SOME PROBLEMS in RELATION to the HEALING of WOUNDS.

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I. INTRODUCTION. *

No subject of scientific research better illustrates how unfathomable is truth than that of Inflammation. To it some of the greatest surgeons and pathologists have directed their attention. These facts, however, need not unduly deter one from entering on so difficult a field of study. In the past, it is the mere patient accumulation of seemingly trivial facts that has contributed not a little to the advance of science. For some master mind, laying hold on these, has, by the added light thrown on them by his own observations, often given them their true interpretation.

No subject, again, could better illustrate the truth that every step of advance in knowledge has been built on the steps preceding. The history of Inflammation and that of Wound-healing have run parallel courses. Both take us back to the ancients; both may be divided into the same two main divisions. The/

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* For the greater part of this Introduction, I am indebted to the works of Marchand, Metchnikoff, and Adami.

The earlier lasts from the time of Galen to the invention of the miscroscope (about 1590), and the discovery of the Circulation of the Blood. (1628). The later reaches to the founding of the doctrine of cells (Schwann) and the new views of the formation of tissue. Successive stages are marked by the names of great men, among whom may be mentioned Boerhaave, Hunter, Bichat, Schwann, Virchow, Lister.

Hippocrates distinguished between the healing of wounds with and without suppuration. Celsus (ist Century A.D.) gave us our clinical conception of inflammation, and from his time the four cardinal symptoms "redness and swelling with heat and pain" have been recognised. Galen (2nd Century A.D.) in the "Ars Medica" enunciates principles on the healing of wounds wnich have been the foundation of all later discussions on this subject. He also, was the first to speak of the primary and secondary healing of wounds. During the later Old Ages and the whole of the Middle Ages, observations were naturally restricted to those made by the naked eye; a really great change could set in only with the use of the mi$croscope. The discoveries of the Circulation in the capillaries and of the red blood corpuscles by Malpighii in 1661 and 1665 were specially important, followed up as they were by the knowledge of the more intimate/

intimate structure of the various organs and tissues of the body. Towards the end of the 18th Century the nature of the small blood-vessels became more definitely known, and discussions arose as to the nature of the substance which first joins together the severed edges of a wound. In every wound small vessels were cut across with loss of blood which coagulated between the wound-edges. This coagulable and plastic lymph was said to change into cellular tissue and be traversed by vessels.

The teaching of John Hunter laid the foundation for the more modern conceptions of wound-healing. As far back as 1790 Hunter pointed out that blood and lymph were living substances and as such could bring about the natural union of the severed parts. In his famous work Hunter distinguished three kinds of healing of wounds:-

(1) By primary union,
(2) Through inflammation, which he called Adhesive.
(3) By Granulation.

As a special kind of healing Hunter described "Healing under a scab" which stands between primary healing and that by Granulation. Hunter thought it possible that the vessels, whose severed ends lay on each/

each side of the wound, influenced one another by a sympathetic attraction and then inosculated.

Bichat accounted for the formation of new tissue by its development out of existing cell tissue; this doctrine was later developed by Virchow. The conception of healing by Blood Clot (Hunter), however, remained the dominating one till almost the middle of the 19th Century.

The year 1838 marks a very important turning point in the history of all pathological processes. In that year Schwann ennunciated his cell-theory which must be placed beside the evolution-theory as "one of the foundation stones of modern biology." In all the higher forms of life, whether plant or animal, the body may be resolved into a host of minute structural units, known as cells, out of which, directly or indirectly, every part is built. In the following years attention was naturally focussed on the question - How do the cells of the body arise? Living tissue elements had been thought to be produced out of parts previously destitute of shape, such as the formative fluids. Haller held that the various tissues of the body were reducible to fibres as their ultimate constituents, and, later, another element - the globule or granule - was supposed to form the fibres. A cell was supposed to be produced by/

by the globules arranging themselves in a spherical form to constitute a membrane, inside which other globules were arranged and composed the contents. Others held that a number of globules existed scattered throughout a fluid and, under certain circumstances gathered together in a cluster. This was the starting point of all further development, a membrane being formed outside and a nucleus inside by the differentiation of the globules. Schwann, Henle and their fellow-workers believed that cells might arise, either by the division of a pre-existing mother-cell, or by "free cell formation," new cells arising out of a formative substance - the "cytoblastem." The first formed cells Henle called "Primary Cells." In wound-healing Henle accounted for the new formation of cells by their rise from the plastic Exudate, and of the fibres of the connective tissue by a transformation of the "cytoblastem." These views of the spontaneous generation of new cells were destined to give way to the view that no cell can build itself up out of any non-cellular substance, but, where a cell arises, there a cell must have previously existed.

A complete change, therefore, of all the conceptions of the new formation of tissue arose with the/

the formulating of the doctrine by Virchow in 1858 "Omnis cellula e cellula." Remak had already in 1843 observed the division of embryonic cells, and Kolliker and others showed that an essential part of this process is a division of the cell nucleus. Virchow, however, first set forth the conception of a series of cell-divisions extending backwards uninterruptedly. In place of the former blastem and the original plastic lymph Virchow, with small restrictions, put the connective tissue as the common budding-stock of the body. The cells of granulation tissue he accounted for by the connective tissue cells which increase in consequence of irritation.

A further important change in the views on inflammation and wound-healing came with the discovery by Cohnheim in 1867 of the emigration of colorless blood cells from the blood-vessels. Cohnheim held that these cells changed into round cells with one nucleus and then into the spindle cells which formed the connective tissue, but that the tissue cells in inflammation took no active part. If a wound heal by first intention all the emigrated cells become connective tissue cells; if suppuration supervene, part change into pus corpuscles.

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Stricker and others defended the former opinion/

opinion that the new formed tissue arises exclusively out of the pre-existing tissue cells, in accordance with the first law of regeneration that the newly formed cells are derived always from cells of the same kind - "Omnis cellula e cellula ejusdem generis." The discovery of the Indirect or Mitotic division of nuclei in 1873 became of great importance for the understanding of this law and this method of cell division must be regarded as the general expression of the "eternal law of continuous development" on which Virchow insisted. This process is characteristic of all embryonic and actively growing cells, while "mass-division," as shewn in direct division or Amitosis, is equally characteristic of highly specialised or degenerating cells in which development is approaching its end.

Later investigations on the relationship of nucleus and cytoplasm have conclusively shewn the controlling influence of the former. Adami has reviewed the data which together compel the conclusion that the nucleus is the centre of cell-activity, showing that the higher syntheses - those associated with growth and those governing the specific enzyme action of different forms of cells - are determined and initiated by nuclear matter. The nucleus of the fertilised/

fertilised ovum is formed of corresponding amounts of nuclear matter (chromatin) from both parents, each supplying a like number of chromosomes or chromatin loops. The nuclear matter, therefore, conveys and determines and controls the inherited pecularity of the individual. Macallum, Carlier, and others have described the processes seen in gland cells - how the nuclear matter becomes diffused into nucleolar which then passes into the cell-substance from the nucleus and becomes changed into definite secretory granules. The many varied nuclear changes in the cells of many tumour growths need only be mentioned as another illustration of the great importance of the nucleus. In our endeavour to comprehend what life is, Adami holds that the ultimate conception of life must be that it is the function or the sum of the functions of a special order of molecules, and that these ultimate molecules of living matter - the "biophores" - must be relegated to the nucleus. He expresses his conviction that the future will see not merely a cellular but a nuclear pathology and physiology. "From the 'omne vivum ex vivo' to the 'omne ovum ex ovo' and the 'omnis cellula e cellula' of our predecessors we now reach the 'omne chromosoma e chromasomate' of the modern student of development and see before us surely/

surely the conclusion 'omne biophorum ex biophoro ejusdem generis'."

As the work on which the following paper is based is concerned, not with the phenomena of inflammation and their significance, but with the morphological elements which are connected with it, and the rôle ascribed to them, I can only very briefly outline some of the views held regarding the essential features of inflammation. The Clinical conception of inflammation has changed little since the time of Celsus and Galen. At first, 'heat', from which the process has acquired its name, was looked upon as the prominent symptom; later, most importance was attached to the 'redness', and hyperaemia gradually superseded all other essential symptoms; and this in its turn gave place to the 'swelling' - a consequence of the hyperaemia. Definitions of inflammation have failed because they have put in the foreground the primary essential phenomenon from the standpoint of the observer. The old view, however, that inflammation was essentially an injurious process has given way. Now the irritant is regarded as causing cell and tissue destruction, but inflammation itself as "the series and sum of the reactive processes set up in the tissue and bringing about regeneration and repair"./

repair". All the changes are related to the neutralisation and removal of the irritant and the repair of the damage.

Boerhaave, Hunter, and Rokitansky looked upon Inflammation as a series of phenomena essentially connected with the blood-vessels. The vessel changes they thought were due to a paralysis of the Vaso Motor nerves by the irritant causing the inflammation, and the exudation they accounted for by assuming an increased thinning and permeability of the vessels.

Virchow in his attempt to separate the conception of the Inflammatory process from a mere vague connection with the blood-vessels brought it into relation with the phenomena observed in the cells. Inflammation was the result of an irritant which acted on the tissue cells, exciting proliferative changes which attracted more blood to the part. Virchow's theory, known as "The Cellular and Attraction theory", explained the part of the exudate taken up by the irritated cells as the product of an increased attraction of the components of the blood by the tissues, and the part which gathers in the spaces as the result of mechanical pressure in the vessels. A living part cannot be injured without undergoing changes which result in production of young/

young cells, but this is never the first link in the chain of effects. The first local effect is vascular and this change in the vessels shows itself in tissue degeneration. Degenerative changes must play a certain rôle, especially in the early stages. Probably these begin in the vessels and attack first the cement substance between the cells and then the cells themselves.

The discovery of the emigration of leucocytes by Addison and Dutrochet in 1842 and by Waller in 1846, and the re-discovery by Cohnheim in 1867, rendered necessary a reversion to the influence of the vessels.

In 1873 Cohnheim ennunciated his theory of inflammation, since known as the "Alteration Theory"- "the alteration of the walls of the vessels is the cause of the phenomena designated inflammation." Cohnheim's proposition merely states that this is the proximate cause of all the phenomena - the dilatation, the stasis, the exudation etc. The changed state of the vessels is itself an essential part of inflammation and therefore not the cause. For Cohnheim inflammation was not a reaction to extrinsic influences but a primary lesion of the vessels. The attention of pathologists had therefore been concentrated on the/

the part played by the vascular system and the local tissue elements respectively. At first the "rubor," being the most significant sign of inflammation, was accounted the essential part and theories were reduced to an explanation of the hyperaemia. Hyperaemia, however, may often exist without exudation, which others considered an essential condition, and to explain the "tumour" it was held that the tissues had an attractive influence on the blood. This view was more definitely formulated in Virchow's conception of an increased nutritive and productive activity of the cells. The researches of Lister and Burdon Sanderson had given us an explanation of the various phenomena which the vessels manifest, and brought out the fact that the irritant acts injuriously on the tissues. They had also demonstrated the importance of micro-organisms as the cause of the inflammation, but the relationship between this factor and the movement of the leucocytes was not understood till Metchnikoff enunciated his "Phagocytic Theory."

Metchnikoff saw in Inflammation "an aggressive reaction of the organism against pathogenic agents." In "phagocytosis" he recognised this defence of the organism, and from his biological studies in comparative inflammation, he concluded, that this/

this was the essential feature in inflammation and that the reaction to injury exhibited throughout the whole animal kingdom was against Cohnheim's view. In the vegetable world the processes of reaction to injury are not analogous to inflammation. Repair is accompanied by the destruction of the injured cells and a local proliferation of the neighbouring cells. The lowest unicellular organisms react to injury by chemiotaxis and phagocytosis with intracellular digestion, and in the higher forms of life these reactions are performed by certain definite cell-groups. In both the nucleus plays an important part in the reaction to injury.

In unicellular organisms, e.g.- the Amoebae we find that the reaction to injury is two-fold:-

(1) Destruction or removal of the irritant effected by the ingestion of the particle around which a vacuole is formed, the fluid in which becomes increasingly acid and digestive.

(2) The new growth of the organism. The Myxomycetes, - a group representing both animal and vegetable properties and forming large plasmodia with many nuclei - can eliminate dangerous objects by a digestive or excretory process, or, by a negative chemiotaxis, can avoid them.

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In multicellular organisms with tissues more differentiated for separate functions we find the same two processes as in the Amoebae: the role of defence is devolved on moveable cells of the mesoderm, which flow on the hurt point and new growth is affected chiefly by the fixed cells of the part. Amongst the lowest Metazoa - the Sponges, which were for long supposed to be colonies of protoza - the amoeboid cells of the mesoderm digest substances as well as englobe them, or form a mass round the foreign object and fuse around it. The reaction here is confined to these cells of the mesoderm floating in a semi-liquid substance filling the general body cavity: there is neither blood nor plasma, blood corpuscles nor blood-vessels. Higher in the scale among the Worms we find fixed cells lining the body cavity - the peritoneal endothelium; here the reaction is by means of the mesodermal cells of the fluid and these fixed endothelial cells. The incomplete vascular system takes no share: the blood, flowing from the heart sooner or later into the lacunae of the body cavity, may be looked upon as a mesodermal fluid. In the higher invertebrates, e.g.- the Insects, the blood corpuscles are represented by colorless cells with pronounced phagocytic functions. They gather around/

around a foreign body and infiltrate the surrounding tissues but, as there is no closed vascular system, there can be no question of diapedesis - the lacunar circulation aiding the approach of the leucocytes. The cell accumulation consists chiefly of the cells attracted to the injured spot to which they are brought by the blood current. There is later a proliferation of the fixed cells of the part and this may be so great, involving epiblast, mesoblast, and hypoblast cells, that it can reproduce whole segments.

Among the Vertebrates the phenomena are more complex. The blood is enclosed in a complete vascular system, and there is a highly developed nervous system. In the embryo of the Axolott the phenomena of reaction are carried out by the pre-existing moveable cells of the connective tissue alone as the blood-vessels in parts, such as the fin, are too narrow to allow the circulation of the corpuscles. In older larvae, however, with wider blood-vessels the inflammatory reaction takes place in the manner so frequently described. There is first an acceleration of the blood stream, then a slowing with an accumulation of white corpuscles in the peripheral zone, and their emigration and movement towards the injured spot. The wandering cells of the connective tissue also move but their number is unimportant compared/

compared to the mass of the emigrated blood cells. The fixed cells undergo various changes - swelling and vacuolation - and lose many of their processes. The migratory cells soon show marked phagocytic properties, and ultimately change into fixed connective tissue cells, although some perish and others pass again to the lymphatics.

From this rapid survey of Metchnikoff's position we have seen that the determination of amoeboid cells to the region of injury is the most constant early response to injury. At first it cannot be distinguished from a simple intra-cellular digestion effected by amoeboid phagocytic cells of the mesoderm. Among the invertebrates this is shared in by the fixed cells, the peritoneal endothelium, the cells of the perivisceral fluid and of the blood. Among the invertebrates the defending phagocytes emigrate from the vessels. Metchnikoff closes this chapter of his work by stating "we must conclude that the essential factor consists in a phagocytic reaction on the part of the animal organism." The other phenomena are all accessory, to facilitate the approach of the phagocytes. All the acts are measured, adapted to the mode of action of the cause, proportionate to its intensity, and have a beneficial influence. Metchnikoff does not include/

include the phenomena of repair in inflammation, as these begin only where the struggle is over. The key-note of Metchnikoff's doctrine of Immunity "There is only one constant element in Immunity, and that is phagocytosis" might equally be applied to his doctrine of Inflammation. His view is at once too narrow and too wide for it identifies inflammation with what is a universal phenomenon of nature.

Undoubtedly all the views contain an element of truth and are as the different aspects of some complex phenomenon observed from different points of view. To Virchow, the founder of Cellular Pathology, we owe the full recognition of the fundamental importance of the living cell in all the processes of life. Cohnheim's work laid the foundation of most of the later discoveries on the nature of inflammation and his conception comes nearest to the clinical and therapeutic point of view. To Metchnikoff we owe the broad conception of the significance of the phenomena, and through him we have reached our present standpoint regarding not merely Inflammation but the larger subject of Immunity.

As I have before stated my subject is the histology of Inflammation. Different problems will be/

be taken up under successive headings, but I should perhaps, mention here what these histological problems are which will be passed under review. The first and main one is concerned with the vexed question of the cell-forms found in the inflamed area - their origin, function and significance, and their destiny. This is the old question of the cells of granulation tissue, regarding which one writer has said "confusion is worse confounded," and it embraces many of the minor points to which reference will be made. Notice will also be taken of the mode of formation and time of appearance of new blood-vessels; the new formation of the connective tissue fibres; the mode of formation of giant-cells; the changes in muscle and in fat tissue.

II/

II. MATERIALS AND METHODS.

The plan of research was the following:-

I. A series of small incisions in the skin and subcutaneous tissues of various animals. Rabbits and mice were used for these wounds. The animals were killed after varying periods - 6 hours, 12, 18, 21, 24, 27, 30, 33, 36, 42, 48, 60, 72, 84, 96 hours, 5, 6½, 8, 10, 12, 15, 21, 31 days after the incision. A few incisions were made in the rat to trace the differences, if any, in the first appearance of Mitoses and in the new formation of blood-vessels. Incised tissues were kindly given me by Dr Beattie from the dog, and by Dr Milne from the Guinea-pigs.

