# WOUND HEALING AND MANAGEMENT

## A MONOGRAPH FOR SURGEONS

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BY

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# PREFACE

AN informed insight into the processes of healing is important to the surgeon if only because a large part of his life is concerned with making and repairing wounds.

Many monographs on wound healing have been published but for the most part they have dealt with special aspects of the problem and have often taken the form of symposia by several authors. These are fascinating reading for the research worker engaged in the field but are too detailed for the general surgeon.

There seemed to me to be a need for a compact book, especially written for surgeons, in which could be found a concise account both of the biology of repair and of modern wound management—in short a practical account of wound healing to which a surgeon could turn for source material.

I have attempted in this monograph to fill this need. I am very conscious that in summarizing the fundamental work I have been guilty of too much compression and that complexities have often been over-simplified. I do not think that accuracy has suffered to an appreciable extent; if it has I apologise in advance to the individual research workers. The review of the clinical work, though often dealing with technical matters, is much more straightforward. It will, I hope, provide the surgeon with readily available information about wound management and enable him to review his own practice from time to time in the light of original work.

I have dealt entirely with the management of operative incisions and in particular with the subjects of sterilization, theatre design, theatre practice and suture technique because I have had some special experience with these problems. The management of traumatic wounds and burns is equally important but is adequately treated in many monographs.

Wound healing teems with unsolved problems. One may point to our lack of knowledge of the stimulus to repair and its subsequent inhibition, the role of the mucopolysaccharides and of their sulphation, the origin and function of the fibroblast and the control of collagen synthesis. In clinical work, wound infection, dehiscence and herniation make up a formidable challenge.

It may be that not all of these problems are soluble but it is certain that none of them will be solved without a great deal of expensive and time consuming research.

I am indebted to many people for help in the preparation of this book. My secretary, Miss Allison Thomson has been indefatigable in typing and in

proof reading. Mr. Murray Ettle, senior technician in the Department of Surgery has prepared and photographed most of the figures. I am indebted to the following for illustrations: Professor J. L. Pritchard, Queen's University, Belfast for Figs. 66B, 67B and 68B; Dr. A. T. Macqueen, Department of Physiology, Queen's College, Dundee for Figs 1, 2, 7, 8, 9, 14, 15, 17, 18 and 19; Dr. J. Rogers, Department of Dermatology, Royal Infirmary, Dundee, for Figs. 16A and 16B and 46; Dr. Henry Goodall, Department of Pathology, Royal Infirmary, Dundee, for Figs. 11, 12 and 13; Dr. A. Todd, Department of Pathology, Royal Infirmary, Dundee, for Fig. 29 and Miss M. W. Mackenzie, Medical Artist, Queen's College, Dundee, for the drawings of Figs. 66A, 67A, 68A and 70A.

D. M. DOUGLAS.

Dundee, 1963

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# PART I

# THE BASIS OF REPAIR

## Chapter 1

## CONNECTIVE TISSUE AND EPITHELIUM

An incision in the liver or kidney does not heal by the proliferation of liver or kidney cells but by the deposition of connective tissue (Fig. 1). The same is true, at least primarily, for every tissue in the body. Later the specialised tissues may proliferate and may permeate the wound but the primary repair substance is connective tissue. It may therefore be regarded as a kind of universal cement which appears whenever tissues are wounded. It is important at the outset to examine in detail this substance.

### Structure and Function of Connective Tissue

As the name suggests, connective tissue is concerned with supporting the framework of the body. It is therefore found in virtually every part of the organism. Its form is closely determined by the part it has to play. For example, in glandular structures such as the intestinal mucosa, the thyroid, liver and spleen, it is delicate and loose. By contrast, in ligaments and tendons which have to move heavy masses of tissues it is compact and immensely strong (Fig. 2). In the walls of arteries it is distensible and elastic; in the ligaments of joints it is tough and unyielding. The looseness or denseness of connective tissue is related to the ratio of fibres to the unit area. A high fibre-area ratio as in tendon gives great compactness and strength, whereas the reverse, as in the sheaths of nerves, gives laxity and mobility.

