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On Formation of Scar-Tissue

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ON FORMATION OF SCAR-TISSUE.

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In the course of a research by one of us (B.) into the process of occlusion of arteries after ligation, it seemed desirable to form an independent opinion of the part played by the elements of the arterial wall in the production of the fibrous tissue which eventually closes the vascular channel. The following experiments were carried out in the hope of their furnishing, to ourselves at least, some answer to the disputed question of the origin of inflammatory tissue. Are the colorless corpuscles of the blood, capable as we know them to be of passing from the vessels into the intervascular tissue, also the source of the new tissue which the inflammatory process may produce?

Cohnheim's reply to this question was an affirmative, at least as positive as are the discoveries brilliant which it was based upon. For Cohnheim the colorless corpuscles which wandered from the blood vessels in an area of inflammation were not only the source of pus cells when pus appeared, but were the formative cells for the new tissue if any new tissue were formed. From this position in the question he never withdrew. In the edition of the *Vorlesungen* only two years prior to his untimely death again with renewed insistance he supported it. It was from his laboratory that most of the observations emanated which bring evidence in favour of this "leucocytic" view. They are set forth in papers from the Institute at Breslau by Senftleben[2], and by B. Heidenhain[3]; and by Senftleben[4]

[1] The preparations with the drawings were shown in March of this year at the Royal College of Surgeons in illustration of part of the Erasmus Wilson Lectures on "Ligation in Continuity."

[2] *Virchow's Archiv*, Vol. LXXII.

[3] *Ueber d. Verfettung fremder Körper in d. Bauchhöhle*, 1872.

[4] *Virchow's Archiv*, Vol. LXXVII.

and by Tillmans[1] from the Institute at Leipsic. Among observers who independently of contact with Cohnheim published opinions in harmony with his upon this question, may be named especially Schede[2], Aufrecht[3], Bizzozero[4], and Ziegler[5].

Upon the evidence furnished by the researches of Ziegler Cohnheim laid a quite exceptional stress. The conclusions of the then Assistant in the Wurzburg laboratory have had much to do with the ascendancy of Cohnheim's teaching on the point. They have been incorporated by Ziegler himself in the well-known *Lehrbuch der Pathologische Anatomie.*

Ziegler placed little oblong chambers, made by fastening at the four corners two cover-glasses a slight distance apart, under the skin of the dog, and left them for a certain length of time. The cleft between the glasses became filled with cells, which could be examined directly under a microscope. He found these cells to be leucocytes, many of which were fatty, and resembled the ordinary cells of pus. But in many experiments after a time actual formation of tissue took place in the layer of cells between the glasses. Cells were found in all stages from the lymphoid to the epithelioid and giant-cell type. Certain cells seemed to grow large at the expense of their neighbours whose protoplasm was appropriated by the larger growing cell. He judged that his preparations proved that the giant cells of granulation tissue are at least in some cases produced from the wandering colorless corpuscles of the blood, and further that the giant cells produce both blood vessels and connective tissue. "So war dadurch der Nachweis geleistet" "dass bei der Entzündung die ausgewanderten Zellen eine gewebsbildende Rolle spielen."

Ziegler's researches were taken as furnishing experimental proof that not only do migrated white blood cells in certain numbers become pus corpuscles, but that they also in certain numbers are capable of further development, and are the primary source of cicatricial tissue.

Ziegler's conclusions were in fact similar to those of Cohnheim. A considerable number of writers have controverted them. Baum-

[1] *Virchow's Archiv*, Vol. LXXVIII.

[2] *Arch. f. klin. Chir.*, XV.

[3] *Virchow's Archiv*, Vol. XLIV.

[4] *Annali universi di Medicina*, 1868.

[5] *Exper. Unters. üb. d. Herkunft d. Tuberkelelemente*, 1875, and *Unters. üb. path. Bindegewebs u. Gefässneubildung*, 1876.

garten[1], Böttcher[2], Ewetzky[3], Weiss[4], Hamilton[5], all have at various times raised a voice against it, denying to the migrated blood cells any power of further development. According to them the formation of scar-tissue is in no way directly due to elements existing in the blood; the fibrous tissue does not arise from leucocytes. Quite notably have those histologists who have studied the process of occlusion of blood vessels assumed a sceptic attitude amid the general acquiescence in the Cohnheim-Ziegler doctrine. We will point out the writings of Thiersch[6], Riedel[7], Auerbach[8], Pick[9], Heuking and Thoma[10] and most recently and very definitely Hunter[11]; as we follow the opinions of these observers chronologically toward the present time more and more pronounced do we find the tendency to deny to leucocytes an exclusive share in the replacing of coagulum, &c. by cicatricial tissue.

From the foregoing it is evident that a very desirable side light, might be thrown on the question upon which one of us (B.) was engaged, by an examination elsewhere than in a ligated blood vessel, of this capability of leucocytes to produce a fibrous connective tissue. It was determined to repeat the classical experiments of Ziegler. We may remark that we began work with an educational bias favourable to Cohnheim's view.

Methods employed.

Two circular cover-glasses, each $\frac{5}{8}$ of an in. in diameter and ·006 of an in. in thickness, were fastened together so as to form a little flat glass chamber, in the manner employed by Ziegler. A strip of tinfoil placed between them at their edge along $\frac{11}{12}$ of their circumference was cemented by shellac on each face to the corresponding surface of the cover-glass. The tiny chamber thus formed had therefore

[1] *Die sogenannt. Organis. des Thrombus*, Leipzig, 1877.
[2] Ziegler's *Beiträge zur pathologischen Anat.* II. 2.
[3] *Unters. aus der path. Institt. zu Zürich*, III.
[4] *Archiv f. klinische Chirurgie*, Bd. XXIII, 1879.
[5] Spongegrafting. *Edin. Med. Journ.*, 1881.
[6] Pitha u. Billroth. Bd. I. Abth. 2, § 549.
[7] *Zeitschr. f. Chirurg.*, Bd. VI. 1876.
[8] *Ueber die Obliteration der Arterien nach Ligatur*, 1877
[9] *Zeitschr. f. Heilkunde*, Bd. VI. 1886.
[10] *Virchow's Archiv*, Vol. CIX. 1887.
[11] Gold Medal Thesis for M.D. Edin. unpublished.

between the two ends of the strip of tin-foil an opening into the interior. The tin-foil first employed was $\frac{1}{10}$ mm. thick; that thickness was inconvenient, as the depth of the chamber was then too great for higher powers of the microscope to explore. Tin-foil $\frac{1}{20}$ mm. in thickness was subsequently employed. With this thickness membranes were obtained between the cover-glasses that made very satisfactory microscopical specimens. Fig. 18, Plate XXXIII.