II. A short series of abscesses to trace the encapsulation of the abscess and, if possible, its absorption. Sterile and infected catgut was inserted into the subcutaneous and muscular tissues of the back in rabbits and the animals killed after 30 hours, 60 hours, 90 hours, 5, 6½, 8, 11, 15, 18, 25, 31, and 42 days. Two degrees of infection were attempted in the hope of producing a lesser and greater reaction. Sterile catgut and/

and catgut infected with Staphylococcus pyogenes albus was inserted on either side of the Spinal column in one animal, and catgut infected with Bacillus Coli communis in the same position in another animal. The method of preparation was to steep the catgut in broth infected from a fresh culture of the organism and leave it in the incubator for 24 hours. It was then removed and washed repeatedly in sterile distilled water. It was found that the catgut injected with the Staphylococcus pyogenes albus had become practically sterile, for the tissues removed from the same animal showed practically no difference either to the naked eye or miscroscopically. I have thus only the tissues showing the reaction to the sterile catgut and to the intensely active pus-producing Bacillus Coli.

III. A short series of granulating surfaces comparable to ulcers. These were obtained simply by leaving incised wounds to gape.

It may not be out of place to mention some of the difficulties which were met with in the course of this work. In addition to the restlessness of the experimented-on animals interfering with the healing/

healing processes there were many circumstances which altered the sequence of the phenomena. In rabbits the "skin" is composed of epidermis, a very dense corium layer closely penetrated by hairs and with few blood-vessels, and a narrow strip of muscular tissue. This is separated from the dense fascia covering the deep muscles by a very loose layer of areolar tissue; it is in this loose layer that the separation takes place when a rabbit is "skinned". As in many rabbits the dense connective tissue of the corium had almost a cartilaginous consistence the incision produced very slight reaction, while extensive haemorrhage and leucocyte infiltration had taken place into the loose layer. It was found almost impossible to cut these sections with the microtome, and as the most important layer for examination lay between two dense structures, the dense muscle fascia and the still denser corium it was entirely broken up. The above remarks refer specially to the skin over the back but many of the incisions were made through the abdominal wall. Here the chief difficulty lay in the approximation of the wound edges, due chiefly to the separation of the layers during respiration. Owing to the difficulty of cutting the sections, if stitches were superimposed, the whole thickness of the/

the abdominal wall was taken in, by single stitches placed close to one another to prevent hernia of the abdominal contents. The thin "skin" over the abdominal surface however, and the differing tension of its surface caused great incurving of the skin into the wound-edges. The stitches, especially in the wounds of the earlier periods caused great difficulties in the subsequent cutting of the sections.

Many of these difficulties would have been obviated had I been able to make the incisions on the dog, in parts where the fat tissue renders the skin wound more comparable to the conditions in the human skin, and where approximation of the wound-edges might more easily have been obtained without too many stitches. On the other hand, it was found that the skin tissues of the dog are even more difficult to cut with the microtome than those of the rabbit, as these are incomparably more difficult than those of rats and Guinea-pigs. By carrying the tissue through hard paraffin and embedding it in hard paraffin it was found that the dense tissues were better penetrated than when a mixture of hard and soft paraffin was used.

As simple aseptic wounds show little reaction no attempt was made at asepsis. This would in/

in any case have been difficult in the conditions under which the operations were carried out. Many of the earlier of these were performed alone and the catgut or horsehair stitches came much in contact with the surrounding objects in my attempt to keep the animal under chloroform. The result is that many of my specimens show an excessive accumulation of leucocytes around the stitches, while knives, soon blunted by the tough skin of the rabbit, caused greater heamorrhage and excessive degenerative processes.

The further course of the healing process, the intensity and extent of the emigration of leucocytes and proliferation of cells, the time of the new formation of blood-vessels and of the transformation of the cell into fibres all varied in individual cases within wide limits and without one being able always to discover sufficient grounds for it. Such variation Changing mechanical, chemical and bacterial irritation took their part in these differences, even when one had taken care to carry out details in the same operation with the greatest possible equal care and technique.

A further difficulty was met with in the opening up of many of the wounds during the spasms which precede death. In consequence of this a great part/

part of the valuable material for examination, lying between the wound edges, fell away when the tissue was placed in the fixing fluid. It was found necessary to place the whole piece of tissue, enclosing the incision, in the fixing fluid en bloc, as the cutting of the tissue, to obtain better penetration of the fixing fluid, disarranged the soft parts in the wound cleft and around it.

After a trial of a large number of fixing fluids, Lang's Corrosive Solution was finally chosen in all cases. The animals were killed under Chloroform and the tissues immediately placed in warm fixing fluid, at the body-temperature, as recommended by Maximow and Schwarz.

The following staining methods were used:-

1. Haematin and Eosin - as the ordinary routine stain.
2. Alcoholic Eosin and Methylene Blue - specially for cells.
3. Benda's Stain (Saffranin and Licht Grün) also for cells.
4. The Unna-Pappenheim Methyl-Green-Pyronin and Resorcin stain - both for cells and to trace the new blood-vessels.
5. Van/

5. Van Gieson Stain: both as a routine stain and to trace fibril formation.

Mallory's Connective Tissue Stain: Heidenhain's Iron-Haematoxylin Stain: Gram's Stain and Thionin Blue were also used.

III. SUMMARY OF MAXIMOW'S WORK.

I have thought it right to give here a somewhat detailed account of the most recent extensive work on this subject. To do so will not only be convenient for reference in future discussion, but also in a very small measure will enable me to pay the debt I owe to Maximow for the help derived from his very beautiful and minute descriptions. As his articles in Ziegler's Beiträge, however, extend to nearly 500 pages, this resumé can be of only the briefest description.

Maximow induced both an aseptic and a septic inflammation in rabbits and other animals by introducing ingenious foreign bodies into the intermuscular connective tissue of the lateral abdominal wall. By means of these he obtained different varieties of cells almost in pure culture (Reimculturen) according to the ease with which they were able to penetrate into small cavities or interstices.

Maximow begins his exhaustive work by a careful description of the cell-forms normally present in the connective tissue in the rabbit. These are/

are as follows:-

(1) The usual connective tissue cells. These are the best defined elements and have a large oval nucleus with numerous very fine chromatin particles and one or more fine nucleoli. The cell-body is elongated and has numerous processes, and it lies closely connected with the collagan fibres. The network structure of the protoplasm is more evident around the nucleus, while, towards the periphery, the protoplasm stains more faintly and is an almost structureless layer.

(2) The small "wandering cells" - first described in the connective tissue by von Recklinghausen. These cells do not represent any precise conception and their source is unknown. Many regard them as leucocytes which have emigrated from the blood-vessels, and others as free and wandering connective tissue cells. Their number is usually very scanty and they lie irregularly distributed in the meshes of the connective tissue. The smaller forms are almost identical with the lymphocytes in structures. The larger forms have a slightly indented/

indented nucleus and a wider rim of protoplasm: the indentation of the nucleus deepening and the protoplasm border around it widening with the enlargement of the cell. Corresponding to the nuclear depression a clearer "hof" is differentiated in Iron-haematoxylin preparations. This clearer area contains the centrosome apparatus.

(3) Cells which are very similar to the connective tissue cells but have special granules in the protoplasm and the nucleus denser in structure. The cell-body shows a fine-meshed network with many clear vacuoles. These cells have been confounded with the ordinary connective tissue corpuscles but, as distinguished from these, the processes never anastomose and, on account of this, portions of their protoplasm are frequently constricted and cut off, while the cell-body becomes regenerated. This appearance, which Ranvier held to be a secretion and an important factor in the nourishment of the tissues, led him to give the name Clasmatocyte to these cells from the Greek, klasma - a part cut off. These cells are in very scanty numbers in the subcutaneous tissue but/

but are in large numbers in the omentum. Ranvier derived these cells from lymphoid cells and traced all transitions between the two forms. The cells, after emigration from the blood-vessels, greatly increase in size, especially in length; garner in their protoplasm a special tingible substance in the form of granules, send out processes, thereby losing their power of amoeboid movement; and become settled elements in the tissue. Maximow found not only transition forms between the lymphoid cells and the Clasmatocytes , but also transitions from Clasmatocytes to the ordinary connective tissue cells without granules.

Maximow found no polymorpho-nuclear leucocytes nor Mast-cells in the subcutaneous tissue of the rabbit, but in the dog, rat etc., and in man there are Mast-cells especially in the sub-epidermal layer.

In the corium there are few blood-vessels. In the deeper layers, however, vessels are more numerous, the smaller of which especially are surrounded by numerous cells to which Marchand has given the name Adventitial cells. Maximow divides these adventitial cells of Marchand into:-

(1)/

(1) Small a-typical connective tissue cells, lying closely together.
(2) Granule-containing Clasmatocytes or Clasmatocyte-like Adventitial cells.

In the fat tissue each fat cell is surrounded by a ring of capillaries. Between the vessel wall and the cell membrane lie numerous cells closely pressed together, whose nature is very difficult to decide, but Clasmatocyte-like adventitial cells with small dark nuclei and fine granules are specially abundant.

Maximow, on the ground of finding all transition stages between the small lymphocyte and the large mononuclear cells in the blood of the rabbit, classes all together as lymphocytes. He states that the small "wandering-cells" of the tissue cannot be distinguished from lymphocytes, nor the larger forms from the mononuclears of the blood; and asks "Where then arise these 'wandering-cells' of the tissue?" He gives the very natural explanation that they arise by emigration from the blood. Pappenheim, Domenici and others, who will not admit the amoeboid movement of lymphocytes deny this origin.

Saxer has shewn that during embryonic life the "wandering-cells" of the connective tissue and of the blood have a common origin from "primary wandering/

wandering-cells" which arise in the embryonic connective tissue. Maximow sees in this sufficient grounds for asserting that the wandering-cells of the tissue are emigrated lymphocytes, as there is no evidence of the transformation of connective tissue cells into wandering-cells. Saxer further shows how the cell layer which produces the connective tissue elements has the same source of origin - the mesenchyme - as the layer which produces the blood elements, and believes that conditions arise in the mature organism which render it possible for the cells of one layer to pass over into the cells of the other. Maximow thinks that very probably the two layers are not at first very definitely separated from one another and the cells of one may remain in the other, producing such forms as the Clasmatocytes in the connective tissue, with transitions both to wandering cells and to tissue cells.

In discussing the cells present in the early stages of aseptic inflammation Maximow distinguishes three kinds of cells:-

(1) Polymorpho-nuclear leucocytes,
(2) Fibroblasts - derived from the original connective tissue cells.
(3) "Polyblasts" - these are the mononuclear amoeboid cells grouped under one name.

Already/

Already 19 hours after the introduction of a foreign body they were found in great numbers, therefore they cannot be, Maximow says, derived by the division of the pre-existing cells, but must have emigrated from the blood-vessels. They are actively amoeboid and develop progressively on the field of inflammation, producing the most varied forms - hence the name Polyblast. The lymphocytes emigrate from the vessels and rapidly develop into the large mononuclear forms; some mononuclears emigrate as such from the vessels. The great majority of these Polyblasts are derived from the emigrated cells, but a small portion Maximow derives from

(1) the "wandering-cells" of the tissue.
(2) the Clasmatocytes, and
(3) Clasmatocyte-like adventitial cells, which all appear on the inflamed areas as Polyblasts.

Maximow sets out with the object of following in the course of the inflammatory process the destiny of the histogenous and haematogenous cells. It will be observed that he classes amongst haematogenous cells those forms such as the "wandering-cells" of the tissue, the Clasmatocytes, and adventitial cells, which by some authors are classed as histogenous. He describes minutely the changes in the tissue/

tissue-elements at varying periods after the introduction of the foreign body, and at the end summarizes his work.

The cell-forms appearing during inflammation are divided into three groups:-

(1) The polymorpho-nuclear leucocytes - these appear first on the inflamed area and in some way prepare the ground for the other forms. They do not form stable elements of the tissue and are either removed by the lymphatics, or taken up by other cells, or broken up.

(2) The Fibroblasts - are the usual connective tissue cells. They are highly differentiated elements, increase in early stages, and wander into the interstices of the foreign bodies later than the leucocytes and Polyblasts. In a few instances the fibroblasts may finally round off and become histogenous wandering cells. The fibroblasts of the rabbit are incapable of phagocytosis. Maximow regards the fibril substance as an intracellular formation beginning first in the periphery of the cell protoplasm, and traces the origin of new blood-vessels from the pre-existing vessels by the formation of young buds which are hollow from the beginning.

(3) The Polyblasts - are the mononuclear round/

round cells appearing already in the first few hours: the smaller ones resemble the lymphocytes and the larger the mononuclear cells of the blood. Their origin has already been given and Maximow states that this multiplicity of origin is nothing against the unity of conception, as all these cells belong to the same group. In the area of inflammation they undergo a long series of changes, emigrate into the interior of the foreign body, and remain partly in the capsule which surrounds it; everywhere they are active as phagocytes. They develop into large amoeboid cells with a highly differentiated centrosome apparatus. The centrosome group is surrounded by a clear protoplasm "hof" with a darker peripheral layer of cell body, most marked in the Plasma cell. Maximow considers Plasma cells as a specially differentiated group of his Polyblasts, the special staining arising as an expression of a special function of the cell protoplasm.

Regarding the fate of the Polyblasts, Maximow is very definite. Some perish when their phagocytic function is fulfilled; some return to the lymph and blood stream laden with the decayed products of their absorption in the inflamed area; others settle in the tissue, and are transformed into:- (1) Wandering/

Wandering cells of the tissue. (2) Clasmatocytes of the normal tissue with characteristic granules. (3) Clasmatocyte-like adventitial cells. (4) A few become like Fibroblasts and cannot be distinguished from them, yet do not form tissue.

There is thus a complete correspondence between the processes in the embryonic development of connective tissue and the inflammatory new formation of connective tissue. From the great Mesenchyme layer some cells differentiate as connective tissue cells, while, on the other hand, free wandering cells arise to which belong the leucocytes of the blood and the wandering cells of connective tissue. These latter in process of Ontogeny become sessile forming:- (1) The small wandering cells of the connective tissue. (2) The Clasmatocytes, and (3) The Clasmatocyte-like adventitial cells.

In Inflammation, the Fibroblasts react through proliferation and form the great mass of the young tissue. On the other hand the wandering-cells of the tissue, the Clasmatocytes, and the Clasmatocyte-like adventitial cells change again into active wandering cells and join with large masses of new wandering cells - the emigrated lymphocytes from the blood (to which they are related) - and all together form Polyblasts. After their function is fulfilled the/

the Polyblasts remaining in the tissue become sessile as round "wandering-cells" of the tissue, form round the vessels as adventitial cells, and in the tissue as Clasmatocytes.

Maximow claims that his Polyblasts play a very important rôle in inflammation and his contention will be admitted by all. Where others join issue with him, however, is in his assertion that the great majority arise by emigration from the blood-vessels.

One might briefly compare with this work of Maximow's that of Busse in 1878. Busse investigated the healing of aseptic human skin wounds. His long and interesting paper is taken up with proving:-

(1) That the first adherence of the wound-edges is caused not by an exudate, but directly by a fibrinoid degeneration and swelling of the connective tissue at the margins of the wound.

(2) That the countless cells appearing during the first two days in the inflamed area arise through a re-formation of the intercellular substance into cells and nuclei. Busse, who was a pupil of Grawitz, looked upon the intercellular substance as a living tissue element, stating that as it had arisen from the cells, irritations could bring about its reversion/

reversion into cells.

Busse, in the examination of thousands of specimens, was able to trace only one instance of emigration; he, therefore, said that it was impossible that the cells could be derived by emigration. As in the examination of these thousands of specimens he found no evidence of Mitosis before the 3rd day, he, therefore, again denied that the cells could be derived from the pre-existing connective tissue cells. He admitted that after the 3rd day, these cells, by mitotic division, would share in the new formation of cells.

Busse agrees with Cohnheim that the various cell-forms represent various periods of development of one cell - the first cells formed from the intercellular substance are polynuclear, these become mononuclear, and then spindle-shaped. Busse states that Cohnheim's opinion, though correct, is based on a wrong premise - that these cells are emigrated leucocytes!

IV. THE CELLS OF GRANULATION TISSUE.

THEIR ORIGIN, FUNCTION, AND DESTINY.

The structure of Granulation tissue has for many years been the subject of investigation, but the origin, function, and destiny of the cell-forms found in it is by no means decided. Although upon these important questions more research has been expended than on any other branch of this subject, it still remains the battle-ground of pathologists.

When we examine the tissue in an inflamed area, we can distinguish the following three types of cells:-

1. Polymorpho-nuclear leucocytes - these are clearly cells emigrated from the blood vessels.
2. Larger cells with a single round or indented nucleus and a relatively large quantity of protoplasm - the mononuclear cells of the inflammatory exudate. What are these mononuclear cells, and what is their relation to the third type of cell?

3. Cells of varying size and shape, usually large with oval nucleus and abundant protoplasm, frequently elongated and spindle-shaped. These are the Fibroblast cells - the formative cells of the scar tissue.

(1) POLYMORPHO-NUCLEAR LEUCOCYTES.

THEIR ORIGIN. These are the usual granular leucocytes which emigrate from the blood-vessels under the influence of certain stimuli. They appear very early, and are always the first cells present in an inflamed area. The number of leucocytes that collect at the seat of inflammation depends on the intensity of the chemiotactic influence, i.e., on the presence of bacterial irritants or the disintegration of the tissues: the products of tissue disintegration exert a marked attraction on the leucocytes. When large numbers collect they push aside the pre-formed constituents of the tissues and form accumulations in spaces of their own production. Tactile impressions, e.g., from the swollen endothelial cells or their contractile movements, are also said to incite the leucocytes to emigrate through the/

the vessel wall.