Microscopic examination of connective tissue shows that it is composed of fibrils, cells and amorphous elements or ground substance.

### The Fibrillary Elements of Connective Tissue

The fibrils of connective tissue are of three varieties, termed collagen, reticulin and elastin.

1. **Collagen fibres** are composed of an albuminoid substance which is converted to glue on boiling, hence the name collagen. They are strong, resistant to stretching and are made up of large numbers of smaller interlacing fibrils.

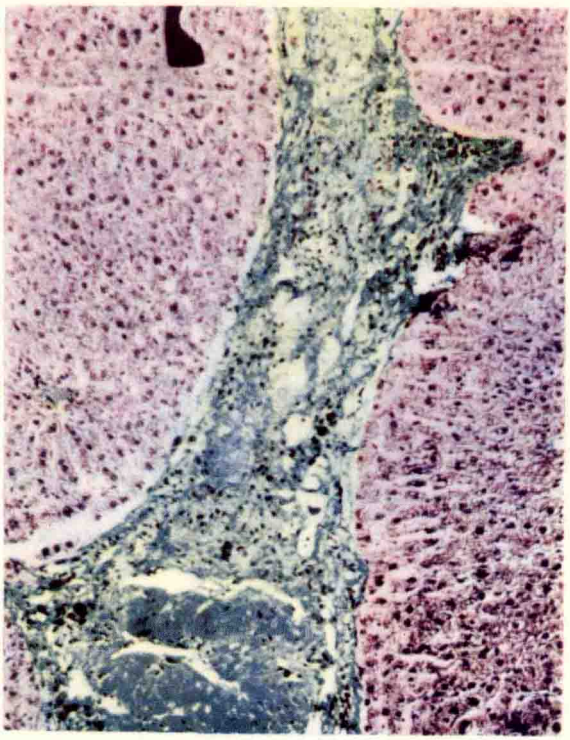

FIG. 1

Incised wound of liver of rat, 7 weeks. (Masson's stain × 150.)
The incision has healed by the deposition of collagen fibres by fibroblasts.

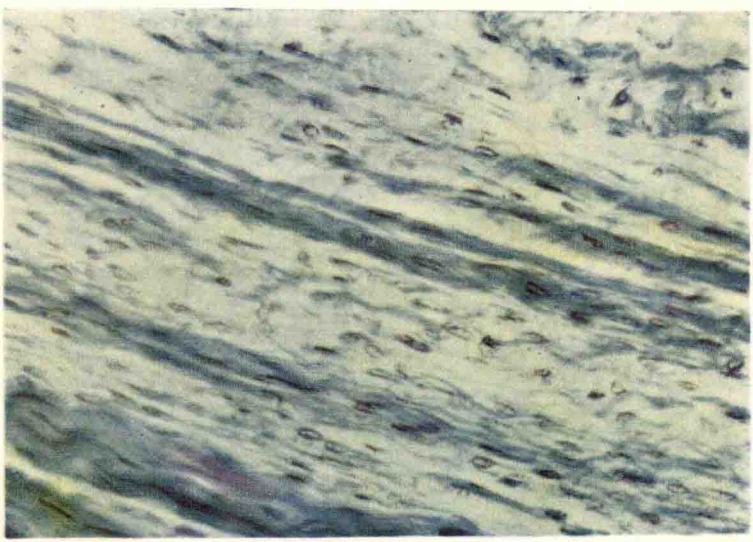

FIG. 2

Collagen fibres and fibroblasts in section of tendon. (Masson's stain × 360.)
The high ratio of fibres to cells gives great tensile strength.

When exposed to weak acid or alkali they swell; when exposed to strong acid or alkali they disappear.

The staining reactions are characteristic. They take up the acid fuchsin of van Gieson's stain and appear as bright red wavy bands with a few fibroblasts interspersed between them (Fig. 3).