These chambers for eight and forty hours before use were emptied of air and filled with distilled water previously sterilized, or with nutrient broth containing peptone according to the recipe of Koch. Both the chamber and the fluid in which it was kept were again sterilized by heat an hour or so before being used for experiment. In a few instances the air was not entirely expelled.

The animals employed by us have been in all cases rabbits or guinea-pigs. During every experiment the animal has been deeply under the influence of an anaesthetic. Antiseptic precautions were vigorously maintained throughout all the operations. No suppuration ever occurred. Had it done so in any experiment we should have excluded the results of that experiment. In our earlier experiments the chambers were placed in the peritoneal cavity; in the later into the subcutaneous connective tissue of the flank.

The chambers were allowed to remain within the animals for various periods, from four hours at shortest to 18 days at longest. When the chamber was removed its contents were examined either fresh upon a warm stage under the microscope, or after appropriate treatment with hardening and staining reagents. The outsides of the chambers were often covered with thin films of young fibrous tissue—these films were examined by the same methods as were the contents. The reagent chiefly employed was osmic acid, either in freshly made ·5% watery solution, or in vapour from a 1% solution. In the former case the chamber taken warm from the body was at once plunged into the osmic acid solution or was rapidly split open, and with the contents so exposed, placed in osmic acid. In the solution of osmic acid they remained for an hour, in the dark. Where osmium vapours were used the same plan was adopted, except that the chamber was always opened unless bubbles of air happened to have got ingress previously, as sometimes happened. Exposure to the vapour was ensured by placing the specimen between two watch-glasses, at the bottom of which was osmic acid solution, or by suspending from the cork of a bottle containing the solution. The action of the osmic acid was allowed about two hours'

play, always in the dark. The preparations with osmic acid were, after washing in water, mounted either in Farrant's gum solution, or after dehydration, in xylol-balsam. They were in some instances after-stained, with picrocarmine, with fuchsin, methylene blue, eosin, or most frequently with haematoxylin (Ehrlich's solution). Other preparations were made without the use of osmic acid. In these after hardening in chromic acid, or in alcohol, or Fleming's solution, picrocarmine, methylene blue, eosin, fuchsin, haematoxylin, etc. were used.

For the observations on living specimens a warm stage of Stricker's pattern was used.

Contents of the Chambers.

One of the first steps which we took was to examine the serous moisture of the abdominal cavity of the rabbit and guinea-pig, and of subcutaneous wounds and the blood in order to ascertain the characters of the cellular elements contained therein. The examination was conducted by the cover-glass method first recommended by Koch and Löffler. Eosin, fuchsin, saffranin and methylene blue were used as stains. Some preparations were made by exposing the moist film on the cover-glass to vapours of osmic acid solution.

The examination revealed the presence in the serous moisture and in the tissue plasma of at least two kinds of cells. The one kind resembled in all respects the leucocyte, the colorless cell of the blood. This kind was in fact indistinguishable from the leucocyte.

A second kind of cell was also present and sufficiently numerous. This cell, which is much larger in size than is the former kind, when fixed by osmic acid vapour, often presents a discoid figure, some 30μ to 40μ across; not infrequently however, and more frequently than not when fixed by plunging into osmic acid solution, or by drying, as in the Koch-Löffler method, the cell outline is irregular, often angular, with especial prominence of one angle or of two; in the last case the cell might be described as fusiform. Whatever the shape of the body of the cell may be, there always lies within it, generally towards the centre, a large oval vesicular nucleus, itself somewhat larger than the red corpuscle of the blood. It does not stain so darkly as does the nucleus of a leucocyte. The substance of the flattened plate-like cell-body is markedly granular, particularly so when prepared by the rapidly drying method. The protoplasm reduced to a flattened flake as it generally is, may be of such tenuity near the margin that in the fresh condition and

in many osmic acid preparations the outline of the cell is somewhat difficult to distinguish. The determination of the limits of the cell is however rendered easier by the fact that the cell substance is very granular, the granules taking a sepia tint with osmic acid, and being readily stained by fuchsin and other aniline colours. A marked characteristic of these cells is their tendency to occur in masses and clumps. In the masses the outlines of the individuals are often hard to recognise. In specimens examined fresh upon the warm stage the granules are many of them brilliant and highly refracting, though not to such an extent as are fatty particles. Granules of various size exist in one and the same cell.

Four Hours.—The shortest sojourn we allowed the chambers was four hours in the subcutaneous tissue. The chambers were found to contain fluid with a few blood corpuscles, not collected into rouleaux, and unaltered in appearance. No fibrin had appeared. In short, mixed with the bouillon was simply a trace of blood, as yet unclotted.

Within the chamber in the neighbourhood of its opening were however a large number of leucocytes, unmixed with red blood corpuscles or indeed with any other kind of cell. None of these wandering cells had apparently penetrated far into the recess of the chamber, because there appeared an obvious gap between the position of their pioneers and the diluted blood elements occupying the chamber elsewhere.

Nine and a Half Hours.—Nine and a half hours after insertion into the abdominal cavity, the diluted blood was also found unclotted—although in this instance the chamber had been filled not with bouillon but with water sterilized. The trace of blood which almost unavoidably found its way into the chambers at the time of their insertion had remained unclotted in all specimens examined earlier than fifteen hours. The bouillon containing peptone, seemed, as indeed one might have expected, to retard the clotting of the blood that entered the chamber. In chambers filled with nutrient peptone-bouillon no clot was ever found earlier than twenty hours after the original implantation. On the other hand we found a very considerable formation of fibrin in a chamber that had been filled with sterilized water and then allowed a sojourn of eighteen hours in the subcutaneous tissue. The nutrient bouillon contained commercial peptones, and we think the peptones may not so rapidly diffuse but that this retardation of the clotting may be explained by their presence.

In the early specimens of fibrin production in the tissue plasma

which filled the chambers we had abundant examples of the formation of fibrin filaments as fine straight or very slightly curved lines, irregularly radiating from granular nodal masses. The nodal granular débris, under various reagents, appeared to consist, as the generally accepted view affirms, of altered blood-platelets, and leucocytes undergoing alteration.