THEIR FUNCTION. Maximow states that "in some way" they prepare the ground for the other cell-forms. They are actively phagocytic for bacteria (Microphages of Metchnikoff), and can also take up red blood corpuscles, dead or useless material such as necrosed cells and foreign particles, e.g., carbon. Many facts point strongly to the production of a bactericidal substance by the leucocytes through a process either of secretion (Buchner) or disintegration (Metchnikoff). These two authors maintain that the alexine or bactericidal complement or microcytase of the serum has its origin in the polynuclear leucocytes. Opie has shown that an enzyme, which is active in the presence of alkali, occurs in the bone-marrow, where the polynuclear leucocytes have their origin; he calls this enzyme leucoprotease. Hahn, Durham, and Beattie have noted that the period of increased leucocytosis in the peritoneum of guineapigs corresponds to the period when the fluid has the greatest inhibitory action on the movements of mobile Bacilli, but Beattie holds that there is not sufficient evidence that/

that the substance causing this inhibitory action is produced by the leucocytes. Leucocytes lead to the solution of tissue and of fibrin by virtue of a ferment action - histolysis. In virtue of this histolysis, leucocytes collect in large tissue clefts, and abscesses are formed.

All these facts lead to the important question, are these secreting cells? Kanthack and Hardy and Buchner hold that they do actively secrete a bactericidal substance, but Metchnikoff maintains that this is produced only when the cells disintegrate. Wright believes that this substance is present in the serum and has nothing to do with the cells. This question is of very great importance, but does not lie further within the scope of my work.

The significance of the multi-lobed nature of the nucleus has been variously construed. Some have seen in it a preparation for cell-division; others, a degenerative process - the cell breaking up; Metchnikoff sees in it a change of form facilitating emigration through the vessel wall. Korschelt, whose work has chiefly dealt with insects, finds a definite correlation between the size of the nucleus and the extent of its surface and the elaboration/

elaboration of material by the cell. By enlarging its surface it may serve to heighten the activity of intracellular processes of nutrition. It is certain that a much divided nucleus seems often associated with great secretory activity; it is possible that metabolic processes of this nature are some of the most important duties of these cells - the products being perhaps certain of the anti-bodies associated with bacterial invasions.

THEIR FATE. Maximow states that they can show unusual duration of life, and even progressively develop. In the interstices of his celloidin capsules he found very hypertrophied forms. They cannot, however, form stable elements in the new tissue, and, when their function is fulfilled, they may:-

(1) Wander back into the lymphatic cells.

(2) Be taken up by phagocytic cells

or

(3) Undergo dissolution and disintegration.

(2)/

(2) THE MONO-NUCLEAR CELLS.

In discussions it is very important that we begin by having a clear idea of the point at issue. In this many-sided question it is difficult to define it in a few words. Many have sought for it in "the haematogenous or histogenous origin" of the mono-nuclear cells. Perhaps this is most all-inclusive but we have seen that there is no unanimity as to what cells in the normal tissues are of haematogenous or histogenous origin. It seems to me that this is only a statement of one side of this complicated subject, and that I must be content to leave its various aspects to unfold as we proceed. The difficulties of the subject are deeper than appears on the surface just as its importance is greater. By trying, however, to trace the evolution of some of these difficulties and the relations proposed, we may reach a truer conception of the points at issue and their importance.

THEIR ORIGIN. The celebrated discovery of/

of Cohnheim had referred all the cell-elements appearing in the inflamed area exclusively to the emigrated leucocytes. That is the one extreme: a haematogenous origin of all the cells - polynuclear and mono-nuclear and later spindle-shaped. At the Berlin Congress in 1890, the three Referees on this question all agreed that "in the reconstruction of the new tissue which builds itself up during inflammation, only the descendants of the tissue cells participate" - that is the opposite extreme. Can the truth lie between these two, and can emigrated blood cells take part in the formation of tissue by changing into connective tissue cells? If this is so, what becomes of the law of the specificity of the cells "omnis cellula e cellula ejusdem generis"?

Cohnheim's discovery was followed up by a long series of experiments by Ziegler. These first made known the processes of development, step by step, of the inflamed connective tissue.

In the glass chamber, far removed from pre-existing connective tissue Ziegler found a new tissue arise out of the penetrated cells. Under the assumption that only leucocytes could emigrate this new tissue must have arisen from the emigrated leucocytes/

leucocytes. Ziegler observed that the multi-nucleated cells (polymorphs) rapidly degenerated or were taken up by other cells which were mono-nucleated. He looked on them as hyaline mononuclear cells and large lymphocytes from the blood which became, later, elongated and spindle-shaped and underwent a further transformation into fibril-forming connective tissue cells. Ziegler, therefore, called these large mononuclear cells of varying shape Fibroblasts.

The discovery of Karyokinesis showed that an active proliferation of the pre-existing connective tissue cells took place and that the progeny of this cell-multiplication consisted of small and large round mononuclear cells (similar to the lymphocytes and mononuclears of the blood) which were capable of active amoeboid movement. Thus another factor was introduced, and it was shown that a portion, at least, of the mononuclear cells (Ziegler's Fibroblasts) were not leucocytes at all, but represented the young cells derived from the pre-existing tissue cells. The pendulum now swung the opposite way and soon it was supposed that not only those mononuclear/

mononuclear cells which subsequently became fixed tissue cells* but also all the mononuclear cells on the inflamed area were the result of the mitotic division of pre-existing connective tissue cells.

These prevailing views had to be modified as new facts were brought forward. The almost instantaneous appearance of such numerous cells on the inflamed area could not be explained by the mitotic division of cells, for proliferative processes were not seen so early. The resemblance of many of the cells normally existing in the connective tissue to small mononuclear leucocytes and the unexplained destiny of the emigrated mononuclear forms in inflammation further increased the difficulty. Ziegler, we have seen, had already suggested a possible development of some of the latter into connective tissue cells, and interest in them was increased, owing to the conception of the term Plasma cells. Unna had derived these from connective/

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* Marchand and Grawitz - two of the Referees at the Berlin Congress - laid special emphasis on the exclusive importance of pre-existing connective tissue cells in forming fixed tissue cells, but Ziegler - the third Referee - though he had come to share these views, suggested a possible development of the emigrated mononuclear leucocytes into connective tissue cells.

connective tissue cells, but v. Marschalks, Krompecher, and others had proved their origin from emigrated mononuclear cells of the blood, and Krompecher had asserted their transformation into connective tissue cells. Doubt was thus cast upon the old teaching of the absolute non-participation of blood cells in connective tissue formation.

Marchand widened the field of controversy regarding the origin of these cells in deriving them from special cells accompanying the blood-vessels - expecially in the omentum - as adventitial cells. These cells could under any irritation rapidly proliferate and produce elements with the characters of lymphocytes and large mononuclear leucocytes. The whole group of these cells so formed Marchand called "Leucocytoid" cells.

Ribbert interpreted the appearance of round celled infiltrations around the vessels as an increase in the cells of normally existing minute lymph nodules intercalated here and there in the perivascular lymph channels, expecially in the lungs and liver. Beattie has noted the presence of these perivascular lymphoid sheaths around the vessels in the omentum of the Guineapig and rabbit. Ribbert maintains that these cells, rapidly proliferating at the onset of inflammation, provide the main/

main source of the mononucleated cells of the inflamed area. With this origin must undoubtedly be allied the cells reaching the inflamed area by the lymph stream.

There remain to be mentioned two further possible sources of these cells. The first of these needs no serious consideration, but is interesting as the fanciful interpretation of one whose opinion is usually of great value. Grawitz' theory, known as the "Slumber-cell" theory, accounted for the countless masses of cells in the inflamed area by a redevelopment of cellular remnants which had almost lost their cellular character. Grawitz believed that everywhere in the tissue lie hidden cells, not stainable by our ordinary methods and therefore not recognisable.

The other possible source of origin is, however, a very important one. The endothelial lining of the blood-vessels and lymphatics (and possibly the lymph spaces) and, in inflammation of serous cavities, the serous endothelium are regarded by many as the most important sources of the mononucleated cells. Adami, Beattie and others hold that by this view a reconciliation can be found for the haematogenous origin of certain fixed connective tissue/

tissue cells. In inflammation the endothelial cells proliferate and pass out from the blood and lymph-vessels into the tissues. Adami believes that the large hyaline cells of the blood are themselves derived from the vessel endothelium. This view alone shows how difficult it is to separate the haematogenous and histogenous cells of the exudate. As some of these are derived from the endothelial lining of blood-vessels and lymphatics and lymph spaces, they must be classed as histogenous cells: others derived from the hyaline cells of the blood must be classed as haematogenous.

The possible origins, therefore, of these cells as reviewed in the previous pages are the following:-

(1) The pre-existing connective tissue cells.

(2) The emigrated blood cells. (Maximow. Helly. Schwarz.)

(3) The Adventitial cells. (Marchand)

(4) Perivascular lymphoid nodules and the lymph stream. (Ribbert. Beattie. Foa.)

(5) Endothelium of the blood-vessels and lymphatics and serosa. (Adami, Beattie and others)

The/

The names attached to these merely indicate that these authors claim the chief source of origin to be the cells named.

Before discussing these theories, it may be well to refer to two circumstances, pointed out by Beattie and many writers, which greatly increase the difficulty of coming to any decision regarding the origin of these cells. The very great difference in the staining properties of the cells in various stages of their development and activity has led many authors to make quite artificial divisions. Variations may be due to a very slight deviation in the activity of the protoplasm. Maximow looks upon the staining as an expression of a special living function of cell-protoplasm. Gulland, from his embryological studies, notes that all young cells tend to be basophile in their cytoplasm, and that the nuclei and protoplasm stain less deeply with the increase in size. The other difficulty arises from the great resemblance of the young cells derived from the proliferation of endothelial and connective tissue cells to the emigrated lymphocytes and mononuclear cells of the blood, and from the very varied cell-forms which connective tissue cells may assume during/

during their development.

The arguments used by the upholders and opponents of the various views must now shortly be stated.

(1) DERIVED BY EMIGRATION FROM THE BLOOD-VESSELS and progressively developing in the tissues, Ziegler, Maximow, Helly, Schwarz, and K.Ziegler. are the chief upholders of this view on the grounds (a) that the simultaneous appearance of such masses of cells, so soon after the irritation, can be accounted for only by emigration, for proliferative appearances have not been observed in the fixed tissue cells. (b) the first appearance of the cells is always closely associated with blood-vessels. (c) That there is a complete morphological harmony between the cells inside and outside the vessels.

Amongst those who have most strenuously opposed this view is Pappenheim, whose arguments, however, fall to the ground as they are based on the assumption of the incapability of lymphocytes to emigrate. Observations have rapidly accumulated which finally establish the possibility of movement, and therefore of emigration of these cells. On the warm/

warm stage Maximow has observed their movements - at 38°-39°C. only the mononuclears show (a sluggish) movement, but at 41°-42°even the small lymphocytes show amoeboid movement. Arnold, V.Marschalko, Maximow, Helly, Councilman, and Mallory have all seen them in the vessel walls. Even those, however, who admit the emigration of these cells see the weak point of Maximow's argument in the great want of proportion between the emigration forms and the countless number of cells. Borst has laid special emphasis on this point, and has also noted that the cells are frequently accumulated round large vessels from which there could be no question of emigration. Borst further thinks that the progressive development of these cells is against their haematogenous origin. To Borst's strictures Maximow has replied that Borst had not observed sufficiently early stages of the inflammatory process (4-6 days), for the lymphocytes and mononuclears emigrate in greater numbers during the early stages than later on. Schwarz also indicates how many circumstances must combine to make possible a sure demonstration of cells in this condition, and also that the tissue must be fixed immediately after removal in a fixing fluid/

fluid - preferably Zeuker's fluid - at body heat. Macallum refers to a case of acute Meningitis occurring in the course of a typical attack of typhoid fever. The meninges were infiltrated with lymphocytes: which process occurring in a tissue ordinarily devoid of lymphoid tissue, could be explained only by an emigration from the blood-vessels in response to a chemiotactic influence.

(2) ORIGIN FROM THE ADVENTITIAL CELLS

(Marchand). Marchand states that the cells of the adventitia begin to increase very early after the onset of inflammation, and thus large numbers of cells similar to lymphocytes and mononuclears are produced around the blood-vessels. These "leucocytoid" cells do not, Marchand thinks, develop into connective tissue cells, nor is it justifiable to derive them from the connective tissue. These cells are genetically nearer to the blood cells than to the tissue cells. Their nucleus is usually round or oval and rich in Chromatin, while the cell-body is round or spindle-shaped. Maximow criticises Marchand's position by asserting that there are no proofs for the division-direct or indirect - of these cells, which alone could give rise to the large accumulations of cell-forms by this view. We have seen that Maximow divides the adventitial/

adventitial cells in normal tissue into a-typical connective tissue cells and Clasmatocyte-like Adventitial cells; in inflammation these produce Fibroblasts and Polyblasts, but appearances of increase in these before 24 hours is rarely seen. This source is certainly insufficient to supply the great multitudes of mononuclear cells. K.Ziegler also criticises Marchand from the same point of view as Maximow; he points out also that the vasa vasorum of the larger vessels are engorged, and, by emigration from these, the adventitial tissue can become infiltrated by small round cells. It is probable that the "leucocytoid" cells participate later in the formation of mononucleated cells.

(3) DERIVATION FROM RIBBERT'S PERIVASCULAR LYMPH NODULES AND THE LYMPH-CELLS COMING TO THE AREA IN THE LYMPH STREAM. Ribbert bases his claim for these sources as the main supply of the mononucleated cells, on the grounds that the round-cell accumulations are mainly around larger vessels from which he thinks there could be no emigration, and, also that it is around these larger vessels that the larger perivascular lymph channels run. He also states that, if these cells emigrated from the vessels, the round cell infiltrations would consist of polymorphs/

polymorphs as well as lymphocytes and mononuclears, and that there is no reason why the lymphocytes should not emigrate as early as the polymorphs. These perivascular lymph nodules have the significance of small lymphatic glands, and have a definite reticulum. Maximow's criticism of this view is that the lymph nodules are very inadequately represented in subcutaneous tissues and could scarcely account for any large proportion of the mononucleated cells; he makes no mention of the cells coming by the lymph channels. Lubarsch mentions the frequent presence of acidophile in these perivascular lymph nodules - these must have emigrated from the blood-vessels.

(4) DERIVATION FROM ENDOTHELIAL CELLS OF BLOOD-VESSELS AND LYMPHATICS AND OF THE SEROSA.

Metchnikoff was the first to suggest that the large mononuclear leucocytes seen in vessels and lymph-glands are identical with the endothelial cells. From their property of ingesting the leucocytes, he named them "Macrophages". Mallory in typhoid fever describes a diffuse proliferation of endothelial cells giving rise to large phagocytic cells. Beattie in/

in peritoneal inflammation in guineapigs derives a large proportion of the mononucleated cells of the exudate from the serous endothelium. He also describes the living cells of the veins as swollen and detached. The proliferated endothelial cells of vessels have been observed by both Councilman and Mallory in the process of emigration into the surrounding tissues. There is an increasing mass of evidence that endothelial cells form a very large source of the mononucleated cells in an inflamed area. Maximow's objection to these as a source during the first 19 hours is again valid, for he has been able to trace no signs of proliferation earlier than this.

(5) DERIVATION FROM PRE-EXISTING CONNECTIVE TISSUE CELLS. Almost all authorities agree that the young connective tissue cells are indistinguishable from the mononuclear leucocytes. This origin, therefore, of the mononucleated cells of exudates must be added to the others, but not in the case of the countless cells appearing in the first hours. Kiener and Duclert in the production of abscesses in the guineapig, have observed appearances in 6 hours which point strongly to the amitotic/

amitotic division of the pre-existing connective tissue cells. The nuclei became irregular and indented and the frequent appearance of double nuclei were taken as indications preceding division. One finds it difficult to understand the purpose of this cell-division, for cells dividing by amitosis cannot fulfil their function and produce new cells, but Kiener and Duclert regard this cell proliferation as a degenerative process, depending on the irritation causing the inflammation. Klemensiewicz is also of opinion that a great increase of the cells in an inflamed area takes place by direct division of connective tissue and other cells.

We have now seen how varied is the possible origin of this "chaotic mass of countless, not well-characterised cells". Ziegler and Maximow name them "Polyblasts". K.Ziegler suggests the name "Polymorph-lymphocytes". This standpoint coincides very largely with that of Ziegler and Maximow. They derive them in great part from the blood and in small part from similar cells in the tissue:- "Wandering cells of the tissue, clasmotocytes, and clasmatocyte-like adventitial cells".

Borst/

Borst looks upon this latter group as the chief source of the early cell-forms through direct division, and does not understand why Maximow, considering the lack of emigration forms, ascribes such slight importance to these cells. The name "Epithelioid" was at one time given to the mononucleated cells by Virchow, from their likeness to the cells lining the blood-vessels, which at that ti e were supposed to be epithelial. These cells by an enlargement of their cell-body and a clearing up of the nucleus became "epithelioid", but the name is now rarely used and "endothelioid" is taking its place. Beattie derives mononucleated phagocytic cells from endothelial cells of the serosa, blood and lymph endothelium, hyaline cells of the blood, and cells of the perivascular lymphoid tissue. Adami derives them:-

1. From "lymphoid tissue, from vascular and other endothelia" - these may reach the inflamed area by migration from blood or local proliferation.
2. From "connective, muscle, and other tissue wandering cells" originating locally as the result of local tissue proliferation. The muscle nuclei, which have separated with a certain amount of cytoplasm/

cytoplasm cannot be distinguished from hyaline mononuclear cells.