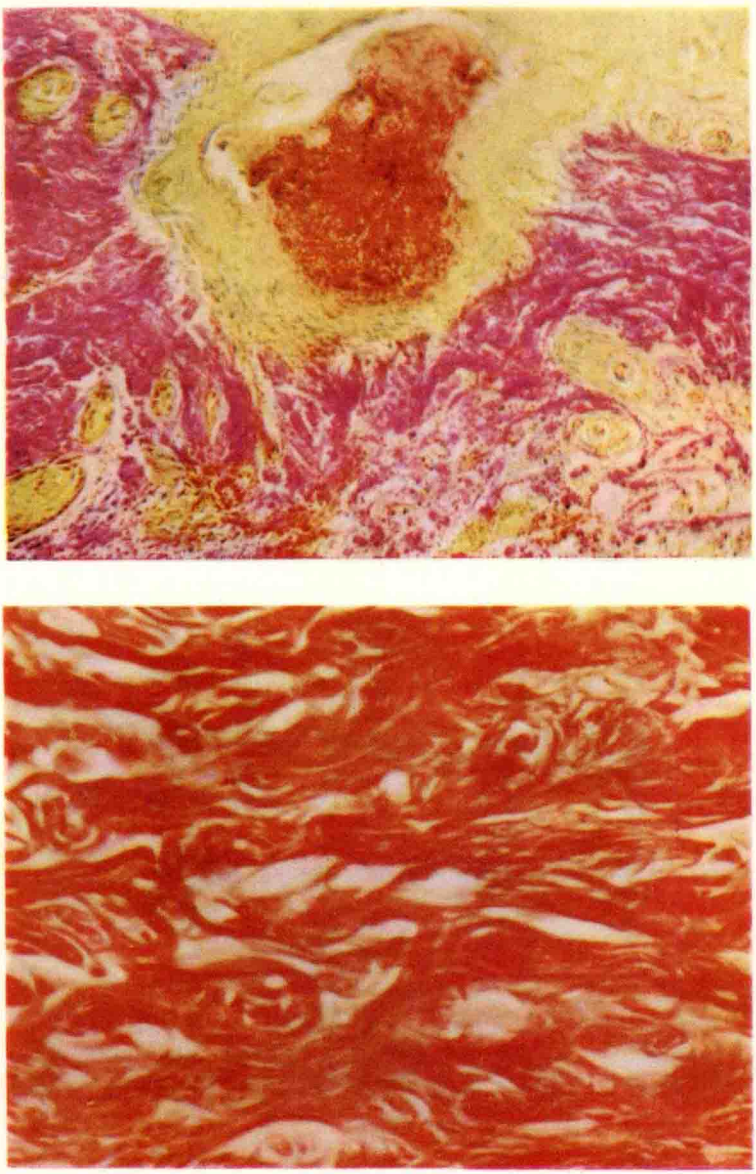

Fig. 3.—Collagen fibres from mouse dermis. (A × 100; B × 600. van Gieson.) The epithelial elements stain yellow, the collagen bright red.

Randall (1953) and his colleagues of the Medical Research Council Biophysics Research Unit, King's College, London, have studied the collagen fibre in great detail using X-ray crystallography. The principle of this method is that the denser parts of a molecule absorb X-rays (in much the same way as bones do) and thus shadows are cast on a sensitive plate. The configurations of the shadows are then studied and molecular composition deduced. Randall came to the conclusion that the collagen fibre is made up of globular units each containing an ordered arrangement of polypeptide chains. Each globule is joined to its fellow by a slow process of aggregation, not by sudden crystallization (Fig. 4). A sheet-like structure of polypeptides within the globule is possible