About the nodal points of the network of fibrin the leucocytes were grouped. Both on the warm stage and after fixation by osmic acid these leucocytes displayed only rarely any deviation from the spheroid form. There were indeed some instances in which they presented a fusiform outline, or possessed a tiny process jutting from the cell body. Such examples were extremely sparse, and occurred only in the neighbourhood of the opening of the chamber, and in certain situations to be specified immediately. For the most part the cells seemed in an inert condition, as far as one can judge of their activity from their form. In the short time between extraction of the chamber from the body and the fixation in osmic acid or the observation of the cell upon a warm stage under the microscope, their vitality had suffered sufficiently for the cell to have assumed its zero of shape, the subspheroid figure. Or for some reason existence within the Ziegler's chamber was not conducive to activity of the protoplasm, yet from what we shall relate there is no good reason for thinking that the leucocytes within the chamber are in a very different state from those invading the inflamed tissue without. The latter supposition is favoured by the fact that the larger cells, plasma-cells as we shall term them, also found in the chambers under similar circumstances, although subjected to the same *technique* of preparation as these leucocytes, showed well-marked amoeboid movements.

Indeed if the latter of the two suppositions just suggested be not accepted, it becomes necessary to assume that of these two kinds of cell the leucocyte is much the more perishable and delicate, and was practically annihilated by a simple procedure that did not appear to interfere with the vitality of its co-occupant the plasma-cell.

Here must be mentioned another sign of degeneration in the leucocytes examined in these chambers[1]. Many of them showed the triple and multiple nuclear bodies that are universally regarded as evidence of the lethal disintegration of the nucleus—as Fleming names it, the "fragmentation" of the nucleus. On the other hand the cell-

[1] Kuss, Paris, 1846. Paget, *Surgical Pathol.* p. 151.

body of the leucocyte was not granular or fatty, but fairly evenly though deeply tinted by the osmium. These points are seen in Fig. 1, Plate XXXI.

EIGHTEEN HOURS.—In chambers removed after the appearance of fibrin within them, but before the stay within the body had exceeded eight and forty hours, it was usual to find a number of areas in which leucocytes were present in much greater numbers than elsewhere. Fig. 2, Pl. XXXI.

The tendency to collect to certain points which the leucocytes evinced in even very early specimens was more marked in these later preparations. About the nodal points of the fibrinous network crowds of them were present. The outlying individuals were frequently arranged in lines along the converging filaments of fibrin. The older within certain limits these films of coagulum the more obvious the aggregation of the leucocytes into certain groups. For convenience on account of their prominence and apparent importance in subsequent stages we have been accustomed to refer to these groups shortly as the cell-islets. Cf. Fig. 3, Pl. XXXI. They are little collections of cells, occurring constantly, scattered about in the thin cellular membranes which grow over and within the glass chambers. Some are obvious to the naked eye, especially when the film has been treated with carmine or with haematoxylin, which show them as deeply colored points. They vary in size from a small pin's head downward. The larger islets are often compounded of smaller ones. The smallest display best what we believe to be the structure originally characteristic of all:—a centre of amorphous albuminous débris surrounded by leucocytes; less frequently one or two altered red blood corpuscles form the centre.

It was in specimens of this date that the first evidence of the presence of another cellular element than the leucocytes and red cells of the blood was found. Cells similar to the large flattened plate-like forms of the peritoneal moisture, already adverted to, began to be found in the chamber. The time of their advent varied within narrow limits when the chamber rested in the subcutaneous tissue; when in the peritoneal cavity there was much greater variation in respect to time. This we believe was due to the chamber not coming to rest in one particular spot for some time after introduction into the abdomen. The movements of the viscera seemed able to shift it and prevent its forming adhesions. When put into the abdomen we always placed it about an inch to right or left of the little wound in the linea alba through which it was inserted. But we never found it anywhere

near that situation after a sojourn of more than a few hours. In the course of four and twenty hours the chamber had nearly always passed toward or actually into the position which it almost constantly came permanently to take, that is, a little distance from the median line in front of the psoas muscle at the very root of the mesentery. Once there it appeared in a few hours to contract adhesions, and become fixed in a permanent fashion. Until bound down by adhesions, the full complement of cells did not reach the interior of the chamber.

In one instance, in a chamber exposed for eighteen hours in the subcutaneous tissue, plasma-cells were found in considerable numbers near the opening. They were indistinguishable in appearance from the plasma-cells of the normal subcutaneous tissue, except that a greater variety of individual form was to be seen in them. In later specimens the plasma-cells were found scattered throughout the whole chamber, although most numerous near the opening.

SEVENTY-TWO HOURS.—In a preparation from a chamber which had been seventy-two hours in the subcutaneous tissue, plasma-cells entered into the formation of the islets even in the portions furthest removed from the opening. At the opening however no other kind of cell was mixed with them, which was not the case elsewhere. No forms intermediate between the leucocyte and the plasma-cell were to be found; they were repeatedly expected and repeatedly looked for, but the search was unsuccessful.

The preparations gave an almost bewildering number of examples of the infinite variation in shape of the large amoeboid plasma-cells, which also varied very considerably in size, and as to granules. The body of the cell was for the most part plate-like, being in many instances extended into so thin a film that its exact limit was hard to determine, especially when, as occasionally happened, the granules of the cell-body were less pronounced towards the periphery. Some idea of the wide diversity of outline exhibited by individual cells may be gathered from our figures. Cf. Figs. 1, 4, 5, 6, 7 and 8, Plates XXXI. and XXXII.

It must not be thought, however, that in any of its forms the plasma-cell could not be distinguished with certainty from the leucocyte. In the same way as in the peritoneal moisture and in the plasma of subcutaneous tissue, the former is here also on the warm stage and in osmic preparations, characterised by larger size, coarser granules, the constant presence of a single clear nucleus of oval figure, and by the differences in staining qualities and mobility already

referred to. We may here add that these differential characters may be obscured by faulty methods of examination.