THEIR FUNCTION:- Varied as is the origin ascribed to these cells, their function is almost universally admitted to be a phagocytic one. Metchnikoff first named them "Phagocytes"; von Recklinghausen called them "contractile cells" for their capacity of independent movement and their ability to take up foreign bodies depends on the contractile property of their protoplasm. For more than 50 years the existence of cells which contained red blood corpuscles (blutkörperchenhaltige Zellen) has been recognised. All observers are agreed that the chief function of these cells is the clearing away of the waste products on the inflamed area and the preparation of the region for the fibroblasts to fulfil their function. That observers differ so greatly as to their origin and their destiny makes the unity of conception of their function on the area of inflammation all the more remarkable. They are not so active to most species of bacteria as are the polymorphonuclear leucocytes, but are specially active in regard to other cells and tissue-debris.

These cells have both an intracellular and an extracellular activity. The ingestion of bacteria and other foreign bodies, especially red blood corpuscles/

corpuscles and leucocytes, is by a process of intracellular digestion analogous to that observed in the Amoebae. The cells elaborate a ferment named by Metchnikoff "macrocytase", and Opie has shown that this enzyme is closely related to, if not identical with, the autolytic enzyme which is contained in various parenchymatous organs. As the mononuclear phagocytes have their origin, according to Opie, so largely in lymphoid tissue, he has given the name "lympho-protease" to this ferment from its power of digesting proteids. Metchnikoff regards the vacuolation of these cells as a result of an abundant secretion of digestive fluid and looks upon them as uni-cellular glands. Beattie has suggested that, when the protoplasm is stained darkly, there is possibly an accumulation of some substance - probably a secretion of the cell. When the cell begins to function actively this secretion is discharged either as a nutritive agent or as some special ferment or possibly antitoxic body, and the cell becomes clearer and vacuolated. It is possible that these cells take an important part in the early stages of infection by the production of a substance which renders the bacteria capable of being taken up by/

by the polymorpho-nuclear leucocytes, and it has been suggested that this is the origin in the serum of the opsonins, which Wright says have nothing to do with the polymorpho-nuclear leucocytes. Adami has shown that, with the increasing activity of the cells, the chromatin of the nucleus becomes used up and discharged into the body of the cell, to combine with certain bodies and thus form the specific secretion of the cell. Metchnikoff holds that "macrocytase" as also "microcytase" are free in the fluids of the body only after phagolysis, whereas Ehrlich thinks the "cytases" are always free in the body fluids. Metchnikoff explains many experiments, which have been adduced to show that extracellular destruction of bacteria may take place in the fluids of the body, by maintaining that the shock associated with the introduction of the experimental material caused leucocyte death and the throwing out of cytases into the serum. This phenomenon is closely comparable to the formation of fibrin ferment, which is not formed from the white blood corpuscles, till they are damaged, as in the blood withdrawn from the animal body. In regard to the "macrophages" it is certain that, as pus phagocytes, they not only digest/

digest in their cell-bodies the products of disintegration which they have absorbed, but are able to dissolve the mass which surrounds them. This is specially noticeable in the later stages where they often occupy special cavities in the thickened pus.

THEIR FATE. They may -

(1) undergo disintegration locally.
(2) a few may return to the circulation.
(3) may form giant cells by fusion or by direct division of the nuclei.
(4) may be factors in the formation of new tissue; this view will be discussed in the next section.

We have already seen that Maximow believes that many of his Polyblasts become constituent elements of the cicatricial tissue: corresponding to the "wandering-cells", the clasmatocytes, and clasmatocyte-like adventitial cells, and that some become like their fibroblasts. Adami states that their fate is a matter of environment, of relative position, and that they may form endothelium of spaces or the adventitial cells of blood-vessels as there is need. He adds, however, that only those derived from histogenous cells, fixed or wandering, can develop into connective tissue cells.

(3). THE FIBROBLASTS.

THEIR ORIGIN.

(1). THEIR RELATION TO PRE-EXISTING CONNECTIVE-TISSUE CELLS. There is no doubt that the large proportion of Fibroblasts are developed by mitotic division from the pre-existing fixed cells. The time of the first appearance of new cells by Mitosis seems very uncertain, but Maximow takes as his first period for investigation 19 hours, "because the possibility of a multiplication of pre-existing tissue elements by division can be absolutely excluded". The young Fibroblasts are said to be difficult to distinguish from the other mononucleated cells of granulation tissue. They begin to proliferate rapidly after the first day, and from these cells is derived the great mass of the new tissue. The young forms are actively amoeboid and penetrate into the fibrin between the wound edges or into porous foreign bodies when, through the activity of the Leucocytes and other cells, the ground has "in some way" been prepared for them.

(2)/

(2). RELATION TO ENDOTHELIAL CELLS.

According to the law first formulated by Bard new formation begins, always and exclusively, in tissue of the same kind. In the group of the connective tissues a mutual substitution of one kind of cell for another may take place and, therefore, connective tissue may be formed from endothelium. Derivatives of the mesenchyme, which have undergone differentiation into endothelium may return in inflammation to their original indifferent stage, and take part in the formation of undifferentiated germinal connective tissue, later forming true fibrous tissue. In spite of the above facts and the increasing mass of evidence to prove that endothelial cells can form connective tissue, there are many authors who think this question not yet decided. Borst, Baumgarten, Cornil, and Duval and many others have traced in serous inflammation and in the organisation of a thrombus the transformation of the endothelium of the sewsa and of blood-vessels into connective tissue. Maximow says that he is forced to the conclusion that, during very intense inflammatory irritation, the endothelial cells of the blood-vessels change into Fibroblasts. Marchand, in holding out against/

against this view, believes that the behaviour of the young vessels, which grow by the continuous formation of buds, speaks against the identity of the vessel endothelial and the connective tissue cells.

(3). RELATION TO MONONUCLEATED CELLS.

This subject has already been extensively dealt with in the section on these cells. We have seen that Maximow and Ziegler consider all the mononuclear cells as developmental forms of a mesenchymal cell-group which is to be separated from the tissue-forming cells. In the tissue they are derived originally from the blood. Of the forms which settle in the tissue a few may resemble Fibroblasts so closely as to be indistinguishable from them; yet Ziegler and Maximow do not admit that they can form fibrous tissue. Borst argues that, if by the most careful histological technique two cell forms cannot be distinguished, there is every ground for assuming that there is no distinction between them, and that the consequences must be deduced from Maximow's researches that blood cells have a share in the formation of connective tissue. Borst and Lubarsch both hold that connective tissue cells may arise from

those/

those mononucleated cells derived from histogenous wandering cells. These include the group "wandering cells of the tissue, clasmatocytes, and clasmatocyte-like adventitial cells" which Maximow claims to be of haematogenous origin. Adami agrees with Borst and Lubarsch in admitting this origin, and includes amongst histogenous wandering cells the endothelial cells. Adami further states that other cells of the same order, the haematogenous mononuclear leucocytes, which are presumably of like endothelial, i.e., connective-tissue, origin, when they migrate can also form connective tissue cells. And thus Adami reconciles the origin of connective tissue cells from blood elements - i.e., from the mononuclear cells of the blood which he derives from endothelial cells. Adami similarly finds a <u>via media</u> between opposing views on the development of tubercles. Baumgarten regards the proliferating fixed cells in the tissue as the source of the epithelioid cells. Borrel would look upon these latter as modified blood cells. Adami, assuming that hyaline mononuclear cells have a common origin whether passing to the part from the blood or developing from pre-existing cells, in other words, that hyaline mononuclear cells are derived/

derived from endothelial cells in the main harmonises the opposing views of Baumgarten and Borrel. Miller has observed, in tracing the Histogenesis of the Tubercle, that the cells arising by division of the fixed cells are quite indistinguishable from the mononuclear cells of the blood, a fact which, he suggests, proves if not a common origin, yet a closely allied nature and function.

THEIR FUNCTION. The Fibroblasts produce the fibrils of the intercellular substance and so build up the great mass of the new cicatricial tissue. Maximow has not observed in the rabbit any phagocytic fibroblasts. In common with many other cells, however, fibroblasts are actively phagocytic, and many observers, Marchand, Kiener and Duclert, and others claim that they are the chief phagocytes in the inflamed area. Their possible relation to the formation of new blood-vessels will be considered later.

THEIR FATE. After the inflammation has run its course and the cicatrisation is complete, the Fibroblasts are left as the ordinary connective-tissue cells of the part. They have become usually spindle-shaped and greatly diminished in size.

(4). PLASMA-CELLS.

THEIR ORIGIN.

The literature on Plasma-cells is a very extensive and confusing one for the name is used by various writers for different forms of cells.

In 1891 Unna first described special cells occurring in Lupus and the more chronic lesions of tubercle and the other infective granulomata. These, Unna said were characterised (1) by an abundance of protoplasm - as distinguished from the connective-tissue cells: (2) by the presence of very fine granules (amorph-granules) in the protoplasm: (3) by the specific staining. (The protoplasm stains deeply with basic aniline dyes especially in the peripheral portion.) Unna further stated that these cells were pathological products with no embryonic analogues and were derived from the poor-in-protoplasm connective-tissue cells by an increase of granoplasm. Together with this increase there was a drawing in of the processes of Spongioplasm and a consequent rounding off of the cell. The ultimate fate of these cells was degeneration or division into lymphocyte-like daughter Plasma-cells. In the former/

former case, Unna said, by a "homogeneous swelling" of the protoplasm they became the epithelioid cells of tubercle nodules.

Waldeyer in 1875 had described cells with abundant protoplasm and very definite basic granules, to which he had given the name Plasma-cells. Unna, believing that his cells were identical with Waldeyer's, had also called them Plasma-cells, but later studies indicated that Waldeyer's cells were more nearly related to Ehrlich's Mast-cells. Waldeyer, therefore, advised the restriction of the name Plasma-cells to Unna's cells.

Marschalko has called in question each of Unna's statements. He recognises they are characteristic cells but denies the presence of granules, the specific staining, the derivation from connective-tissue cells, their being purely pathological entities, and the derivation of small lymphocytes as the Plasma daughter cells. Marschalko derives these cells from the blood lymphocytes. He states that they occur normally in the spleen and lymphatic glands, and may occur on the field of inflammation as early as 24 hours, and can, therefore, not be derived from connective tissue cells. Judassohn, Enderlen/

Enderlen and Justi, and Krompecher to a certain extent agree with Marschalko. Krompecher states, however, that they may form true connective-tissue cells, and by degrees fibrillar intercellular substance. Pappenheim and Hodara alone side with Unna; the former very largely on the grounds that lymphocytes cannot emigrate and form Plasma cells. He therefore is driven to the conclusion that they are derived from connective-tissue cells.

Marchand derives Plasma cells from his "Leucocytoid" adventitial cells; with this origin from histogenous leucocytes Adami agrees. Ribbert derives them from his perivascular lymph nodules and Adami also agrees with this source of origin.

Maximow looks upon Plasma-cells as a specially differentiated group of his Polyblasts. He states that they occur only in the late stages of inflammation and that the lymphocytes which emigrate in these late stages, instead of developing into ordinary Polyblasts, develop into Plasma cells; all transitions from lymphocytes to Plasma-cells can be traced, he thinks, in the round-celled infiltrations. They cannot form permanent elements of the tissue and finally either degenerate or become ordinary Polyblasts.

Whitfield/

Whitfield has found cells similar to Plasma-cells arising directly from subdivision of the endothelium of the central blood-vessel in germ-centres of lymphatic glands, and suggests that an endothelial source may be the origin of Plasma-cells in inflammation. This origin would remove many difficulties. If Plasma-cells are derived from the endothelium of vessels containing only endothelium for their walls, it is obvious that some of these cells might be carried on in the blood-stream - explaining the frequent presence of Plasma-cells in the blood-vessels, and that others pass out externally into the tissues. This would also explain their being found chiefly round vessels. Around the larger vessels they may be derived from the endothelium of the perivascular lymph spaces.

Herbert, working at the pathology of Trachoma, accounts for the rise of new lymph follicles by a change of the connective-tissue cells into Plasma-cells, and then into lymphocytes forming the lymph nodules of follicular conjunctivitis. Herbert therefore agrees with Unna in deriving Plasma-cells from connective-tissue cells, and in recognizing lymphocytes as Plasma daughter-cells.

Councilman/

Councilman has found the small-celled-intertubular infiltrations in Acute Interstitial Nephritis to be composed largely of Plasma-cells. Councilman derives them from the blood-vessels. The principal seat of formation is the spleen where they are derived from the lymphoid cells. In acute interstitial nephritis occurring after scarlet fever and diptheria, sections of the spleen show the whole of a Malpighian nodule converted into plasma-cells.

Mallory has found numerous Plasma-cells in the intestines, spleen, and lymph-glands in typhoid fever. Both Councilman and Mallory have described undoubted indirect division, and Councilman has observed this inside the vessels.

Plasma-cells are normally present in very large numbers in the subepithelial tissue of the alimentary canal in the spleen; in the lymph-glands, and in the bone marrow. They are now acknowledged to be frequent in infectious granulomata and in the stroma of carcinomata, and are in the main characteristic of a chronic process. In acute inflammations Marschalko has found them within 24 hours, and Porcile within 30 hours, after the injection of turpentine into the liver of rabbits, but most observers/

observers much later. Justi describes them in granulating wounds in dog and man after several days, while Foa says the proportion increases as the inflammatory process becomes exhausted. We have seen that Maximow describes them in the later stages of the inflammatory process.

THEIR FUNCTION OR SIGNIFICANCE. These cells are regarded by many observers as the ordinary mononuclear cells which are derived from lymphocytes; if so, they will have the function and significance ascribed to these cells in a former section. But there are many who look upon them as a specially differentiated group of mononuclear cells. Their distinctive attributes are therefore the attributes of the mononuclear cells emphasized. Maximow describes the specially differentiated centrosome apparatus in a well-marked Protoplasm-"hof", the eccentric position of the nucleus, the accumulation of chromatin masses arranged specially at the borders of the nucleus with radiating lines to the central chromatin masses, forming the typical wheel nucleus (Radkern). The special tingible substance in the protoplasm is not specific for Plasma cells but arises as the expression of a special living function of the cell-protoplasm, and is present to a lesser or/

or greater extent in the other mononuclear cells and in the Fibroblasts.

Unna regarded the Plasma cells as an evidence of degenerative change. Marschalko again differs from Unna, and finds in Plasma-cells evidence of progressive activity in the protection of the organism. Joannovics suggests that their staining properties may be due to their having taken up chromatin from other and degenerated cells. Justi believes in the existence of some substance, which is specially present in the peripheral parts of the protoplasm, accounting thus for the deeper staining. He thinks Plasma cells have the task of transporting a certain substance - the nature of which seems at best problematic - either to the proliferating formative cells for their nourishment, or transporting out of the tissues a substance by their removal in the lymph-stream. The presence of degenerating vacuolated Plasma cells in the lymphatics is very suggestive of this latter possibility. Whitfield believes these cells to be closely connected with the question of immunity, possibly local immunity, and Mallory has suggested that in typhoid fever the Plasma-cells may produce the antitoxin.

THEIR FATE. We have seen that the two main views of their origin are (1) from connective tissue cells, (2) from lymphocytes. Unna believes that they either degenerate or divide to form daughter Plasma-cells. Krompecher thinks they may form true connective-tissue cells and convincing proof of this would greatly modify existing ideas, for Krompecher, in common with nearly all recent observers, derives Plasma cells from the blood lymphocytes. In the tissues very frequent degeneration forms are seen, very suggestive of their having fulfilled some definite function. Maximow states that they form no stable element of the cicatricial tissue, but finally degenerate or are changed into ordinary Polyblasts.

(5)/

(5). MAST-CELLS.

The term "Mast-cell" was applied by Ehrlich to large cells occurring in the connective-tissues, the protoplasm of which contained numerous large basophile granules. In size they vary from that of a large leucocyte to a size many times as great. They may be round or irregular. The nucleus is frequently obscured by the large granules; these stain metachromatically especially with Thionin Blue, which stains the nuclei blue and the granules violet to red. Mast-cells are widely distributed in the normal tissues, specially under epithelial surfaces, around blood-vessels, hair follicles, and glands of the skin.

THEIR ORIGIN. (1). Ehrlich held that they arise out of connective-tissue corpuscles under specially favorable conditions of nutrition. (2). Others that they are a cell "sui generis" (Maximow, Jolly). (3). Others that they are of haematogenous origin. Many writers do not distinguish between Plasma-cells, Mast-cells, and Clasmatocytes, but Maximow holds that they are three distinct cell-forms which probable all arise from an original cell layer early differentiated in the mesenchyme. To this/

this layer also belong the mononuclears of the blood.