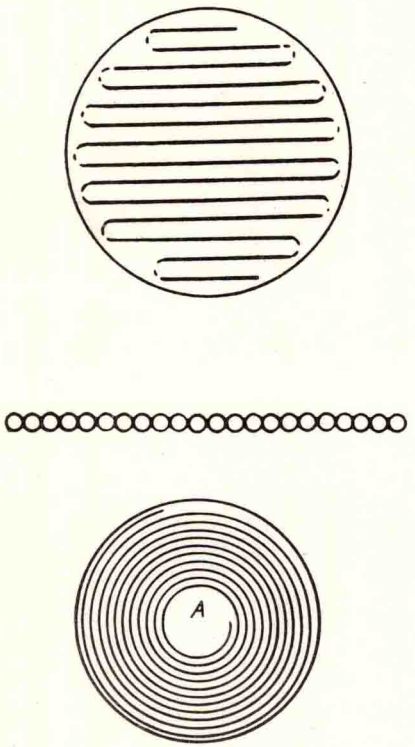


FIG. 4

Fig. 4.—The arrangement of collagen fibres as suggested by Randall from X-ray diffraction studies. They are built up of globular units (middle figure) joined together by covalent linkage. The globular units may be made up of a long convoluted polypeptide chain or series of chains parallel to the fibre axis (upper figure). Alternatively a sheet-like arrangement with polypeptide chains running parallel to the fibre axis is possible (lower figure). This should be compared with the hollow fibrils of Figure 6. (Randall, J. T., *Nature and Structure of Collagen*. 1953, Butterworths, London.)

either azimuthal to the axis of the fibre or helical with the plane of the polypeptide sheets parallel to the fibre. The linkage of the corpuscles must be of the covalent linkage type. Salt linkages would lead to immediate solution in liquids which are known not to dissolve collagen. Sugars or polysaccharides may play an important part in this union.

Electron microscopy has recently shed a good deal of light on the fibrillary structure. It has shown that the typical collagen fibre is a striated structure

not unlike striped muscle. The striations are of two types; in the first the interval between the striations is 640 Ångstrom units or about a hundred millionth of a millimetre. In the second the interval is 210 Å (Fig. 5). In general the 640 Å fibrils are more conspicuous in adult connective tissue and their diameter is greater in adult than in younger tissues.

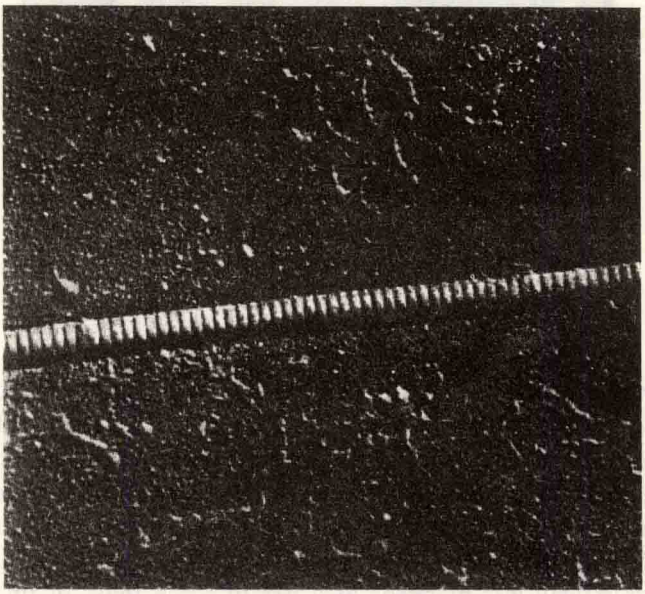

FIG. 5

Collagen fibre from rat tail tendon. Electron microscopy. The transverse striations are well seen, the intervals between the raised ridges being 650 Ångstrom units. (Wyckoff in *Connective Tissues*, ed. Charles Ragan, Josiah Macy Jr. Foundation. New York, 1952.)

The periodicity of the striations is taken by electron microscopists to reflect a regular repetition of molecular arrangement of the polypeptides. When the fibrils are particularly clearly seen (Wyckoff, 1952) the individual transverse striations can be seen to be made up of finer striations of a periodicity of 210 Å. Hence the molecular arrangement of the coarsely and finely striated fibrils may not be very different.