In the specimens obtained from chambers that had rested for seventy-two hours in the subcutaneous tissue of the guinea-pig, we found individuals among the plasma-cells, which showed well-marked vacuolation, Figs. 1, 4, 5, Pl. XXXI. For the most part the matter within the vacuole was a granular débris that furnished no sufficient clue as to its nature. But in a few it was indisputable that the vacuole contained, more or less altered but still perfectly easily recognisable, a leucocyte or red blood corpuscle. In Fig. 4 is shown the appearance presented by one of these cells. A large vacuole contains a somewhat faintly stained body, which is finely granular and indistinctly nucleated. It is a little smaller than is the nucleus of the plasma-cell itself. Fine threads seemed to pass from the sides of the vacuole across the cavity to the substance of the included leucocyte. Taken with the context afforded by examination of other cells in the neighbourhood we believe that this and other similar instances were examples of leucocytes lying in vacuoles in the plasma-cells. Many stages of ingestion could be found. Cf. Figs. 1, 4, 5, Pl. XXXI. Simple approximation, the hollowing out of a little bay in the side of the plasma-cell into which the leucocyte was as it were drawn, partial inclusion, total inclusion—all these were exemplified. And further there were many vacuoles in which mere granular débris lay. This débris was, we think, probably the still undigested remnant of the ingested leucocyte or red blood cell. We doubt whether without very special apparatus the cells of the tissues of mammalia can be kept in sufficiently normal condition for sufficient length of time to compass observations on ingestion by living cells; we were however much assisted in the interpretation of the appearances of the osmic fixed preparations by the processes described by Miss M. Greenwood for the Rhizopoda. Her observations[1] were conducted on living specimens of Amoeba proteus and Actinosphaerium, and she was able to follow in these animals under the microscope all the visible phenomena accompanying the ingestion of prey. In our preparations we had as it were a number of amoebae, many of which had been actively engaged in ingesting living prey, immediately before the reagent had been used that killed them so rapidly as to allow no time for any great departure from their previous aspect.

[1] This *Journal*, Vol. VII. p. 253, Vol. VIII. p. 263.

Nor were leucocytes the only bodies to be found within the substance of the plasma-cell. Red corpuscles of blood were recognisable in them. Very frequently along the border of the space in the chamber, the plasma-cells lying in great numbers near the cement (shellac glue) which fixed the strip of tin-foil to the glass, were filled with tiny droplets of oil that became deep black under the treatment with osmic acid. Sometimes the entire cell was dotted, except just round the nucleus, with fine fatty particles of a fairly equal size: sometimes the oil was collected into a few much larger globules. The cement itself turned deep black under osmic acid treatment. There was little room for doubt that the black particles in the plasma-cells were derived from the cement near the cells; whether the cells took up the particles without altering them, or whether the particles were in any degree a food for the cells are points we can give no answer to.

Contiguous plasma-cells or even those a little distance apart were often connected together by their processes (Figs. 1, 5, 7 and 8, Plates XXXI. and XXXII.). The bands of connection might be short thick arms or long gossamer threads of protoplasm. By similar arms and threads the cells seemed to adhere to the most diverse objects in their surrounding. The surface of the cover-glass, a filament of fibrin, a hair, a fibre of cotton, a lump of the cement fastening the sides of the chamber together, all afforded points to which the processes from the plasma-cells would cling (Figs. 14 and 15).

There were present also in chambers of eighteen hours', twenty-two hours', twenty-six hours', forty-eight hours', and seventy-two hours' standing, as also in others of older date containing well formed granulation tissue, many giant cells (Fig. 6)—huge multi-nucleate cells, that obviously in many instances were cell-fusions. Congregations of large plasma-cells as before mentioned were frequently met with. They adhered one to another in groups. And here many collections of them existed intermediate in character between those groups in which the individual cells were agminated but easily distinguishable from one another, and giant cell masses in which the nuclei were the only guides to the individual position of the coherent members. Some appeared to be cell-fusions; many did not. In these latter the nuclei were gathered together into an irregular heap. The ring-like arrangement of the nuclei frequently found in the giant cells of tubercle was never observed in these membranes by us.

Of nuclei in these giant cells there existed apparently two kinds. One was large, clear, and oval, having all the characters of the nucleus

of the separate plasma-cell; it was invariably present in all the giant cells. The other sort was smaller, round, more darkly tinted by osmium treatment, and was not invariably present, that is, did not exist in every giant cell, but was in some cells even more numerous than the larger oval variety. We doubt very much the accuracy of describing the latter smaller bodies as true nuclei. We incline to believe, from their great similarity to some of the leucocytes observed in the plasma-cells, that they are nothing but leucocytes surrounded by the substance of the giant cell and somewhat altered in appearance. Against this supposition is the fact that there was often no indication of a vacuole-space around the ingested cell, but in support of it the substance of the plasma-cell was seen sometimes very closely applied to the ingested leucocyte in instances in which there was very little doubt as to the nature of the included body. In osmic preparations there is generally a light space free from granules immediately around the oval nucleus of the plasma-cell that simulates somewhat closely the appearance of a vacuole about the nucleus itself.

Advancing further into the chamber, in the specimens of more than forty-eight hours' duration, the plasma-cells begin to apply themselves to the islet-groups of leucocytes. Cf. Figs. 8 and 10. They surround the leucocytes. The islets come to consist of a central portion made up of leucocytes, and an outer zone of large and granular plasma-cells. In this way the islets seem to increase rapidly in size. Neighbouring islets appear to become merged together. Giant cells are frequent in them, especially, it would appear, near, although not actually at, the centre. Most of the growth that went on in the membrane appeared to consist in enlargement of individual islets, and the fusion of neighbouring islets. The islets appeared to be the chief growing points of the tissue. But it is true that gradually a more or less continuous sheet of plasma-cells is formed over the intervening space between the islets. When very thin the inflammatory membrane consisted of a layer of scattered cells lying separated by considerable but fairly regular distances one from another. Each individual cell was of a discoid or fusiform figure, and granular, with a large clear nucleus. The edge of the disc was thin and often deeply scalloped; it merged, under all methods of staining used by us, at certain points quite imperceptibly, in a tenuous film which composed the bulk of the membrane proper. When fixed with osmic acid and after-stained with haematoxylin (Ehrlich's), this membrane is shown to contain, if not to be entirely made up of, a feltwork of filaments, like filaments of fibrin. These

cross in every direction in the plane of the membrane, without prominent arrangement in any one particular sense. The individual filaments vary a good deal in size. Fig. 16, Pl. XXXIII.

It was among the plasma-cells of the fringe of the islets that we noticed the earliest regularly fusiform cells, the immediate precursors of fibrous elements in the new tissue. It is true that plasma-cells of an irregular spindle-shape were observable not rarely among even the earliest of the plasma-cell swarm entering the chamber. But in those instances the outline was probably but one of many which the amoeboid cell successively assumed, and generally it was not of the same character as the regularly fusiform type prevailing among these plasma-cells in the outskirts of an islet. In that latter the majority of the cells lay in lines concentrically set about a core of ill-stained, broken-down matter that composed the centre of the mass. Cf. Fig. 11, Pl. XXXII. The fusiform fibroblasts began in fact the encapsulation of the *débris* of the breaking-down blood cells, &c. The lengthening out and assuming of a regular spindle form took place also very early in those cells that had become attached to hairs and cotton-fibres, and lumps of the shellac glue. They were soon found adhering there in rows of regular disposition, the rows consisting entirely of typical young fusiform fibroblasts.