THEIR FUNCTION. Concerning the significance of Mast-cells we are almost wholly in the dark. According to Ehrlich's theory, the granules are products of the specific activities of cells, but Arnold doubts whether all granules are secretory in nature. The granules are very soluble in water, and a dispersion outside the cell bodies, with a distinct pericellular metachromatically-stained hof, frequently occurs, as if from a secretion. The cell is sluggishly amoeboid, and may detach portions of its cytoplasmic processes with their contained granules along the track taken by its pseudopodia. The significance of the granules, however, is wholly uncertain. We have seen that Ehrlich thought the cells occurred in greater numbers when nutrition was increased, and other writers have looked upon Mast-cells as "reserve" cells. Maximow also believes that the granules represent a stored up substance, which is given to the surrounding tissues, but the use made of it is quite unknown. Hayer has suggested that the granules consist of mucin. Metchnikoff thinks it possible that the Mast-cells act as scavengers, clearing away inflammatory products, disintegrated masses of chromatin &c. K.Ziegler found them in/

in oedema in specially large numbers, and thinks the granules point to an accumulation of pigment. They were found specially underneath the epithelium and in the cells of the hair papilla.

THEIR FATE. In the early stages of inflammation the changes of the Mast-cells are wholly regressive. The granules are dissolved or are taken up by leucocytes and mononuclear cells. Regeneration of the mast cell does not occur, as they are wanting in the scar tissue.

(6)./

(6). THE COARSELY GRANULAR EOSINOPHILE CELLS.

THEIR ORIGIN. They are clearly of haematogenous origin and are attracted to the inflammatory focus, but are never present in very large numbers, at least during the early stages of inflammation. They obey only certain chemiotactic stimuli, and are then called out in place of the ordinary polymorphonuclear leucocytes.

THEIR FUNCTION. Their rôle on the inflamed area is quite undecided. The observations of Kauthack and Hardy, Mesnil and Durham, and Beattie show that they are rarely phagocytic. Kauthack and Hardy have ascribed to them a secretory activity, associated with the reduction in number and the discharge of the granules. Metchnikoff has noted that under the influence of phagocytic action ingested micro-organisms frequently become eosinophile. He believes that some of the granules are derived from foreign bodies ingested by the phagocytes, while others are the result of the transformation of soluble substances which have been absorbed.

THEIR/

THEIR FATE - is similar to that of the ordinary polymorphonuclear leucocytes in the early stages of inflammation. They seem frequently to form settled elements of the scar tissue though they take no part in tissue formation.

(7)/

(7). GIANT-CELLS.

Giant cells usually accompany every inflammatory process especially where foreign bodies of any nature are present.

THEIR ORIGIN. The two main views regarding their mode of origin are that they arise through blending of isolated cells, or through the repeated division of the nucleus of one cell, without subsequent division of the protoplasm. The type of cell from which they arise is also undecided: whether connective-tissue or haematogenous. Langhans in 1866 first described giant-cells in tubercles, and advanced both the unicellular and the multicellular view as to their origin. Ziegler, Maximow and Arnold state that they arise by confluence of mononucleated blood cells. Marchand and his followers trace them to connective-tissue cells and endothelium. The mode of formation in tubercles cannot be taken as evidence in wound-healing. No experimental proof has been given of their origin from lymphatic or blood-vessel endothelium such as has been demonstrated in tubercles. The fully formed giant-cell does not show its mode of formation: it is either

homogenous/

homogenous or extensively vacuolated, and contains granules of different kinds. Appearances also which might point to fusion could be equally explained by a disintegration into separate cells.

Maximow derives giant-cells from his polyblasts and states that mitosis has nothing to do with their formation in inflammation, while amitosis plays quite a subordinate part. When the polyblasts blend to form giant-cells the centrosome groups of the single cells take first a position together in the centre of the new undivided mass of protoplasm, while the nuclei are at the periphery. Later, the inner structure is obliterated and the centrosomes lose their inherent power of regulating the function of the protoplasm. To this may be due the large size to which the protoplasm enlarges and the marked increase of the nuclei.

THEIR FUNCTION. In inflammation the giant-cells are usually foreign-body giant-cells with a definite function - that of removing the foreign body. They may be compared to Osteoclasts which make use of some definite function in the resorption of bone. Metchnikoff regards giant-cells as living, active, and defensive cells (Phagocytes) formed for the distinct purpose, like plasmodial masses in general/

general, of isolating and removing foreign bodies. The foreign body must be removed by an extracellular action by excretion. This may be compared to the formation of plasmodial masses from mesodermal cells around foreign bodies which penetrate into the body cavity of the lower metazoa.

Giant-cells have also an intra-cellular action. Cell-inclusions and bacteria are taken in by means of pseudopodia; for this purpose the most serviceable part of the cell is the peripheral, hyaline, exoplasm, layer.

There is an increasing tendency to regard giant-cells from the point of view of Metchnikoff. Baumgarten and Langhans thought the cell was necrobiotic from the outset. Weigert first pointed out that in a single giant-cell regressive and progressive processes might be at work. Metchnikoff asserts that they are defensive, active, products of tissue reaction - phagocytic and reparative in function.

THEIR FATE. When their function is fulfilled they either disintegrate and undergo absorption, or separate into individual cells, which revert to the original form of cell from which the Giant-cell arose. The resulting small cells assume an oval/

oval or spindle shape and resemble in early stages mononuclear cells. The mode of formation must involve some process which is not incompatible with the viability of the small cells, and the fact of this new formation of cells from giant-cells favors the teleological view of Metchnikoff. Rindfleisch has observed the tubercle giant-cells break up into spindle cells, one for each nucleus, and, later, these formed fibrous tissue. Hektoen has observed this in giant-cells in healing Tubercular meningitis. Many of the giant-cells showed degenerative and necrotic processes with evidence of solution of the nucleus or of nuclear disintegration; many, also, showed part undergoing disintegration and absorption, and part forming viable cells. In the older cells the retrogressive changes were more marked; in the younger the progressive. Where extensive vacuolation, with Karyolysis and Karyorrhexis, had occurred, it was primary to the disintegration of the whole cell.

Giant-cells occurring during muscle changes will be referred to later.

V./

V. ORIGIN OF THE FIBRILS.

The development of the fibrillar intercellular substance is of great importance in the process of wound-healing, and has been the subject of much controversy. Consideration of earlier views - e.g., that the fibrous tissue was by some obscure chemical process elaborated from the fibrine lying in the wound - may be passed over for discussion of the modern theories. These resolve themselves into three groups:-

(1) That the connective-tissue cells secrete a homogeneous substance, which is subsequently rendered fibrillar by the action of mechanical tension. (Virchow, Kölliker, Ranvier, von Ebner)

(2) That the surface layers of the protoplasm become homogeneous, and that the fibrils originate in this homogeneous layer. (Ziegler, Levoff Maximow, Rollett).

(3) That there is an unravelling of the cell-processes into fibrils together with a direct transformation of the peripheral layers of the protoplasm/

protoplasm into fibrils. (Schwann, Boll, Flemming, Marchand).

Schwann described the origin of fibrils in the foetus through a lengthening of the actual cell-bodies, and a breaking-up of the two principal processes into a bundle of finer fibrils.

Virchow held that the intercellular substance was formed first as a homogeneous secretion in which later fibril formation appeared. This intercellular substance remained dependent on the cells and was influenced by them. Donders assumed the reverse of this - a dependence of the cells on the intercellular substance.

Virchow divided the body into a series of cell-territories (Zellenterritorien), and believed that any given portion of intercellular substance was ruled over by the cell which lay in the middle of it. According to Schwann, the intercellular substance was the cytoblastema, destined for the development of new cells.

Flemming first claimed a nearer relation of the fibrils to the cells, and not merely an indirect participation of the cells in their formation. He showed the appearing of delicate fibrils in the cell-bodies/

cell-bodies of young connective-tissue cells in the salamander.

Marchand, in the foetuses of cows, showed the presence of irregular cells, with fine fibrillar processes in all parts of their circumference.

Ziegler first described the appearance of a homogeneous substance in the peripheral layers of the protoplasm, out of which fibrils later developed.

Leo Loeb has demonstrated that a transformation into fibrils can be produced by traction upon the protoplasm of cells. The cells become spindle-shaped and their protoplasm may form long threads, or fine fibrillar threads may be seen actually passing through several cells. These observations favour the view that the conversion of the protoplasm is the result of tension and traction. Leo Loeb has shown that the same is true of the development of the threads of fibrin. If this is so, it is difficult to draw a sharp line of distinction between the intra- and extra-cellular process.

Marchand, Maximow, and others have studied the migration of the connective-tissue cells into the interstices of foreign-bodies, and the formation of fibrillar substance in these isolated cells. Marchand holds that the formation of the fibrils in

a/

a previously amorphous intercellular substance cannot altogether be excluded. As the intercellular substance is derived from the cells, it forms a living tissue together with the cells, and Marchand thinks the same process may go on in this as in the cells - the fibrils once formed becoming thicker and more numerous, without a direct further participation of the isolated cell-body. Maximow has noted that in the more loosely-arranged granulation tissue, fibrils are found parallel to the blood-vessels, while there are no fibroblasts lying in this direction. This circumstance seems to have a proof that fibrils can to a certain extent differentiate themselves from the homogeneous intermediate material secreted by the cells.

The increase of the fibres, however, is usually held to take place by a continuous cleaving off from the sides of the cell-body, and the first formed fibrils advance further and further from the cells. In a fully-developed condition the tissue is formed of broad bundles, known as Collagen bundles, in which we can no longer recognise fibres. Between these lie the long flattened cell-bodies with narrow nuclei.

VI./

VI. THE NEW FORMATION OF BLOOD-VESSELS.

The formation of new vessels is one of the earliest and most essential steps in all new tissue-formation.

The early conception of the mode of new formation of blood-vessels was that it occurred just as in the vascular area in the embryo of the chick. The first network of capillaries in the embryo arises either:-

(1) INTRA-CELLULARLY - the mesoblastic cells become fused in certain districts, vacuole-like spaces are developed in them, which later become confluent and form the lumen. (Billroth).

or (2) INTERCELLULARLY - the mesoblastic cells in the vascular area become arranged in strands or rows, and the spaces between them later open into one another. (Thoma).

After the first capillary network is formed and throughout post-embryonic life, the belief in an intra-cellular and an intercellular formation has each its adherents. By the intercellular formation spindle-shaped cells are believed to arrange themselves in rows, so that an intercellular/

intercellular canal is formed, the lumen of which then communicates with the lumen of pre-existing capillaries. (Cornil and Ranvier).

The so-called intracellular formation of post-embryonic life is a process of "Budding". The first appearance is a solid more or less conical bud, with a long tapering process, on the outer surface of an endothelial cell. This joins to a similar process and buds from a neighbouring capillary, and the protoplasmic bridge thus formed between two capillary vessels becomes hollowed out from the lumen at either end. This formation by a process of vacuolation and hollowing out of the solid bud is, to a certain extent comparable to the vacuolation of the mesoblastic cells in the embryo, and has therefore been called "intracellular". There are many, however, who claim that "budding" should really be termed an "intercellular" process, as it consists of a division and separation of cells lying side by side. The "intercellular" mode of Thoma consists in the solid bud of protoplasm becoming nucleated; as this nucleus multiplies by mitosis, a nucleated bud of protoplasm - differentiating itself into separate cells - is formed. The lumen of the capillary is pushed out into this bud, the cells of

which/

which re-arrange themselves to form the wall. When this has taken place in two buds joined by their processes, the protoplasmic bridge has almost become a new capillary loop. This "intercellular" process of Thoma and other writers is quite distinct from the "intercellular" process of Cornil and Ranvier, which is analogous to the similar process in the embryo.

In the adult, therefore, the possible modes of formation of new vessels may be (1) Intercellular, (2) Budding. This latter may be (a) Intracellular - the hollowing out of the substance of a cell, or (b) Intercellular, the division and separation of cells, forming a nucleated bud, and their re-arrangement to form the lumen.

The new capillary wall soon becomes thickened by proliferation of the endothelial cells, or by the apposition of new cells from the surrounding tissue. The method of the new production of the muscular and elastic coats is little understood.

Meyer was the first who carefully described the new formation of blood-vessels by budding from the pre-existing vessels.

Arnold/

Arnold, in Keratitis, saw the vessels arise through the formation of delicate, at first solid then canalised buds, from the existing capillaries. He supposed that isolated spindle cells attached to the vessel-walls have the significance of an adventitial covering.

Ziegler believed that multinucleated masses of protoplasm became connected with the projections from the cells of the vessel-walls and that these later, were hollowed out.

Yamagiwa stated that spindle-shaped cells derived from the surrounding tissue formed bridges between the processes of two vessel buds, and were later incorporated in the vessel-wall as endothelial cells of the new vessel.

Maximow cannot agree with Yamagieva that the fibroblasts which seem to unite two vascular anastomoses with each other ever become absorbed into the cell system of the latter and change into endothelial cells.

RELATION/

RELATION OF OTHER CELLS TO THE FORMATION OF VESSELS.

Rindfleisch and others have described vaso-formative cells in the tissue external to the vessels, and Ziegler at one time thought that cells in the granulation tissue became hollowed out and gain attachment to pre-existing vessels. The appearances which led Rindfleisch and Ziegler to this view were (1) the presence of cells which had taken up red blood corpuscles, or(2) the presence in the section of only a portion of the new vessel containing blood cells, or (3) in places where the young vessels were undergoing involution, the interruption of communication with the main vessel through the constriction on either side.

The participation of the elements of the surrounding tissue, as assumed by Yamagiwa is, as yet undecided. It is more likely they have the significance Arnold gave them of adventitial elements. Maximow considers that the vascular endothelial cells are specifically differentiated cells which are not to be confused with connective tissue cells. In this Marchand agrees with Maximow. We have/

have seen that most authors believe that endothelial cells can form connective tissue, e.g., in the organisation of a thrombus, but there is far from the same preponderance of opinion in favour of the view that connective-tissue cells can form endothelium.

THE CAUSES OF NEW VESSEL FORMATION.

The vessels do not always as Hamilton stated grow in the direction of least resistance; the new growth cannot, therefore, be due to a decrease of outer pressure leading to the formation of capillary loops. The appearance of a bud of protoplasm at one side of the outer wall cannot be explained by an increased inner pressure. Mechanical explanations are, therefore, insufficient. The vessel buds do not spread in all directions but along quite definite paths, all merging to a certain focus. They, therefore, seem responsive to the chemiotactic influence which acts on all living protoplasm. The endothelial cells are contractile and phagocytic. The assumption, therefore, of a chemiotactic sensibility of the endothelial cells, to explain also the remarkable powers of reciprocal attraction possessed by the protoplasmic processes

of/

of developing capillaries, which enable them to meet and form new capillary loops, seems quite legitimate.

Maximow, in his exhaustive work, has an important chapter on the new formation of blood-vessels. He thinks that the term "hollowing out" is inapplicable, whether referring to the substance of a single cell, or a nucleated mass of protoplasm already differentiated into cells. Maximow claims that all the young buds are hollow from the beginning. The whole processes he traces to an active development and proliferation of living, moveable, endothelial cells through a positive chemiotactic stimulus. He believes that the endothelial cells retain their specific differentiation, and remain connected with one another in layers as a tube.

Thoma has shown that even in the first rows of capillaries in the chick, there is an inner more condensed layer in the endothelial cells. Kolossow distinguishes, in the endothelium of the blood-vessels, an inner condensed membranous portion bordering the lumen, and a protoplasmic portion containing the nucleus. This latter portion is less differentiated, and from it the processes go out which join the cells together, but Kolossow states that/

that in some way the inner portions of successive cells cling closely together. It is the outer protoplasmic layer which reacts more quickly to the chemiotactic stimulus and, in doing so, draws with it the inner membrane. This forward movement of the two layers may take place uniformly, and then the wall of the young growth remains all over the same as that of the mother-vessel, but the outer layer may move itself independently of the inner membrane. This results in the protoplasm being collected at the end in the form of a swelling with processes. The nucleus may or may not be in this swelling. It can remain lying at the root of this young growth, ready for its rôle later on.

The presence of this thin inner membrane, intimately connected with the protoplasmic layer and following it closely everywhere, results in the lumen being in the growth from the beginning as a direct consequence of the pre-existing lumen. The growth is simply a protrusion of a membrane composed of a flat, moveable cell, the protoplasm of which may be collected at some part in greater mass where the chemiotactic stimulus is strongest.

Maximow has never seen in the rabbit, buds which presented a solid conical mass with numerous nuclei. Even if they were found, it would mean only/

only that the protoplasmic part of the endothelium develops more strongly, and that its power of motion is more energetic in response to the chemiotactic influence, while at the point of growth the protoplasmic parts of several endothelial cells may collect for a while until the inner membranous layer follows.

Maximow describes several shapes in these young vessel growths. There are all transitions between broad, blunt, finger-shaped ones and narrow pointed ones, but all agree in having their lumen limited by the inner dense layer.

The young growth lengthens itself gradually, and numerous endothelial cells, connected the one with the other, contrive to push their way into its wall, starting from the mother-vessel. The endothelial tube grows on, partly by means of independent additions to the endothelial cells already in existence here, and partly (Maximow thinks chiefly) by the removal of new cells attracted hither from more distant parts.

VII. REGENERATION OF CROSS-STRIPED MUSCLE.

Regeneration of muscle fibre was for long regarded as impossible. Zenker (1864) was the first to give proof of the regeneration of muscle fibres from muscle cells, in typhoid fever. Waldeyer confirmed his results, and described the development of irregular cells, many of which were multinucleated, within the sarcolemma. Both these authors thought that the old fibres completely disappeared, and that the new cells were derived from the sarcolemma (Zenker), or from the connective tissue cells (Waldeyer). Zenker believed that these cells became elongated and ultimately striated while Waldeyer thought they were destined to degenerate.

