Royal Postgraduate Medical School, London. Demonstrations were given.

Meeting March 7 1967

A laboratory meeting was held at King's College Hospital Medical School, London. Demonstrations were given.