Later than seventy-two hours.

Older specimens revealed further progress in the formation of a fibrous-tissue membrane. After a stay of eight days, or ten days, or fourteen days in the subcutaneous tissue in many instances the islets consisted of plasma-cells alone. The leucocytes had disappeared. The pigmented remnants of the red blood corpuscles were much longer traceable. In many places along certain lines the spindle-shaped cells had become attenuated, and formed distinct bands and often long and delicate cords (Figs. 12, 13). In many places in the tenth day specimens, and in some of the eighth day ones an inter-cellular substance showing fibrillation exists (Fig. 12). This extra-cellular matter is well seen where, as occasionally happens, a single chain of fusiform fibroblasts, set in end-wise series, has produced a thread-like tiny cord. Each fibroblast appears to lie in a sheath of fibrillated matter. The delicate lines marking the fibrillae run parallel to the contour of the cell. The fibrillated matter was not tinted by osmic acid or by any of the stains employed by us to the same depth as the granular substance

of the cell itself. The granules of the cell-body, the clear oval nucleus, were still marked characters of the plasma-cell, although it might be considered at this stage to have become a fixed corpuscle of connective tissue.

We were unable to satisfy ourselves on the question as to whether the fibrillated extra-cellular matter had been formed by direct transformation from the surface portion of the cell-body, or whether it had arisen as a secretion from the protoplasm of the cell. But the latter view appears to us the most probable, if only for the reason that the fibroblast-cell and its new capsule of fibrillated matter are when taken together much larger than, so far as we have observed, the individual naked fibroblast ever is.

From the islets the bands of spindle cells spread away in various directions. The determination of the direction of the earliest-formed chains of spindle cells seemed to us greatly due to the lines taken by the filaments of the original fibrin-network; the radiation from the same nodal points, the interlacing not always at acute angles but frequently in rectangular fashion.

In membranes of ten, fourteen, and even eighteen days' growth, not all the cells nor even the majority were spindle-shaped. A vast number were triradiate, and multiradiate; some had but one process; very few were rounded. Many recalled to mind the branched fixed corpuscles of the cornea. Long tapering branches united cell to cell, not only the cells of one plane one with another, but the cells of different planes also (Figs. 8, 9 and 17). A meshwork of infinite variety and complexity was thus established. But in all these examples of plasma cells in the stable as well as in the previously described labile forms, the granular nature of the cell substance and the clear oval nucleus were characters never lost.

In the same manner as did the more delicate strands of fibrous tissue, larger, broader sheets and beams arose. In all the spindle cells side to side as well as end to end are separated by intervening matter fibrillated in a direction parallel to the longer axes of the cells.

It may have been noticed that no mention has been made of any developing blood vessels in the membranes examined. It is a striking fact that in none of the preparations, not even in the preparations of eighteen days' growth, taken from the peritoneal cavity, did we find in any instance any trace of a formation of blood vessels. Nowhere were capillaries to be found; although the chambers were bound by adhesions and in the later specimens encapsuled in cicatricial tissue. This obser-

vation seems to furnish a negative to the view advanced by Creighton that the giant cells of granulation-tissue are exclusively vaso-factive. Here we had giant cells in abundance, but never any capillary formation. Perhaps the film of tissue in the chamber was thin enough to allow sufficient nutriment to reach the cells by fluid soakage only.

Abstract of some of the Notes of the Experiments.

The number of hours mentioned corresponds to the time during which the chambers rested in the bodies of the animals.

1. 4 *hours.* Subcutaneous. No fibrin. No rouleaux of red cells. A large number of leucocytes in the neighbourhood of the mouth of the chamber.

2. 18 *hours.* Subcutaneous.

High power. Fibrin network very extensive. Crowds of leucocytes at the nodal points of fibrin. Cells circular in outline, nuclei crescentic or trilobed. At the periphery of the islet a few leucocytes still in an active state and of irregular form. At the mouth of the chamber are a few large plasma and giant cells. Fig. 2, Pl. XXXI.

Low power. The islets of cells in the fibrin film are well seen. Indeed they are visible to the naked eye, about the size of pins' heads.

3. 72 *hours.* Subcutaneous.

Islets more marked. Fibrin network very extensive. Numerous large plasma-cells encircling the islets of leucocytes at the nodal points. In the neighbourhood of the mouth of the chamber the plasma corpuscles are more numerous, and the islets are partly made up of these cells. Moreover, numerous red and white blood cells are visible in the vacuoles of giant cells and in those of separate plasma-cells.

72 *hours.* Peritoneal cavity.

The chamber was quite *free.* No trace of an adhesion.

The chamber seems to have escaped the leucocytic immigration. The islets are formed almost entirely of plasma-cells.

4. 8 *days.* Peritoneal cavity. Fixed by an adhesion; how long fixed?

The islets are formed of plasma-cells alone. The leucocytes have all disappeared, a few only are visible in the vacuoles of the larger cells. The cells at the circumference of the islets are lengthened out along the lines of fibrin, and joined with others free of the islets to form a giant cell field, or plasmodium. Every cell is connected

by long processes with others, so that looked at in one way the whole field is one giant cell. The islets consist often of one giant cell together with numerous plasma corpuscles. It is only at the periphery of the islets that the contour of the individual plasma-cells can be made out. See Fig. 8. Foreign bodies such as blood-clot, shellac, etc. are surrounded by a capsule of spindle cells. Fig. 11.

5. *Several chambers.* 8 to 18 days in peritoneal cavity. Some fixed, and some *not* fixed by adhesions.

Fibrillation advanced. Every stage can be observed, from the simple fusiform elongation of the plasma-cell to the development of a perfect fibril. Fig. 12.

The above experiments seemed to point to a certain definite period at which the migration of leucocytes and connective tissue corpuscles occurred.

In order to examine somewhat further the behaviour of the leucocytes and of the plasma-cells respectively toward the chamber, a slight modification of the mode of experiment was used on two occasions. Two chambers (or four) were placed side by side in the subcutaneous tissue. At the end of twenty-two hours they were taken out, one was dropped into osmic acid, and the other was sealed with warm paraffin. The sealing was done by dipping the mouth of the chamber into a soft paraffin melting at 107° Fahrenheit. Only the part of the chamber immediately next the opening was touched by the paraffin, which was just above the temperature of solidification. After being sealed the chamber was placed in the abdominal cavity of a second guinea-pig, there to remain for incubation.