Most of these observations were made on collagen fibrils teased out of areolar tissue or tendon. But when sections are cut from dense connective tissue another point emerges. The fibrils have a tubular form with hollow cores, though the striations still show the same periodicity. Of course, during the preparation for electron microscopy the material from the hollow core may have been removed (Fig. 6).

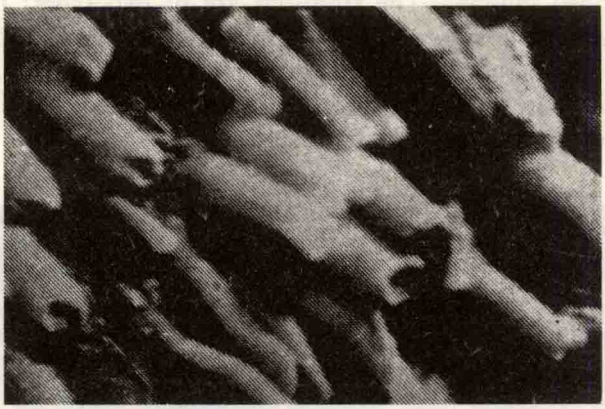

FIG. 6

Collagen fibres from tendon showing the apparently tube-like hollow character. Electron microscopy. (Wyckoff in *Connective Tissues*, ed. Charles Ragan, Josiah Macy Jr. Foundation. New York, 1952.)

Electron microscopy has also been used to study the formation of collagen fibres *in vitro*. If rat tail tendon is treated by dilute solutions of a weak acid like acetic, formic or citric acid, a solution of collagen is obtained. If buffered sodium oxalate at a pH of 4·6 is added, the collagen precipitates out in the form of fibrils which show characteristic striations of the 640 or 210 Å type. If the pH of the precipitating solution is altered towards alkalinity the striae become progressively less marked; at a pH of 5·5 a mass of unstriated fibrils appear. The process of solution and re-precipitation may be repeated on three or four occasions, is extremely rapid and seems analogous to crystallization. These observations lend support to the view that fibrillogenesis in connective tissue is an extracellular process.

2. **Reticulin fibres** are found abundantly in the supporting structures of the haemopoietic system, bone marrow, liver and spleen. They are much finer than adult collagen fibres, show many branching processes and have little tensile strength (Fig. 7). They take up silver stains, unlike adult collagen, but many authorities believe they are identical with young collagen fibrils (Gross, 1950).

3. **Elastin fibres** are yellow in colour, branch and anastomose freely with one another and have the power of returning to their original form after stretching (Fig. 8).

Like collagen they are composed of an albuminoid substance but with different properties. It is highly resistant to strong acid or alkali and is unaffected by boiling. The staining property, too, is different.

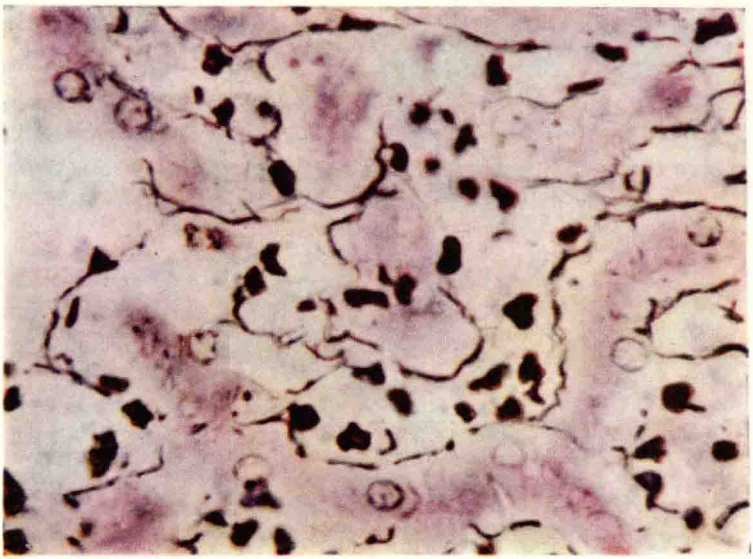

FIG. 7

Reticulin from mouse liver. (Silver stain with gold toning × 720.) The delicate structure of the fibres, the fine branching processes and their argyrophilic character is demonstrated.