Hoffmann (1866) and Weber (1868) recognised the muscular origin of these irregular cells but, as they found young fibres appear in parts where there had been no muscle elements previously, they thought that the participation of connective tissue cells could not be excluded. Later writers have shown that these muscle cells can emigrate; thus the appearance of new fibres, where no striated element had/

had previously existed, was explained. It is now recognised that Muscle cells are developed from the sarcoplasm around the proliferated nuclei of the sarcolemma, and that connective-tissue cells take no share in their formation.

Neumann in 1868 described another mode of development of new muscle fibres by the "budding" of the fibre fragments. He regarded this as the sole method of regeneration. Gussenbauer, Kolliker, and others agreed with Neumann.

Zeuker, Volkmann, and others, on the other hand, maintained that the muscle cells are the most active, if not the sole agents in regeneration.

Barfurth in 1891 expressed the view that these two processes were not mutually exclusive, and Volkmann in 1893 showed that Neumann's "buds" and the "muscle-cells" were, both alike of purely sarcoplasm origin.

From this time the origin of cells appearing in the sarcolemma was admitted to be wholly muscular: the active agents in regeneration might be "muscle-cells" or buds" - the two processes being practically identical. In the one case the sarcoplasm is individualised into distinct cells; in the other it remains in plasmodial indivision. Volkmann has pointed/

pointed out that the "bud" is only a more or less large muscle cell that has remained in contact with the contractile substance of the preserved segment. When the sarcolemma is preserved and the striated fibre alone is degenerated, e.g., in typhus, the regeneration is effected chiefly by muscle-cells, i.e., Embryonic type. When the sarcolemma is ruptured, e.g., in wounds, the "buds" take the prevailing part.

(1) Embryonic type - first described in typhus. The first change is an increase of the nuclei, which multiply by direct division and become surrounded by an increasing layer of protoplasm. This may at once divide into separate cells or form a more or less extended mass of protoplasm with several nuclei - "les bandes et les plagues à noyaux multiples" of Zeuker and Waldeyer - which later separates into distinct cells. During this time the striated substance has undergone degeneration, and is broken up into hyaline masses which are surrounded and invaded by the proliferating cells. These changes are disseminated in areas throughout the degenerated muscle. Regeneration begins only when defervescence has set in, and arises solely from the muscle cells. These now distend the sarcolemma sheath and can be distinguished/

distinguished into central more round cells and peripheral more elongated ones. The central cells have only a phagocytic function towards the remaining degenerated muscle substance, and sometimes they form multinucleated masses around the degenerating muscle fragments. These central cells, whether unicellular or multicellular, finally disintegrate and disappear. It is the peripheral ones which are concerned in the regeneration of muscle fibres. They increase in size and length and their nuclei multiply by direct division - forming long strips of nucleated protoplasm. When these have reached a length 8 or 9 times their diameter (Volkmann) they acquire a striation at first longitudinal, then transverse. This striation appears first in the periphery, leaving the nucleus still surrounded by a layer of finely-granular protoplasm. The sarcolemma eventually disappears, and the fibres become striated, at first longitudinally and then transversely - the nucleus gradually reaching its position on the outer surface of the fibre. At the extremity of the preserved muscle substance a process of "budding" takes place.

(2) Regeneration by "Budding" (Neumann) - occurs specially after wounds. All budding arises directly/

directly and solely from the non-differentiated protoplasm which increases with the formation of an indivisible plasmodial mass, in which the fibril formation will later be differentiated. For this formation it is necessary that a segment of muscle be preserved intact from which the "budding" can take place. At the extremity of the intact fibre or at the extremity of the fibril into which it has become disassociated, the muscle nuclei multiply and the sarcoplasm increases around them. These so-called "buds" become elongated, form multinucleated bands or ribbons of protoplasm, more or less homogeneous or fibrillated. The striation of these buds becomes apparent at its junction with the old fibre, and the free extremity is simply a multinucleated mass of homogeneous protoplasm. The buds may be lateral or terminal - usually terminal. These masses now undergo a longitudinal division, independent of the position of the nuclei, so that the nuclei are frequently in the centre of the new fibres but ultimately come to the surface. Later a transverse striation appears. The new cells having acquired their striation, both longitudinal and transverse, the new sarcolemma sheath is now formed. The origin of the new sheath is unknown. Galeotti/

Galeotti and Levi consider it to be an envelope which the non-differentiated protoplasm secretes around the fibre to protect it. Sometimes the new sarcolemma must be derived from the old. The muscularisation of a cicatrix does not take place more than 1 or 2 m.m. on either side, so that a cicatrix of more than 3 or 4 m.m. will not be perfectly muscularized.

Where the Sarcolemma sheath is extensively ruptured as in a wound, and foreign elements, e.g., red blood corpuscles, leucocytes, and migratory cells, have penetrated the evolution of the muscle cells is very different. These scatter in the adjacent tissue, where they are for a long time recognisable but ultimately most atrophy disappear. A few may become elongated and develop into young muscle fibres.

Dividing muscle cells that are not in connection with living sarcoplasm change also into multinucleated protoplasm masses which frequently contain hyaline substance. Such muscle "giant-cells" are found frequently in great numbers in the early scar after wounds.

Kirby and Foinitzki have studied the influence of nerves and found that deprivation of nerve influence in no way hinders muscle regeneration.

The/

The experimental introduction of muscle grafts into muscle tissue has as yet failed. The transplanted muscle is soon re-absorbed.

VIII. CHANGES IN FAT TISSUE.

The source and genetic relation of fat cells has not yet been made clear. In embryonic development, the first fat cells arise from round, finely granular, cells which make their appearance in small groups in the neighbourhood of the vessels. The fat cells are normally pressed closely together, and between them are fibrils of connective-tissue and a zone of capillaries. Many Clasmatocytes and Mast-cells are found in relation to the vessels in the fat tissue.

In inflammation, the changes observed in fat cells are two-fold according to most observers:-

(1) Atrophy of the fat out of the fat cell.

(2) The appearance of numerous, small, cells or multinucleated cells in the place of the large fat cell. Borst believes that the synthetic origin of fat through cell-activity may play an important part in inflammation, in regeneration of tissue, and in wound-healing.

Crajewiez/

Crajewiez first made an investigation of fat tissue in inflammation. On the 2nd day after the injection of Iodine solution, he found the space between the fat drop and the membrane filled up with young cells, which frequently bordered the membrane like an epithelial ring. Sometimes 12 such cells were found in one mother-cell. These brood-cells were sometimes very isolated but frequently whole lobules were transformed into such cells. Crajewiez called this an "endogenous" formation of cells.

Flemming and Cornil and Ranvier describe the disappearance of the fat, the division of the nuclei and a change of each fat cell into a nest of embryonal cells.

Marchand notes the heaping up in the former fat cell of large, round, cells, some with 2 or more nuclei, and, in some cases, the presence of a single multinucleated mass filling up the fat cell. In some parts the membrane of the fat cell is destroyed. The young cells are finely granular or vacuolated. Marchand believes that these appearances point to a new formation of cells, inside the membrane of the original fat cell, from the protoplasm of the original cell. He, therefore, thinks it justifiable to speak of/

of an "endogenous" cell-formation. To this conclusion the occasional appearance of Mitosis points. Direct division is not excluded. According to Marchand the young cells can later become new fat cells. The membrane, as far as it remains intact, represents the mechanical surrounding of the new-formed cell.

Maximow differs from those who believe in an "endogenous" cell-formation. The fat cell first disperses its fat and becomes a fibroblast cell. Only after the disappearance of the fat, can cell-division take place. If the fat cell cannot disperse its own fat, numerous polyblasts come forward, surround and destroy the membrane, absorb the fat, and finally take the place of the former large fat drops. The part of the cell-body which surrounds the nucleus becomes free from fat and changes into a Fibroblast. In this way, Maximow thinks, endogenous cell-formation is simulated. After the absorption of the fat by the polyblasts is over, they separate from one another and are distributed in the tissues. The nucleus of the original fat cell enlarges and assumes the characters of a Fibroblast nucleus, while the protoplasm surrounding it increases and cell-division takes place.

K./

K.Ziegler also opposes the view of an "endogenous" cell-formation and agrees with Maximow. Ziegler describes single fat cells encircled by a ring of rounded or oblong "polymorph lymphocytes." When the continuity of a fat cell is broken up, these penetrate into its interior. The frequent presence of the characteristic cell-nucleus of the fat cell contradicts the view that these penetrated cells could have arisen by an endogenous cell-formation.

IX. THE HEALING OF WOUNDS.

(1). HEALING BY FIRST INTENTION.

Healing of aseptic incised wounds, accompanied with a minimum of reaction, is known as healing by primary union or first intention. In a former section I have stated why it is impossible that such healing can occur in experimented-on animals, and the wounds to be considered later are those where a considerable irritation was set up by the varying causes already enumerated. Yet parts of the wound frequently healed by first intention. When the adaptation of the wound edges was good the dense corium layers seemed to unite very rapidly, so that in a few days the strip of cellular tissue uniting the former wound edges was so narrow as to be almost indistinguishable from the parts adjoining the wound. The reaction of the vessels and of the leucocytes had been slight and the main part in the healing process had been taken by the connective-tissue cells immediately adjoining the incision. In a very short time the narrow strip of fibrin uniting the two cut surfaces/

surfaces together had been replaced by the proliferation of the pre-existing tissue cells adjoining, together with the new formation of a few capillaries from the slightly dilated and congested vessels near the margin of the wound. This, however, had occurred only in the dense, comparatively non-vascular, corium tissue.

Plate VIII. illustrates such a wound, $6\frac{1}{2}$ days old, where the epithelium had perfectly covered the surface and the line of incision was recognised, under the low power, only by the break in the continuity of the dense collagen bundles and their replacement by a cellular fibrous tissue. Other sections, at an even earlier date, show complete healing with a want of vascular reaction, exudation, and leucocytosis. This result was obtained only where the margins of the wound had been very little injured and where adaptation must have been perfect. Such favorable cases from a surgical standpoint were admittedly rare. The tissues were rather incited to reaction, short of suppuration, so that the changes might be better followed.

A new formation of tissue, however slight, is a necessary condition for the firm union of the margins/

margins of a wound. Where the loss of tissue is greater, and the new formation greater in amount, the process is called healing by second intention.

(2)/

(2). HEALING BY SECOND INTENTION.

Formerly this method of healing was always associated with suppuration, and suppuration was regarded as its essential feature. Since Lister showed how this could be avoided, even large losses of tissue may be replaced by connective-tissue without real suppuration appearing.

There are no essential differences in the two types of healing for Nature frequently shows a remarkable uniformity in her methods. In the following paragraphs I will briefly describe the changes which occur in an incised wound leading to its complete healing.

STAGE OF 6 HOURS. By the extravasation of blood and the exuded serum the defect is filled up and fibrin forms. This fibrin not only bridges the gap but agglutinates the wound edges, and is a consequence of the pouring out of the blood and the distinction of leucocytes with liberation of fibrin ferment.

A certain amount of tissue damage has been caused by the incision and this necrosed material together with the fibrin act chemiotactically on the/

the leucocytes. Already in six hours there are innumerable leucocytes in the inflamed area adjoining the incision. These first-appearing cells are the ordinary polymorpho-nuclear leucocytes of the blood; they densely border the fibrin and infiltrate the wound edges. Many have penetrated the fibrin which is very granular, with many red blood corpuscles still recognisable; it has not yet assumed the trellisated or fibrillated appearance of later stages. The vessels in the neighbourhood are dilated and many are filled with leucocytes. Slide shows a markedly dilated vessel, filled with leucocytes, which seems as if its open end bordered the fibrin. Other vessels show a peripheral distribution of the leucocytes and many cells in the vessel wall which may represent leucocytes emigrating. The endothelium is swollen and projects into the lumen.

The tissues in the neighbourhood of the wound show a marked oedema. The collagen bundles are swollen, homogeneous, and separated from one another, and the connective-tissue cells are distinctly separated from the bundles. Those in the area bordering the wound show an increase in size of the cell body and frequently a drawing in of the processes: farther from the wound there is little change./

change. Many authors, e.g., Kiener and Duclert, hold that, in the early stages of the inflammatory process, amitosis plays a considerable part in the production of the cells present in the inflamed area. In none of my specimens at this, or indeed at any stage, have I seen distinct evidence of amitosis; the appearances of division have all suggested mitosis.

Where muscle fibres have been severed by the incision, the muscle bundles show extensive degenerative processes. The bundles, widely separated from one another by fibrin, are granular or homogenous, frequently penetrated by leucocytes and red blood corpuscles.

I have not been able to trace many mononucleated cells in the exudate at this stage. In the oedematous tissue bordering the wound, especially in the mouse, many of the cells are very similar to the large mononucleated blood cells. Their nuclei is darker than that of the connective-tissue cells, has a less open chromatin network, and no true nucleoli. Stained with haematin and eosin the protoplasm takes the eosin stain, while the protoplasm of the connective-tissue cells is purplish, giving the impression of having taken both haematin and eosin stains.

Already/

Already, then, at six hours we see (1) a dilatation of the vessels with peripheral arrangement of the leucocytes; (2) an active emigration of leucocytes to the injured area; (3) a swelling of the tissue elements through the transuded lymph, and formation of fibrin between the wound edges and in all the tissue interstices; (4) degeneration of tissue elements bordering the line of incision.

The next stages investigated were twelve and eighteen hours, but it is unnecessary to enter fully into them. -

The vessels are still more dilated and in a wider area; the grouping of the leucocytes inside and outside the vessel and in its wall is very marked; the whole capillary mesh-work of the tissues is made evident; an increasing massing of leucocytes infiltrates the wound edges and penetrates the fibrin. The fibrin has now a distict fibrillar appearance on the borders adjoining the tissue, and a more open network character in the interior. The connective-tissue cells in a wider area have become affected; many show star-shaped forms and some rounded shapes. More mononucleated cells are present with a quite evident rim of protoplasm and indented nucleus.

It is in these early stages that Maximow, Schwarz, Helly, and Ziegler describe the numberless mass of cells, many of which are mononucleated - so many that they can account for them only by emigration. Although my tissues were taken from animals newly dead and immediately placed in warm fixing fluid, thus fulfilling the conditions Schwarz considers necessary to obtain emigration pictures, in none of my specimens have I been able to find decided instances of this emigration and very few of leucocyte emigration.

Maximow has pointed out that the objections to his claim of the emigration origin of the mononucleated cells are equally valid against the origin of the polymorpho-nuclear leucocytes for emigration pictures are few. In the examination of thousands of specimens Büsse found only one example. Borst, on the other hand, writes as if such pictures were a frequent occurrence. Vessel-walls certainly seem frequently studded with cells, many of them at right angles to the circumference, the nuclei are of very varied shape; this might be interpreted as leucocyte emigration but whether of lymphocytes or polymorpho-nuclear forms is very hard to decide.

The/

The capability of lymphocytes to emigrate is, however, almost universally admitted. And it is on the grounds that his ascription of the origin of the mononucleated cells almost exclusively to the blood in these early stages is unnecessary, that I think Maximow's arguments built on a wrong foundation, considering how few these emigration forms undoubtedly are. The number of mononucleated cells present "up to nineteen hours" seems not so large that it cannot be accounted for by a local origin from the histogenous wandering cells of the tissues, the nucleus and cell-body of which rapidly increase in size. Together with these may be a few emigrated cells and cells brought to the part by the lymph stream. In my specimens up to eighteen hours, most of the mononucleated cells are around vessels, glands, hair follicles, and nerves, where they could easily have arisen from the enlargement and rounding off of the adventitial cells. A few are present in the fibrin meshwork along with a few enlarged connective-tissue cells.

STAGE OF 24 HOURS. It is acknowledged that by this time proliferative appearances can occur in the fixed tissue cells. During this and later stages large numbers of small lymphocyte-like cells appear/

appear in the inflamed area. These have their source, I think, in the lymph-stream and in Ribbert's lymph-nodules when present. Maximow states that the blood-vessels are filled with numerous cells in every way identical with the mononucleated cells of the exudate. My specimens do not bear out this statement; the vessels are filled with red blood corpuscles and numerous leucocytes, and the perivascular channels at the borders of the inflamed area frequently show rows of lymphocytes.

The connective-tissue cells, bordering the immediate neighbourhood of the wound, show enlargement and darkening of the nucleus, preparatory to division. In slide I have found two instances of mitoses but the numbers are as yet very scanty.

The endothelium of the vessels is very swollen, granular, and turbid, and shows an increased activity which has to do with phagocytosis and new formation of vessels. One can understand from the appearances in the vessels that, whatever part is taken by the endothelium in relation to lymph, transudation is altered.

The mononucleated cells are beginning their phagocytic function and already show much vacuolation and many cell-inclusions - both leucocytes/

leucocytes and red blood cells. This vacuolation has been shown to be not a sign of degeneration but evidence of an increased activity.

The Fibrin forms at the wound edges an almost homogeneous layer, densely infiltrated with leucocytes in all stages of decay, the interior of the fibrin shows the more open network with cells - connective-tissue, mononucleated, and leucocytes - filling the meshes.