In this way it was possible to compare the contents of two chambers which had been placed side by side in the subcutaneous tissue, and whose contents were presumably the same at the time of withdrawal. One was then fixed for histological examination. The other incubated for a longer period, no new cells being allowed to enter during this second incubation.

The imperviousness of the chambers after sealing was tested in the following two ways:

(*a*) A little 1% hydrochloric acid was introduced in the chamber, the outer edge of the opening carefully dried, and then the paraffin applied by dipping the mouth just as in the experiment above. The chamber was then placed in blue litmus. No change took place in the litmus, although the chamber remained a week in the solution.

(β) A little of an active culture of Spirillum Finkleri was introduced into the chamber, the edge of the opening cleaned, and then sealing performed as before. The chamber was then placed in nutrient broth (in another case in nutrient gelatine) for a week, at a temperature of 35° C. No growth appeared in the broth (or gelatine). In a control tube the broth was turbid in two days.

In the experiments performed in this way the appearances observed in the chambers were alike. The chambers were withdrawn at the end of twenty-two hours, one was then incubated further for another forty-four hours.

The contents at end of twenty-two hours were as follows, viz.:—
a large number of leucocytes and several patches of red corpuscles. Plasma-cells are also present but very sparsely; they are most numerous at the mouth of the chamber. They are scattered at long intervals. In one place a few plasma-cells are collected around some red cells, and a fibre of wool. Fibrin filaments are present in the chambers taken from the rabbit, but none in those from the guinea-pig.

Contents of sealed chambers after forty-four hours' further incubation:

Fibrin network extensive. The leucocytes lie around the red cell masses. The leucocytes possess for the most part "fragmented" nuclei. The plasma-cells are far more numerous. They exist in patches and groups quite apart in many cases from the clots or the leucocytes. Many plasma-cells lie mingled with the leucocytes around the little blood-clots.

Remarks.

We have pointed out that there appears to be a definite sequence of events in the processes induced within the tissue by implantation there of the experimental chamber—processes which must according to ordinary terminology be designated as inflammatory in nature. At a definite time and in a definite order occur the immigrations respectively of leucocytes and of the daughter cells of the tissue corpuscles. The former had commenced at the end of four hours. In our experiments the fibrin within the chamber was crowded with leucocytes within eighteen hours of the time of insertion in the subcutaneous tissue of rabbit or guinea-pig. But in those eighteen hours scarcely a plasma-cell could be found to have penetrated into the chamber. On the other hand after the lapse of seventy-two hours the nodal points, which had been previously the centres of aggregation of leucocytes, had been

transformed into islets consisting chiefly of plasma-cells. This primary leucocytic invasion and the subsequent appearance of "larger cells with clear vesicular nuclei" has also been noted by Ziegler and by others. W. Hunter[1] passed by transfusion all the blood of one rabbit into the peritoneal cavity of a second. At the end of a few hours he was able to find scarcely a white corpuscle in the circulating blood; the amoeboid cells had migrated into the peritoneal cavity where the foreign body—the fluid blood or the coagulum—was resting.

This observed order in the occurrence of events serves to explain "the periods of repose" that are known to the surgeon. The fluid which oozes from the surface of a wound is at first blood tinged, but soon becomes pale, until at the end of a few hours the surface is covered with a whitish film. This film is a fibrinous network, containing within its meshes leucocytes in enormous numbers, and ever increasing as the first few hours pass by subsequent to the development of the film. "Such a calm continues from one day to eight, ten, or more, according to the nature and extent of the wounded part, and the general condition of the body." "The calm may be the brooding time for either good or evil; whilst it lasts the mode of union of the wound will in many cases be determined." "Moreover in open wounds the time at which on each tissue granulations are produced is determined by this calm; for they begin to be distinctly formed at its end[2]." The share which we think the white corpuscles have in the constructive process of repair will be evident from what we mention elsewhere in the paper. "Apparently they do not hinder it[3]." And previous to the advent of aseptic surgery it was believed by many that to leave the cut-surfaces of a wound exposed until they bore a whitish, glassy film, and not to put them into contact until then, was to give a condition favorable to union by primary adhesion.

Indeed, whatever view be adopted regarding the fibroblastic value of the leucocyte, certain other purposes which it may subserve in the process of repair were in our experiments extremely obvious. It was the pioneer of all the wandering swarm of cells that visited the intruding occupant of the tissue. Whatever causes, intrinsic or extraneous, guided its early voyaging, the route it traversed and the position it

[1] *Journ. Anat. and Phys.*, Vol. xxi., 1887. "In 6 hours scarcely a white corpuscle was to be found in a field of several hundred squares (instead of 4, 5, or 6 in every 100 squares) though the red cells were much increased in number."

[2] Paget, *Surgical Pathology*, Ed. iv. p. 151.

[3] *Ibid.* Paget.

assumed seemed to determine almost absolutely the course of after-coming plasma-cells that appeared in great measure to be simply followers along the track thus broken for them. Where the intruding body was of penetrable nature, as in the case of blood-coagulum, these leucocytes entered it in the van of a destroying army, that in turn attacked it from channels that leucocytes had prepared. By leucocytes the mass to be absorbed was in part previously divided up and made to offer a greater surface for absorption by plasma-cells. Where larger masses of clot are concerned cracks and fissures occur from chemical causes, as shown by one of us elsewhere (B.)[1], which in the same way allow of the entrance of the plasma-cells among whose functions in the clot mass are absorption and substitution. The filaments of fibrin when they were present appeared to direct to a certain extent the path travelled by the cells. Certainly to group themselves about the granular nodal points of the fibrinous network was quite characteristic of the distribution of the leucocytic swarm, and this directly influenced the formation of islets in the cellular membrane, the islanding being the direct outcome of the original grouping.

And leucocytes served also as a pabulum for the active plasma-cells[2]. Just as, in the extremely interesting observations given by M. Greenwood[3], little monads, Euglenae and Algae coexisting in the same water with Amoeba proteus were by it ingested, so leucocytes become the prey of the plasma-cell, and are by it included and ingested. And if the growth and proliferation of the plasma-cells be of importance in the

[1] Erasmus Wilson Lectures of the present year.