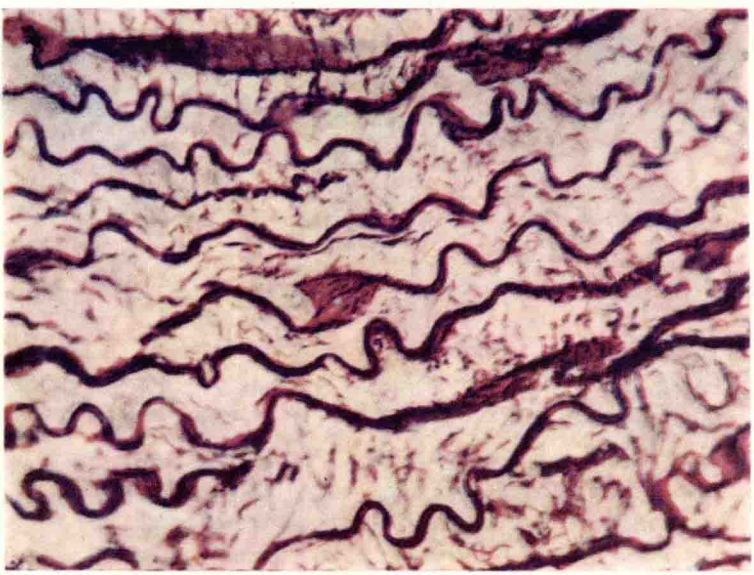

FIG. 8

Elastin fibres from aorta. (Weigert Resorcin-Fuchsin stain × 360.) The characteristic wavy fibres which stretch and return to their original form is well seen.

Elastin is found most typically in the walls of arteries, where it is admirably suited to take up the lateral thrust of the pulsatile flow from the heart and convert it into an even flow in the capillaries. When the elastin layer of an artery is destroyed, as in syphilis, the wall may give way and an aneurysm result. Elastin fibres are also plentiful in the ligamentum nuchae of grazing animals where they are important in taking the strain off the cervical muscles.

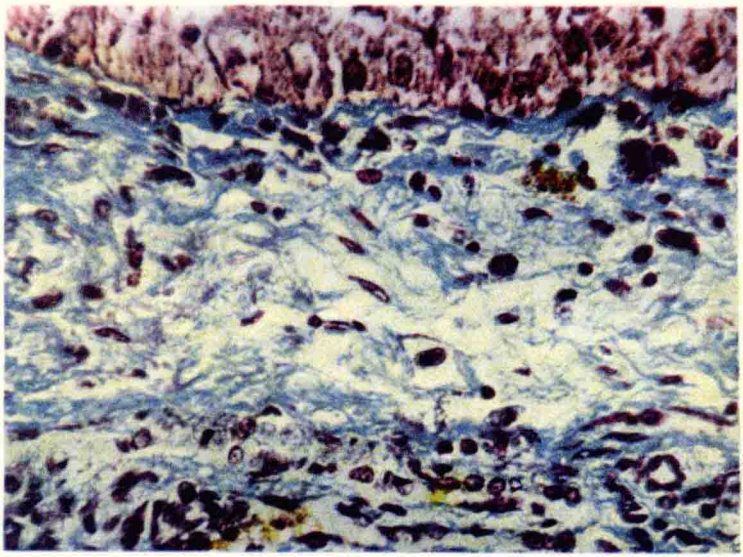

FIG. 9

Fibroblasts and collagen from healing wound of rat liver. (Masson's stain × 360.) Their elongated shape, as it were compressed between the fibres, is demonstrated.

## CELLULAR ELEMENTS

The two chief cells of connective tissue are the fibroblast and the macrophage but many other cells are commonly found. These include lymphoid cells, mast cells, plasma cells, fat and pigment cells.

1. **Fibroblasts** The fibroblast is an elongated cell measuring about 50 $\mu$ with a central ovoid nucleus (Fig. 9). The cytoplasm contains prominent mitochondria which probably contain the specific enzymes.