The muscle fibres bordering the wound are now hyaline, homogeneous, irregular clumps, closely surrounded by numerous cells. In the contiguous portion the muscle nuclei are undergoing rapid multiplication, forming long chains of nuclei. These are often accumulated at the ends of stumps of fibres giving rise to the muscle "buds" of Neumann. More frequently the Sarcolemma has been extensively ruptured and the substance of the muscle bundle punctured by cells - leucocytes, red cells, and mononucleateds. The solvent action of the cells removes the muscle by a process of solution. This process is aided by the phagocytic action of the mononucleated cells, which are very frequently found with small portions of muscle forming granules in their protoplasm, or with larger portions enclosed in a vacuole. It/

It is quite possible that many of these round mononucleated cells in this muscle area are really "muscle-cells", derived by a proliferation of the nuclei of the sarcolemma, which have become surrounded with sarcoplasm and have separated from the remainder of the muscle fibre.

The wound-line has been considered in the above description as a well-marked, more or less straight, strip of fibrin of varying breadth. This is not, however, the usual result of an incision. More frequently the wound edges do not lie in apposition. The severed epithelium of the deeper edge is agglutinated with the opposite wound surface, and the projecting wound edge is bordered by a thin layer of fibrin and is infiltrated with leucocytes. In the tissue the wound is followed by a long, undulating line, at times forming a fairly wide irregular fissure, which broadens below to a triangular space. The course of the wound is marked by irregular fragments of connective-tissue bundles and muscle fibres. A marked heaping up of leucocytes in the wound edges and in the triangular space is present and the muscle fragments are embedded in fibrin containing many leucocytes. With the high power the edge of fibrin shows intimate connection with the severed tissue elements. The vessels are dilated and filled with leucocytes.

The/

The connective-tissue in the inflamed area bordering the wound becomes swollen, hyaline, and homogeneous, but can be distinguished from the fibrin by van Gieson stain. Later it becomes dissolved and disappears.

A reference to Plates VII. and VIII., which illustrate wound-incisions 4 and 6½ days old, will indicate the appearances in a typical healing wound. Naturally, for purposes both of illustration and description, the best specimens available have been chosen. Adaptation of the wound edges was rarely so perfect as in the two cases here illustrated.

STAGE 30-36 HOURS. There are now extremely marked changes.

The fixed cells, both connective-tissue and endothelial, show evidence of active proliferation. In the surrounding connective-tissue the young elongated connective-tissue cells - fibroblasts - are seen with their long axis radiating in one direction, the focus of attraction being the wound-fissure. Some of these young fibroblasts, assuming the most varied shapes, migrate into the fibrin. There is a great proliferation of young vessels. These capillaries form a network at the margin of the/

the wound, and also penetrate the fibrin. The older vessels of the part are becoming closed by a swelling and proliferation of their endothelium.

Large numbers of mononucleated cells, both lymphocytes and hyaline cells, are now present. The larger forms are undergoing vacuolation and may be mistaken for the young capillaries. In the larger mononucleated cells the indented nucleus is becoming more marked, and with Iron haematoxylin a clearer "hof" becomes differentiated (see slide).

Few red cells are now seen, unless small areas of haemorrhage are present. Some have been taken up by the phagocytic mononucleated cells, some are broken up and the products of their disintegration similarly removed. Large numbers of the Leucocytes show all stages of nuclear disintegration - pyknosis, karyorrhexis, etc. At the margin of the wound, and on its surface, disintegrated chromatin lies forming darkly-stained masses.

A typical wound fissure, in the rabbit, at this stage would be represented by a more or less broad, irregular strip of fibrin in the centre. In its meshes, and in the interstices of the tissue at the wound edges, lie numerous cells, mononucleated, fibroblasts, and leucocytes. The same cells are found/

found in the tissue layers in numbers gradually diminishing as we proceed from the wound. Mitotic figures are most frequent in the tissue a little distance from the wound, and the resulting young cells stream, as it were, to where they are needed. The fibrin strip is broader as it passes between the superficial muscle bundles owing to the contraction of the muscle at the time of the incision, and in the loose layer between the superficial and deep muscles it becomes distinctly triangular. This loose layer is densely infiltrated with cells, the variety of the forms of which is very remarkable, and almost impossible to describe. Slides show many of these cell-forms. The old collagen bundles in this layer are almost dissolved and their place is occupied by a countless mass of cells - fibroblasts with numerous mitoses, mononucleated phagocytic cells, leucocytes, and red cells. Many of the leucocytes have newly emigrated from the vessels; others show all stages of degeneration. The red cells, disintegrating in the tissue spaces have the resulting yellow and brown haemosiderin granules taken up by the cells to form pigment granules. (see Plate I. Fig. 2.). Around the vessels and nerves, a little removed from the wound cleft, lie/

lie numerous lymphocyte-like cells, probably brought by the lymph stream; and around the glands, hair follicles, and vessels in the subepidermal layers are numerous cells derived probably from the adventitial cells which surround these structures. The vessels in the subepidermal layers throughout the whole inflamed area are widely dilated, and show evidences of new capillary formation. Plate VI. shows new capillary formation, and illustrates the appearances under high power of the numerous new young vessels formed.

The mononucleated cells during this stage are derived probably from lymphocytes brought by the blood stream. These rapidly enlarge by an increase of their cytoplasm, and an opening up of the nuclear framework. Many also have the same source of origin as in the former stage. The rounder forms of the Fibroblasts can be distinguished from these cells by the characters of the nucleus and the presence of true nucleoli. By careful staining with Eosin and methylene blue, Iron haematoxylin, and Methyl-green pyronin, and by comparing the results, a differentiation of these cells can be made.

STAGE/

STAGE 60-72 HOURS. If the wound is quite aseptic we find the vascularity and the leucocyte emigration rapidly diminishing; the connective-tissue cell proliferation results in an enormous increase in the mass of cells present; the absorptive processes are being actively carried out by the phagocytes; and the new vessel formation is very marked. The epithelium also at this period in a small wound may have covered the surface. The fibrin is penetrated by new vessels growing in on either side, accompanied by fibroblasts and mononucleateds. In the meshwork the latter are very phagocytic. The lymphatics are often already filled with numerous cells laden with decay products. Slides and numerous others illustrate these points.

The muscle fibre shows in a very marked manner the phagocytic action of the cells. Leucocytes have penetrated its substance, and mononucleated cells which frequently fuse into a multinucleated mass surround its remains.

We have now reached the formation of a vascular and cellular tissue which almost completely replaces the fibrin that first filled up the gap, and occupies all the interstices between the tissue elements/

elements. To this young tissue, composed of blood-vessels and numerous cells, the name of "granulation-tissue" has been given, a name now applied to all cellular proliferation including those of the granulomata. As the fibrin was only a temporary formation for the purpose of bridging the gap, so this mass of granulation tissue is only a temporary scaffolding by means of which the firm tissue will be built up. The majority of the young vessels will ultimately become obliterated; many of the cells, when their function is fulfilled, will be removed; and a fibrillated tissue, formed from the fibroblasts and possibly other cells, will be laid down.

STAGE 4-5 DAYS. A remarkable change is now revealed by the microscope. Those who refuse to the phenomena of inflammation the teleological significance ascribed to them by Metchnikoff must find it hard to defend their position. In the former stage we saw that the leucocytes had markedly diminished in numbers (except around areas of special irritation). Their function was to a large extent over, though a few still emigrated, and the reign of the phagocytes had commenced. This has now to yield to/

to the sway of the proliferating tissue cells. These, instead of being irregularly arranged, now lie in a very definite manner parallel to the blood-vessels. A few in the surface parts of the wound are interlacing across the original wound-fissure and becoming parallel to the surface. The leucocytes are in still more diminished numbers, and the phagocytes are found in large numbers in the lymph-stream undergoing disintegration. Many still lie scattered in the tissue between the fibroblasts, removing the remaining products to be absorbed, and some will ultimately settle in the tissue especially around the vessels.

Some of the fibroblasts show distinct fibrillation. Plate V. illustrates this from a wound five days old. The former wound cleft and the surrounding necrosed tissue is now filled up by a young tissue very vascular and cellular. The epithelium has entirely covered the surface. Fibrin may still remain in the deeper parts of the wound. This is ultimately removed by a continued process of solution and also by the phagocytes.

The changes in the muscle tissue are also very definite. Where the muscle fibres had degenerated into hyaline masses, these have been almost

wholly/

wholly removed by the combined extra-cellular and intra-cellular activity of the cells. Their place is taken by a cellular tissue, and here also a similar re-arrangement of the elements is taking place. These are the changes which have occurred in the zone of degenerated muscle tissue. Where, farther from the wound, the preserved muscle fibres have shown regenerative changes, numerous round "muscle-cells" with a large nucleus and true nucleolus, occupy this zone along with many muscle giant-cells. The end of the stumps of fibres shows a rounding off with a great increase in the nuclei - these are evidently the muscle "buds" of Neumann.

STAGE 6-8 DAYS. Much of the structure formed temporarily has now been removed. The fibrous laminae are being laid down between the cells - the cells themselves diminishing in size. Comparatively few other cells are found between the blood-vessels, many of which have become obliterated and form cords of endothelial cells which must help to form fibrous tissue.

LATER/

LATER STAGES. For a long time the site of the original incision, in even a small wound is recognisable by its cellular character. Gradually, however, a condensation of the fibrous laminae takes place; the blood-vessels become obliterated, the fibroblasts form small spindle-shaped connective-tissue cells attached to the collagen bundles, and the remaining mononucleated cells settle in the tissue as wandering cells of the tissue and adventitial cells.

In the neighbourhood of the vessels are frequent "small-celled infiltrations", many of which contain Plasma-cells. The scar tissue is much closer than before, and delicately fibrillar. The close fibrillar arrangement advances far between the old cutis bundles, so that an interweaving of old and new takes place.

For a long time in the scar tissue, the muscle "buds", muscle giant-cells, and "muscle-cells" are recognisable. Fusiform, multinucleated cells are also present which show a distinct fibrillation (Slides). In later stages these are said to be absorbed, but, for a long time, giant-cells, "muscle cells", round and spindle-shaped, are seen/

seen in the scar tissue, especially in the guinea-pig. In my specimens of 21 and 31 days, the former zone of muscle degeneration is occupied by cicatricial tissue, into which round "muscle-cells" have migrated. The former zone of muscle proliferative changes shows all the elements of muscle regeneration, but these can be of little value in the muscularisation of the scar tissue.

Where extensive irritation in a wound has resulted in great loss of tissue and in new formation, the process of healing is very much delayed. Where the stitches have become infected, small abscesses form; these, in process of time, are encapsuled, and the contents absorbed. see "Abscesses".

ABSORPTION/

ABSORPTION OF CATGUT.

To trace the absorption of catgut and the changes which occur during this process, sterile catgut was inserted into the muscular and subcutaneous tissues in the rabbit. The process of absorption in the wounds was also traced. In noting the sequence of the phenomena, many circumstances have to be taken into account; these depend on the vitality of the tissues, and the accompanying processes. In a wound the rate of absorption was much more active than in the muscle substance unassociated with a wound. My specimens show one further difference: in the wound, numerous giant-cells formed around the catgut stitches, and in the stages of 15 and 21 days, the site of the former catgut was occupied by very large syncytial giant-cells. In the catgut isolated in the muscle, I was able to find only one giant-cell in the whole of the series. In neither case was there any definite encapsuling of the catgut.

In a wound the catgut is first surrounded by fibrin and a dense accumulation of leucocytes. These/

these, later, penetrate its substance; still later are added mononuclear cells. Giant-cells form from these mononucleated cells (Plate IV.Fig.1.) and isolate fragments of the catgut. There seems to be very little penetration of vessels - simply a penetration of long rows of cells between which the catgut is gradually dissolved and absorbed. A cellular tissue, composed of mononucleated and giant-cells, for a long time occupies the site, and ultimately must give way to fibrous tissue formation.

In muscle, not in relation to any wound the catgut after the first days lies as an almost inert foreign body for a considerable time. Dense masses of leucocytes, increasing in 30 - 60 hours, accumulate around it. By 90 hours these had penetrated, and formed long lines marked by the dense chromatin, fragments of their disintegration. The immediate zone of muscle tissue was broken up into small fragments, many of which were included in the dense accumulation of leucocytes. The surrounding muscle fibres showed granular and hyaline change, and many formed numerous round muscle-cells. The vessels in the muscular septa were dilated and numerous mononucleated cells and fibroblasts surrounded and infiltrated/

infiltrated the zone of muscle change. A definite encapsulation by fibrous laminae was never present. By 8 and 11 days the radiating lines of cells had become much broader especially at the periphery of the catgut, and blood-vessels entered the base of these wedge shaped projections. The appearance under low power frequently suggested a tree with a short trunk breaking up in the catgut into numerous branches (See Plate xii.)

My specimens of 15 21 and 31 days show very slight differences. Why sometimes it should be penetrated by lymph and young cells and, at other times , be surrounded by them with no immigration but only a cell-encapsulation, I am unable to explain.

(3)/

(3) - HEALING UNDER A SCAB.

Hunter emphasised that this was Nature's method. Wounds left to themselves in animals heal in this way. Since Hunter's time numerous attempts have been made to attain nature's method of treatment. Paget recognised the great advantage of it, but laid stress on its uncertainty and the possibility of pus forming under the scab and accumulating.

When the scab consists of blood and discharge from the wound, the surface is thus placed ~~in~~ on the level of a clean wound with agglutinated edges; we could, therefore, call it primary healing under a scab. Sometimes the scab is composed of necrotic tissue firmly connected with the living tissue, and there is no wound surface. Such a free surface arises only by demarcation and loosening of the necrotic part. The healing takes place by the epithelium gradually growing over the surface just as under the blood scab. Healing under a scab is of great importance in relation to Reverdin transplantation of skin, and the healing of excoriations. The infiltration of the leucocytes marks off the necrotic/

necrotic layer, and causes its severance without a proper suppuration. The purely regenerative processes in the connective tissue go ~~in~~ on below.

In the advancing fringe of newly-formed epidermis, the cells are frequently very long and spindle-shaped. These penetrate the line of demarcation and, eventually join the cells growing from the opposite edge. When the surface is fully covered with epithelium the scab falls off. The manner in which epithelium is regenerated is undoubtedly by division of the young cells at the edge. Rindfleisch supposed that the epithelium was regenerated from the connective tissue elements below it. Certain appearances seem to support this view but, looked at from an embryonic aspect, it is untenable. The epithelium is at first loosely attached to the organising connective tissue; soon, however, processes are given off from the deepest epithelial cells.

Plate IX. and X. illustrate small incised wounds in the mouse left to heal by nature's method. The blood lymph effused has formed a ~~deux~~ clense crust infiltrated in its under surface by leucocytes. The leucocytes are seen streaming to the inflamed area.

Plate XI. illustrates "Healing under a scab" with formation of granulation tissue.

(4)/

(4) A B S C E S S E S.

FORMATION OF THE ABSCESS MEMBRANE AND ABSORPTION OF THE PUS.

"Suppuration is only an extension of emigration" - such was Cohnheim's view. Inflammation was, in the first degree, diapedesis of leucocytes; in the second, formation of pus. In the former case the leucocytes scattered in the tissue are living and capable of fulfilling their function; in the latter, they are dead and can work no more. Where the chemiotactic irritation drawing out such an intense emigration has a peptonising influence on the tissues, they are softened and liquefied, and the incoming leucocytes, with the fluid in which they are suspended, constitutes pus.

We have seen that Cohnheim separated the phenomena of inflammation from those of repair. The destructive stage in the evolution of an abscess is a long one; its duration depends very greatly on the virulence of the organism. The leucocytes war against the organisms causing the suppuration, partly/

partly, through phagocytosis, and partly through dissolved substances. In so doing they are destroyed in immense numbers and become pus corpuscles. At an early stage the lymphocytes become mixed with the leucocytes. Their appearance in large numbers indicates a commencing absorption of the pus.

The abscesses from which the following descriptions are taken were produced in the manner described in Section III. The series represents varying periods from 30 hours to 2 months. I pass over the earlier stages; in these there is chiefly a disintegration of the tissue elements, and a breaking down of the leucocytes by a fatty degeneration of the cell and a nuclear disintegration. My attention will be confined to the period when the reactive phenomena have commenced, and a distinct wall encapsules the pus, though, as yet, it is only a cellular one with no fibrous tissue formation.

STAGE OF 3½ DAYS. Immediately around the area of necrosis and disintegration of tissue elements there is a zone composed of innumerable cells. In the midst of these lie small fragments of muscle in process of absorption, which have not yet lost

every/

every trace of structure. This cell accumulation forms a wall around the necrosed mass. The surrounding better preserved muscle substance shows many of the bundles broken up into groups of "muscle cells" still within the sarcolemma sheath; at other parts the muscle substance is penetrated by leucocytes and surrounded by phagocytic cells. The intermuscular septa are studded with leucocytes, the vessels are dilated, and numerous young connective tissue cells accompany them. Thus a broad cellular zone is formed between necrotic tissue and degenerated leucocytes on one side, and the more or less healthy muscle substance exterior. In the outer layers of this zone, which passes into the normal tissue, the fibrous tissue laminae which encapsule the abscess will be laid down; in the inner zone very important processes occur, and these lead to the absorption of the pus.

STAGE OF 6 - 7 DAYS. The cellular wall is becoming more definitely formed into a membrane. To the cells in the outer zone are added numerous fibroblasts, and gradually the outer layers become laid down as connective tissue elements; while numerous/

numerous young vessels arising from the vessels in the surrounding tissue, penetrate the cellular wall which surrounds the debris. The cells in the inner zone bordering the pus mass begin to show active phagocytosis and penetrate further into the pus.