[2] The plasma-cells are considered by Metschnikoff to be among his group of "phagocytes." He writes: "Die weissen Blutkörperchen bilden einen allerdings ansehnlichen Theil aus der Summe der Phagocyten, indessen gehören zu diesen, wie ich in meinen sämmtlichen Arbeiten ausdrücklich erwähnt habe, auch amöboide Bindegewebs-zellen und manche andere zellige Elemente." *Virchow's Archiv*, Vol. cvii. p. 239, 1887. He proposes to call "grosse in der Regel mit einem einfachen (nicht gelappten) Kerne versehene Phagocyten," in which "der Kern ist rund oder häufiger oval," by the name Makrophagen, no matter what their origin may be. This in contradistinction to the Mikrophagen, "mit stark tingirbaren, zum grossen Theil gelappten oder fragmentirten Kernen und sehr blassem Protoplasma," which are for the most part, he affirms, leucocytes. The plasma-cells are therefore included in his Makrophagen. Cf. also Wyssokowitsch, *Koch's Zeitschrift*, Bd. i. Lief. 1, p. 39, 1886. Metschnikoff studied the phagocytic power of plasma-cells in subcutaneous tissue in cases of Erysipelas in the human subject. In some preparations we have obtained lately in the Ziegler chambers from an experiment in which the wound was allowed to suppurate, there are abundant instances of bacteria within the plasma-cells. Cf. also Hess, *Virch. Arch.* Bd. cxix. Hft. 3, on "Gland cells destroying bacilli."

[3] This *Journal*, Vol. vii. p. 253. Vol. viii. p. 263.

process of repair, what circumstance more propitious than the presence in abundance of nutriment so delicately adapted and so highly organized as the substance of the leucocytic cell? Of Amoeba and Actinosphaerium it was remarked that the food most suitable to these forms is unshielded non-coagulated proteid matter. A low degree of vitality, a diminished activity of its protoplasm, renders an organism easier prey, more readily captured and more readily absorbed. The plasma-cell may in some respects be taken as a hothouse variety of amoeba; it finds its unshielded non-coagulated proteid in the dead or dying leucocyte. It will be remembered that within the chambers the leucocytes revealed striking signs of lowered vitality.

Not that the number of instances in which we could detect an actually included or a partially ingested leucocyte would, we think, account for the large disappearance of them that does actually occur. Is it not probable that the plasma-cell can exert digestive action upon material which it does not incept? Suppose a proteolytic ferment secreted by the plasma-cell, and leucocytes that are dead or dying as in the above experiments; a gradual solution of their substance in the tissue plasma will occur, yielding to it an abundance of rich food for other cells that are in a thriving condition.

Passing in review the chief points observed in regard to plasma-cells, it became clear enough to us that in the study of their origin and development lies the best key to the problems of the formation of tissue of repair. We found them traceable up from forms of an amoeboid kind, different in many ways from the amoeboid cell-forms of blood and lymph, through individual types of almost endless diversity of figure with the utmost variety of combination and interdependence, onward finally to the fixed corpuscle of fusiform or of stellate shape imbedded in fibrillated material.

As to giant cells, often it was obvious that the large cell had resulted from a fusion more or less complete of the bodies of several smaller cells, the nuclei of which remained distributed regularly through the substance of the aggregate. In other instances a massing of the nuclei of the giant cell about one point appeared to denote a mode of origin from a single cell that had grown and undergone nuclear multiplication without actual separance of the daughter cells from the parent as they had been produced.

Again, by the union of cell with cell, by means of long pseudopodium-like processes, it was sometimes found that a whole field under the lens was occupied by the net-like ramifications of one huge multi-nucleated

cell—better described perhaps as an unbroken sheet of anastomosing cells. The characters of the giant cells in the implanted Ziegler-chambers resembled in this particular those of such giant cells as occur in marrow, growing bone, the splenic pulp, myeloid sarcoma, and in granulation tissue. In no cases did the arrangement of the nuclei in them bear resemblance to the ring-like or other regular disposition often seen in the giant cells of tubercle.

Upon the position of the giant cells depends partially the arrangement of the fibrillated tissue which is ultimately produced. The run of the bundles of fibrillae is often from and between giant cells. The cells range themselves previous to fibrillation in lines spreading for some distance from the giant cells; in fact in many ways the resemblance of giant cells to cell-islets is a close one. Just as in some cases, if not in all, the so-called giant cell is really but a congeries of smaller coherent cells, attracted to one and the same spot for the purpose of participation in a common prey, so is it with the cell-islets also. The groups of leucocytes from which the cell-islets arise appear to be originally formed under the common attraction which is offered to these cells by the albuminous *débris* present at the central nodes of the fibrinous network. Later, the leucocytes themselves becoming from some cause or another effete and of low vitality, exert a similar attraction upon the wandering plasma-cells, and afford to them a rich and easy quarry. By this arrival of fresh cells the islet is increased in bulk. The more centrally situated individuals feed upon the leucocytes they have surrounded, and the latter rapidly merge to an amorphous kernel for the entire mass.

The outlying cells become disposed along definite lines, and as it were sketch in in its main outlines the general plan which the adult arrangement of the new fibrous tissue will display.

The cell-islets are the centres of most active growth and proliferation in the young cellular tissue. They contain the stores of nutriment that are gradually dissolved and digested. They may contain also innutrient matters, and matters such as are not only innutrient but incapable of solution by the cells or plasma. At first the shape of those cells which are immediately next to the kernel of nutritious matter in the islet is irregular, and suggests amoeboid properties in the cell; later the cell becomes almost regularly fusiform, and is applied by its side to the material which gradually disappears. The material comes to be encircled by chains of fusiform cells set concentrically around it. It becomes encapsuled in the same way as is the ligature placed around an

artery by the surgeon, or as is any foreign body placed within a wound which heals around it. The fusiform fibroblasts slowly exert the same solvent action upon the imprisoned material as did their amoeboid ancestors. No doubt the more easily affected portions of the material are the first to go into solution and disappear, leaving a constantly less amenable residue and a less nutrient one; and perhaps it is in accordance with the decreasing supply of food from this source that the cells in contact with it undergo gradual change and lose their pristine elasticity of form. They assume the spindle-shape, and a fibrillated intercellular cement substance comes into existence between them. We have already seen reason to think that this "matrix" is a secretion from the cell. Prominent among the conditions under which the young fibroblasts begin to form it is, it would seem, a diminution in the amount of pabulum at hand to support growth. Much as amoeba under adverse conditions assumes an encysted form, so where food is scanty do the inherited tendencies of the fibroblast lead it into states of quietude and encystment. The less nutritious, the more inert the foreign body which the plasma-cells surround, the sooner do they become fixed cells, the earlier do they elongate, and make around themselves the bed of fibrillated matter, which commits them to immutability of form. In the same specimen in which plasma-cells preying upon remnants of blood-clot were still actively amoeboid, it often happened that around innutritious matter as hairs, and cotton fibres, the cells were already perfectly developed into young fibrous tissue.