They are invariably found in relation to connective tissue and are believed to play an essential part in laying down and nourishing connective tissue fibres. It is possible that they also play a part in water metabolism by fluctuating in size during various conditions of hydration.

They are very readily grown in tissue culture and by applying the technique of electron microscopy to growing fibroblasts, their role in fibrillogenesis has been studied in great detail (Porter and Vanamee, 1949). When the edge of a fibroblast growing in tissue culture is examined with a magnification of between 5,000 and 30,000, fibrils can be seen as soon as 24-72 hours after the explant has been placed in culture and after 144 hours many fibrils are

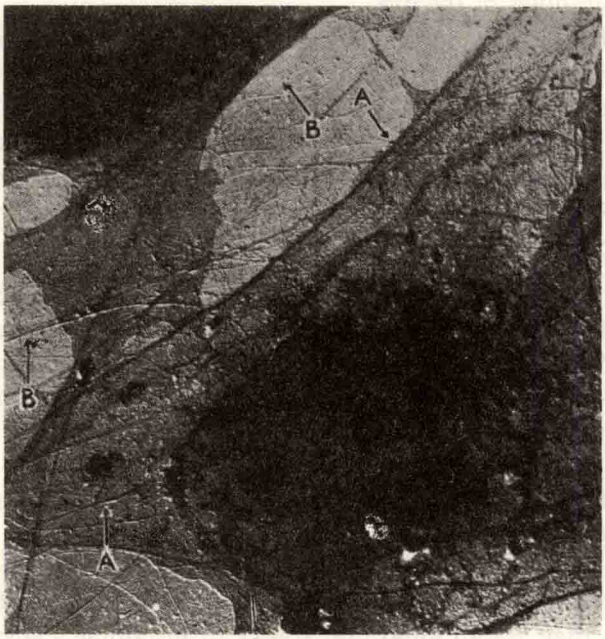


FIG. 10

Electron-micrograph of fibroblast growing in tissue culture of chick embryo skin. (A) refers to tonoglial fibres (B) to collagen fibres. Magnification × 4,500. (From Porter, 1951, in *Connective Tissues*, ed. Charles Ragan, Josiah Macy Jr. Foundation. New York.)

evident (Fig. 10). In the early stages of fibre formation these workers were able to trace the fibres back to the cell. The fibres often look as if they are being pulled off the outer surface of the cell rather in the same way as strands of egg albumen are pulled up by a matchstick dipped into white of egg. In no case were fibres seen forming within the body of the cell.

The very small fibres appear to coalesce together longitudinally to make up larger fibres of a diameter of about 600 Å and with the characteristic 640 Å periodicity. The part played by ground substance in this process will be discussed later.

2. **Macrophages** The name macrophage is applied to cells which have

the special property of ingesting foreign or dead material in the interstices of connective tissue. They have a characteristic round or ovoid shape with a deeply staining nucleus which has an indentation on one side. (Fig. 11).

The easiest way to display them is to inject a particulate dye like Trypan Blue into the areolar tissue of a living animal. A few days later they can be seen in large numbers lying free in the inter-fibrillary substance, their cytoplasm loaded with granules of the dye.

Macrophages may be 'fixed' as in the lining of the sinusoids of liver, spleen or bone-marrow or they may be 'wandering' as in the interstices of connective tissue. Their function is a 'scavenging' one, to pick up and ingest dead material and bacteria from tissues and to dispose of them by cellular digestion.

The relationship between the fibroblast and the macrophage is a matter of dispute. Porter (1951) has no doubt that in tissue culture fibroblasts change into macrophages and vice versa.

3. **Mast cells** (Fig. 12). Mast cells are the subject of a recent fascinating monograph by Riley (1959). The name 'mast' was given to them by Paul Ehrlich (1879) to indicate that they had a 'nourishing' function from the German 'mast' meaning food. They are found in large numbers along blood vessels and in serous membranes such as the capsule of the liver, spleen and peritoneum. They contain granules in the cytoplasm which are metachromatic; that is, they have the property of changing the colour of an ingested dye. For example, when stained with toluidine blue they appear pink.