STAGE OF 11 DAYS. The tissue elements are now still more definitely arranged into two layers. The inner is composed very largely of cellular elements - mononucleated cells and leucocytes. The latter still immigrate into the pus; the former, by a double process of solution and ingestion, are engaged in taking up the debris. Fibroblasts are beginning to find their way into this inner zone. The outer zone bordering the muscle begins to show a very distinct fibrillation; the long axis of the cells arranged circumferentially to the pus. Mononuclear cells and leucocytes are also present in this zone. Numerous vessels are now passing through this outer zone; they penetrate the inner zone and reach the border of the pus. Numerous cells, both fibroblasts and mononucleateds, accompany the vessels. Around the vessels in the outer layer frequent "small-celled-infiltrations" are beginning to appear, and many of these small cells/

cells already show transitions between lymphocytes and typical Plasma-cells.

STAGE OF 18 DAYS. The structure of this new membrane has gradually been modified. The external part is formed of superimposed layers of fibres and cells with fewer blood-vessels. This outer zone of laminated fibrous tissue contains numerous mononuclear cells but few leucocytes. The internal layer is becoming increasingly vascular. These vessels are all penetrating nearly at right angles to the surface of the abscess cavity. In the large endothelial cells of these capillaries one finds numerous Mitoses. (See Slide and Plate VI). Large mononucleated cells and many leucocytes accompany these vessels which are now on all sides penetrating the pus mass. At the junction of the inner zone and the pus mass, the large phagocytic cells form numerous rows of large endothelioid cells all with abundant cell inclusion and vacuolation. The vacuoles must be regarded as dissolved fat drops.

At this stage the activity of the organism producing the abscess has become lessened and, under the influence of the vascularization, the large phagocytic/

phagocytic cells display a marked activity. The processes of absorption of the pus are now actively in progress.

THE ABSORPTION OF THE PUS is by means of a two-fold process:-

1. The large endothelioid cells in the periphery of the pus mass are actively phagocytic. They penetrate into the pus, render it more fluid, and digest the material they appropriate.

2. The tissue of the abscess-membrane on its inner surface thrusts into the pus mass projections containing blood-vessels. These projections contain all the elements of granulation-tissue. As they increase in thickness and length the abscess-cavity - if a small one - is divided up into compartments by these vascular partitions, which are bordered on each side by large phagocytic cells actively carrying on their extracellular and intracellular function.

The rôle of the vessels in the absorption of the pus is the same as the rôle of the vessels which enter the fibrin strip in the wound cleft. Young fibroblasts pass in alongside of the vessels, and soon the vaso-formative network which bridges the cavity is increased in breadth. In this manner the /

the pus is gradually absorbed by the phagocytes. The fibroblast proliferation goes on with the formation of fibrous tissue which gradually replaces the former abscess cavity.

When all the pus is absorbed we find a heaped up accumulation of pus phagocytes, at first with the degenerated remains of cells. As these cell-inclusions disappear the protoplasm obtains a fine homogeneous appearance and the centrosome apparatus reappears. Most of these pus-phagocytes become degenerated, but may remain for a long period in the narrow interstices of the fibrous tissue. During the phagocytic activity of these cells, all the component parts of the pus are absorbed. The chromatin particles arising from their disintegration are to be seen lying in vacuoles in the cell body. Then they diminish, lose colour, and finally fall to pieces. Pus phagocytes can digest substances intracellularly, and dissolve substances extracellularly.

Bardenheuer, Kiener and Duclert, Marchand, and many other writers, derive the large phagocytes of the abscess-membrane from connective tissue cells and endothelium, but Maximow traces them to the Polyblasts/

Polyblasts, which, in suppurative inflammation as in aseptic inflammation, clear away the waste products. The Fibroblasts, together with the endothelial cells, initiate the regenerative processes. Maximow states that between the fibrous laminae many Polyblasts become sessile, and can only with difficulty be distinguished from fibroblasts. Around the blood-vessels numerous Polyblasts are laid down, which become granular and elongated. These are the Clasmatocyte-like adventitial cells. In my specimens of 31, 42, and 60 days, I found numerous cells in the abscess wall which seemed to correspond to Maximow's sessile polyblasts and adventitial cells. In the tissue around the blood-vessels I found, from the 11th day onwards, increasing numbers of Plasma cells. In that of 60 days, the Plasma cell groups form numerous "small-celled infiltrations" in the abscess wall. In these groups are all transitions from lymphocytes to Plasma-cells. I have never found a Plasma-cell phagocytic. The vacuolation must, I think, be ascribed to a degenerative process. In the abscess wall of 60 days I found large numbers of eosinophile cells, in one part of the wall (see Slide) almost/

almost as numerous as the Plasma-cells. With Muir's granule stain the eosinophile granules were intensely coloured.

Slides illustrate the points mentioned in these later stages.(See also separate list.) Plate XIII. represents a low power view of the abscess-membrane (18 days old).

(5)

(5) ULCERS - THE STRUCTURE OF GRANULATION TISSUE.

An ulcer, in the sense of Golding-Bird's definition "an open granulating surface," has been taken to illustrate the structure and arrangement of the individual elements in the granulating surface. The term "granulation tissue" is now applied to any cellular proliferation, but it was originally given to the structure known as a "granulation" which appears on the surface of an open wound. The changes which an exposed wound surface undergo, are those of intense hyperaemia, infiltration of the tissue by cellular elements, and a swelling and liquefaction of the tissues. In two days, in association with these changes , is a tissue proliferation which leads to the formation of the wound granulations. After a few days there has formed in the wound a very vascular tissue, with numerous cells - fibroblasts, mononucleateds, and leucocytes. Small red nodules, quickly increasing in size and number and running into each other, form the granular red surface. Greyish or blood-stained fluid, consisting of blood and lymph plasma with numerous leucocytes, covers the surface/

surface of the wound - the so-called "wound-secretion".

It is to the arrangement of the tissue elements in one of these "granulations" that the following paragraphs will be devoted. Through the kindness of Mr Muir, I have a beautiful series of plates which reveal the structure of the different layers better than any words can do. Plate XIII represents a low power view of a single granulation, which, as the wound could not be kept aseptic, is covered with a fibrino-purulent discharge. Plates XVII, XVIII, XIX, XX, illustrate the different layers in a healthy, aseptic, granulation. Plate XIV gives a low power view of a small ulcer with exuberant granulations, shown by the extremely dense telengiectatic grouping of the vessels, which are all cut in cross-section. Plate XV is a high power view of the same ulcer, showing the phagocytic cells bordering the tissue and fibrin layers.

For purposes of illustration and description, I have chosen granulation tissue of six days. At this time the vascular and cellular proliferation is in full sway, and the growth has the different layers more typically developed than at an earlier or later date.

If we follow the growing tissue from the wound/

wound surface to the deeper tissue, we can distinguish the following layers which I name the Surface, Intermediate, and Foundation layers. The foundation layer borders the mother-tissue out of which proceed the vessels, whose branching anastomosis forms the network scaffolding of the granulation.

I. SURFACE OR FIBRIN LAYER.(see Plate XVII.) This is composed of beautifully reticulated fibrin filaments in the meshes of which lie numerous cells. The amount of the fibrin poured out and the proportion of the cells varies very much. Leucocytes are in large proportion in the meshes, but mononucleated cells and fibroblasts are also present. The fibroblasts form beautiful star-shaped or more elongated cells. Some have a rounder appearance but can always be easily distinguished from the mononucleated cells by their clearer, oval, nucleus; the presence of one or more true nucleoli; and the reticulated meshwork of their protoplasm which stains a deeper pyronin color with the methyl-green-pyronin stain. The mononucleated forms have usually an indented nucleus, no true nucleoli, and a vacuolated protoplasm with frequent cell-inclusions.

Two/

Two questions arise in relation to these fibroblasts. How have they reached this layer, and what is their function there so early? They must have reached the fibrin by migration from the deeper tissues where active proliferation is going on, or have been carried thither by the upward flow of lymph. In their migration they may use the fibrin filaments as guiding lines. But what is their significance there so early? Where this surface layer joins the intermediate layer, the elongated forms are more abundant. Their processes form long, stretching, faint lines - only with difficulty followed amongst the fibrin filaments. The direction of their long axis corresponds to the direction of the growth of the tissue. These long, stretched-out fibroblasts seem now to enter into specially intimate relations with the young vascular buds which are found so abundantly in the next layer. The end of their long processes spreads out and attaches itself to the endothelium forming the young vessel wall. Processes from several fibroblasts may attach themselves to the same bud. Their direction being, as it were, a direct continuation into the fibrin layer of the young vascular buds. It is difficult to resist the conclusion/

conclusion that these cells are later incorporated into the vessel wall as endothelial cells. They certainly serve the young vessel as a guide and possibly as motive power.

II. THE INTERMEDIATE LAYER can be divided into two portions - an upper, more superficial part and a lower, deeper part. These cannot very easily be differentiated. I have therefore named them together the Intermediate layer. The Fibrin layer merges into the next by a grouping of the elongated fibroblasts. The upper portion (see Plate XVIII.) is characterised by the presence of numerous vessel buds, between which lie numerous fibroblasts irregularly arranged. The fibroblast processes here frequently anastomose. Cells, similar to those which deeper in this layer form the capillaries, are being apparently arranged to form a capillary endothelial wall. It is impossible to distinguish between these cells and many of the fibroblasts by which they are surrounded: both have an oval, clear nucleus with true nucleoli and a reticulated protoplasm stained more or less deeply with pyronin. Where the endothelial cells have definitely formed a capillary wall, the protoplasm/

protoplasm stains much less deeply with the pyronin and the nucleus has a deeper shade, but the differences are only relative.

Numerous mononucleated cells, red cells, and leucocytes are found between the vessels; some of the first are markedly phagocytic. If the surface is under special irritation, the number of leucocytes in all the layers is very marked, especially in the surface and intermediate zones; and these layers are occupied by giant phagocytes developed from the mononucleated cells. Plate II represents a group of giant-phagocytes present in the superficial part of the intermediate layer. Numerous cell-inclusions and the products of cell disintegration are found in these cells. If foreign bodies, hairs, dust particles, etc., fall on to the granulating surface and penetrate the fibrin, giant-cells are rapidly formed around them. Plate IV. Fig. 2. was taken from the same specimen as Plate II.

Where there are no such foreign bodies and no special irritation resulting in numerous decayed products, this surface portion of the intermediate layer is transparent. The vascular buds stand out quite distinctly, with the irregularly-grouped fibroblasts/

fibroblasts between them, and the long-stretched-out processes of the elongated fibroblasts in the fibrin layer attached to them.

This portion merges into the deeper by a very gradual transition. The endothelial cells are now definitely grouped to form capillaries, which all take a definite direction in radiating lines. (see Plate XIX.) Between these the fibroblasts, which formerly lay irregularly as star-shaped or elongated cells with branching processes, become definitely arranged with their long axes parallel to the capillaries. Many have to a great extent taken in their side processes and still others lie close to the outer surface of the endothelial wall. These latter are united to one another by their long processes, and give the appearance of forming a second wall to the capillary exactly similar to the endothelial wall. These fibroblasts immediately outside the vessels are beginning to show fibril formation in the layer of protoplasm not in relation to the vessel wall. As this increases the cells begin to separate a little from it, leaving a perivascular space.

The intermediate layer still contains fibrin even in the deeper portion, also numerous polyblasts and leucocytes. The latter diminish in numbers as we reach the deeper tissues.

III. FOUNDATION LAYER: this, I have so named because the tissue, especially in its deeper parts, consists of spindle-shaped fibroblasts which have a direction at right angles to the vessels. It is therefore approximating to the Mother-tissue from which this young growth has arisen. The transition between this zone and the intermediate one is marked by cells running diagonally. Between the fibroblasts lie numerous mononucleated cells which are becoming more elongated, especially those in relation to vessels. This zone contains few leucocytes, and seldom phagocytes or giant-cells.

The young vessels passing up from the foundation layer give off anastomosing branches in the intermediate layer and in the upper part of the latter give rise to vascular buds. This intermediate zone is a perfect network of capillaries. As we near the deeper tissues the anastomosing branches can be recognised as having undergone involution and become endothelial cords. Greenfield has pointed out that the channels of vessels in organising tissue, which fall into disuse, are closed by a natural endarteritis.

The cicatrisation of this tissue is carried out as in granulation tissue in the healing of wounds. In all the three zones, numerous mononucleated/

mononucleated cells are found, a few of which become sessile adventitial cells in the cicatricial tissue. This is more difficult to trace here than in the granulation tissue of the abscess-membrane. Plasma-cells are beginning to be found around the vessels in the Foundation layer and Mother-tissue.

In the deeper layers numerous mitoses are found, some of which, I think, are definitely mono-nucleated cells in mitosis. I have had great diffi-culty, however, in attributing any distinctive char-acters to the Mitoses of the various cells - connec-tive-tissue, endothelial, or mononucleated.

X/

X. CONCLUSION.

I approach this part of my work with more than reluctance. My inexperience in the interpretation of pathological processes, and the incomplete stage which my work has yet reached, give me no warrant to summarize conclusions on a subject beset with so many difficulties. It is customary, however, to end a thesis with a few such remarks. The conclusions to be stated have been gathered from the impressions derived from the direct observation of morphological facts. Sections have been cut from over 140 blocks of tissues representing the processes of inflammation and repair; between 700 and 800 specimens have been stained. The almost insurmountable difficulty experienced in cutting these sections thin enough for the examination with very high powers has left little time for their systematic study, and I am quite prepared on closer observation to modify or withdraw the statements I now make.

The primary object, however, of this paper

was/

was to present, as briefly as is consistent with clearness, a consecutive view of the chief problems in relation to wound-healing. An endeavour has been made to state the various theories which have been offered for their solution.

A study of the histology of wound-healing impresses one with the view that each factor in the inflammatory process not only tends in one direction and that a reparative one, but also that each reaction is purposeful. Here and there I have tried to point this out and I lay stress upon it now as perhaps the one general broad impression that forces itself upon one. Adami has stated that if we recognize physiological purpose, we must admit pathological.

Chemiotaxis is regarded by many as a convenient term to explain much that we do not understand. It may be so, but however much is included in it, it plays a very important part in the phenomena of inflammation. The chemiotactic influence exerted on all living protoplasm accounts for many of the processes we could not otherwise explain, and is, I think, largely accountable also for the cell-proliferation.

Regarding/

Regarding the various problems, a much deeper study than I have yet been able to give my specimens is necessary before I am justified in coming to any conclusions, other than those gathered from reading.

The vexed question of the haematogenous or histogenous origin of the mononucleated cells seems about to be solved by finding a via media between the two main opposing views. This reconciliation has found its chief advocate in Adami. The points at issue will further be much simplified if other workers bear out Maximow's confident assertions in many passages, *regarding the young fibroblasts which arise from the mitotic division of the connective-tissue cells. These, Maximow states, can be immediately recognized as such, and are not similar to the small round cells found in such large numbers during the early stages of inflammation. Almost all other authors hold that young connective-tissue cells are small round cells indistinguishable from the other round cells of the inflammatory exudate. Maximow bases his statements/

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*I have tabulated more than a dozen such definite statements in Maximow's writings.

statements on the character of the nucleus and nucleolus, and the structure of the protoplasm. I think that my specimens bear this out but it is an important point requiring prolonged study and I am not convinced.

If Maximow is correct it certainly clears away many difficulties. In the inflamed area we would find three distinct types of cells:-

(1) Leucocytes - with a definite origin.

(2) Mononucleated cells - with a very varied origin.

(3) Fibroblasts - with a definite origin.

Can these cells be kept apart with a definite function of their own, and be immediately recognisable as such cells in the inflamed area? Or are fibroblasts, at one stage of their career similar to monomucleated cells? And, if so, which of these many mononucleated cells, acknowledged to have a very varied origin, spring from the pre-existing connective-tissue cells? Lastly, do any of the others give rise to cells forming fibrous tissue?

An eludication of the problems regarding the origin of the so-called "histogenous wandering cells" would also do very much to clear the ground.

I/

I think most writers have taken far too little account of the cells coming to the inflamed area in the lymph-stream. The bone marrow has been shown to be a readily available source of leucocytes in case of need. The lymph-glands in the neighbourhood of an inflamed area and Ribbert's lymphoid nodules where present must surely also present a readily available supply in emergency .

Plates V.and VI. beautifully illustrate fibril formation and new vessel-formation. I have not yet succeeded in overcoming the difficulty of getting serial sections of skin tissues, and without these I cannot come to any decision on the mode of formation of the new vessels.

The Plasma-cell question is also important. From the study of my specimens, especially of abscess walls, I am convinced that this is a specially differentiated cell with a specific function. What this function is, and how it is carried out, are not decided. It may be that some substance is produced which reaches the circulation, having an influence general and local on abscesses, granulomata, and tumours.

In/

In conclusion, I gladly thank those who have so often helped me during the course of this work. To Professor Greenfield and Professor Beattie I am specially indebted for the suggestion of the work itself, and for much encouragement during its progress. Dr. Peter McEwan has kindly assisted me in the operations. The work has been carried out in the Pathological Laboratory of the University; to Mr Richard Muir and Mr George Buchanan of this Department I am very grateful, for the carrying out of the technique has been possible only through their help. The drawings are all by Mr Richard Muir whom, as my "guide, philosopher, and friend," I cannot sufficiently thank.

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