For many years the function of mast cells was thought to be the production of heparin but recently Riley and West (1953) have produced convincing evidence that they are an important source of histamine. Histamine releasing substances, such as stilbamidine, cause disruption of the mast cell granules. In addition there is a close correlation between the tissue content of histamine and mast cells (Table 1).

**Table 1.** *Histamine (μg/g.) and relative mast cell content of various tissues*

| *Tissue* | *Histamine* | *Mast cells* |
|---|---|---|
| Rat liver | 0·3 | 0 |
| Pig aorta | 0·7 | 0 |
| Ox-liver parenchyma | 4·5 | + |
| Ox aorta | 10·0 | + |
| Rat subcutaneous tissue | 16·0 | ++ |
| Ox Inferior Vena Cava | 20·0 | ++ |
| Ox liver capsule | 40·0 | +++ |

Riley, 1959.

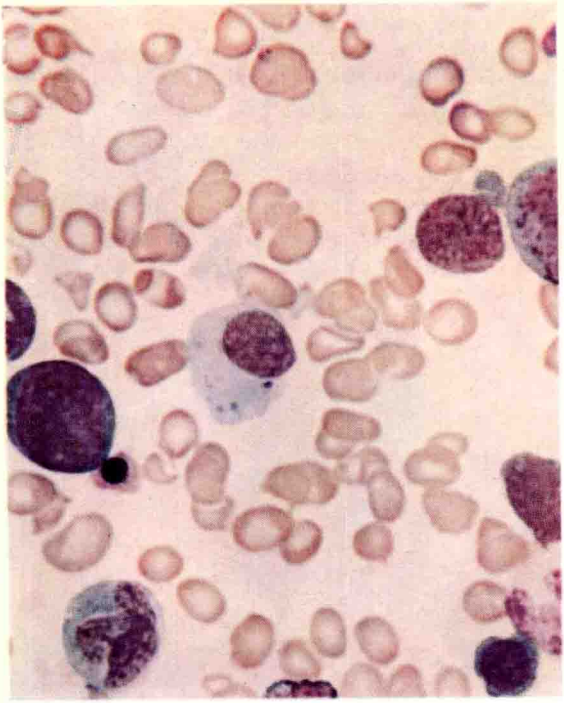

FIG. 11

Marrow smear showing macrophages with typical roundish shape and deeply staining indented nucleus. (H. & E. × 900.)

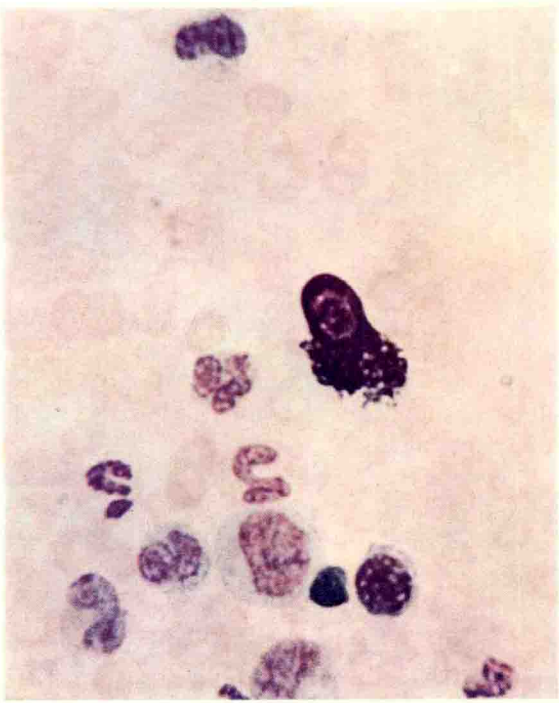

FIG. 12

Mast cell from marrow smear. The cytoplasm is packed with eosinophilic granules. (H. & E. × 900.)