When embedded in the fibrillated secreted substance all digestive and absorptive activities within the cell do not cease. Encapsulation does not arrest absorption. This has been shown by one of us (B.)[1]. It holds even in those instances in which the foreign substance is of such a nature as to resist digestion or chemical solution for a lengthened period. Carbolized cat-gut is quickly split up and destroyed by an environment of living tissue; kangaroo tendon, which is denser and more resistent, becomes encapsuled and continues to be gradually dissolved and absorbed after a dense fibrous investment has been produced around it; it may require a hundred days for complete disappearance. Prepared silk in like manner becomes encapsuled, but is finally absorbed in a period which sometimes is as lengthy as three years. Even silver is slowly destroyed. Gold and platinum appear able to resist indefinitely long.

[1] Erasmus Wilson Lectures of the present year.

Unfortunately it was only until the present experiments had been concluded and the present paper very nearly so that we were able to obtain copies of Professor Ziegler's monographs from the Würzburg Institute. We had been obliged to satisfy ourselves with the results of his work in abstracts of the original papers. As a matter of fact our work has not been a repetition of his quite to the extent we had imagined. A great part of our observations deal with periods which his do not touch or only slightly so. [1]His first communication is based upon observations on chambers implanted in sixteen dogs at thirty-six different intervals. But of these only five, upon four individual animals, refer to the first two weeks after implantation, and he records no observations prior to the seventh day. Of our observations with rabbits the major part refer to the first two weeks after implantation, and our earliest observations were made only four hours after implantation. We imagine too, judging from the beautiful illustrations to the original papers, that the cell-masses that we have so frequently referred to as cell-islets are included by him among the giant cells. It must be remembered also, that his experiments date prior to the acceptance of antiseptic surgery, and eleven times he records pus, either in the implanted chambers or in the wound. In no case did we ever find the slightest trace of pus, as we have said already.

It will have been seen that in most points our observations entirely confirm the original observations made by Ziegler. One particular there is however, and that one of fundamental importance, in which we are in disaccord with the descriptions furnished in his paper. As far as we observed, there are in the tissue-plasma of a part subjected to irritation such as that described in the experiments two kinds of cell. On the one hand there are present leucocytes indistinguishable from and probably identical with the colorless corpuscles of the blood; on the other hand are plasma corpuscles, cell-elements proper to the connective tissue of the part offended. The cell that plays as we incline to believe the only actively constructive *rôle* in all the energetic upbuilding of new tissue that goes forward in the part, is the plasma-cell, a corpuscle absolutely distinct from the colorless corpuscle of the blood. Our cover-glass preparations lead us to believe that these free cells in small number exist in the tissue plasma even under normal circumstances. Where the connective tissue corpuscles are proliferating, as for instance within an inflamed area, there these free

[1] Ziegler's second communication deals entirely with "die Schicksale der eingewanderten, zum Theil bereits veränderten Zellen von dem 25. bis 70. Tage."

tissue-cells are enormously more numerous. In our experiments, out of them arose the permanent membranes, to be designated inflammatory if the ordinary unsatisfactory use of the term be sufficient, which spread themselves over and inside Ziegler's chambers when lying in a subcutaneous space or in the peritoneal sac—membranes composed at first of cells entirely similar to the corpuscles of the normal tissue-plasma. Colorless blood-cells doubtless wandered in the surrounding of the chamber, and doubtless entered in plenty the space within it. But these leucocytes had no permanence of possession. The fibroblast of the new tissue was not of leucocytic origin. Our observations yield no support to Cohnheim's view of the genesis of cicatricial tissue from leucocytes.

When pus is formed in an inflamed focus many of the migrated colorless corpuscles of the blood become, as is well known, pus-corpuscles. It does not appear strange that where pus is not produced those of the swarm of leucocytes, which do not drain off by lymphatic channels from the tissue they have temporarily invaded but remain behind, should not thrive within it. Many circumstances might, we conceive, render their sojourn perilous. The high carbonic acid tension, the comparatively stagnant character of the fluid, the presence of, to them, unwonted chemical bodies, and of others in unwonted percentages —these are instances of conditions which might, we conceive, constitute an environment of disadvantage. And that the migrated leucocytes should rapidly be not merely acclimatized in the new locality, but should actually become fixed elements of the part, and generate the cells of a fibrous tissue is to our minds improbable. The cells of fibrous tissue and the colorless corpuscles of the blood are both of mesoblastic origin, but we have no evidence that they are more nearly related one to another than are the fibres of a striated muscle to the endothelial cells of an artery. No one advances the view that of these latter one can by any means be made to reproduce the other. Even in tumours with their apparent departure from the normal type of growth the principle of heredity is in reality religiously obeyed. "The secondary growths in carcinoma are identical with the primary, for it is the epithelial element which is infected, and it is this element which determines in the normal process of development the general anatomy of the parts around, be they glandular or otherwise. So a columnar celled carcinoma of the rectum produces in its metastatic growths intestinal crypts in the liver; a thyroid cancer produces in its secondary tumours thyroid tissue in the bones; an osteoid sarcoma shows in its

secondary manifestations osteoid tissue; and it might even be conjectured that if the epithelium over the papilla of a hair received the carcinomatous infection hair-like structures would be found in the primary and secondary tumours[1]." In the production of scar-tissue it seems to us of transcendent significance that such tissue is characterised by the possession of cells of which each tends to secrete a collaginous capsule for itself, so that around the cells a more or less solid and fibrillated intercellular matrix comes to be characteristic. Cells with a similar tendency characterise broadly the connective tissues wherever found. It is in accordance to laws of natural descent for the cells of connective tissue, when thrown into renewed and extraordinary genetic activity in what is termed plastic inflammation, to produce a progeny of cells possessed of the same tendencies as themselves. And among all these tendencies which one is more unfailingly repeated in them than to mould a semi-solid fibrillated collaginous capsule, in short, to build up fibrous tissue? But the cells of the blood nowhere show signs of any such propensity. The colorless corpuscle of the blood is conspicuously endowed with a character apparently opposed to, even incompatible with, the formation of a semi-solid circum-cellular test—the whole story of its normal life so far as we know that, is associated to one continuous flux of form.

The term "inflammation" is at present employed to signify a number of phenomena of which some are not only widely dissimilar one from another, but are even not necessarily associated. John Hunter recognized the importance of distinguishing two classes of injuries to tissue, in consequence of the radical differences between the resulting processes of metabolism set up by them in the tissue and comprehended within the one term inflammation. He says[2], "The injuries done to sound parts I shall divide into two sorts, according to the effect of the accident. The injuries of the first division in which the parts do not communicate externally seldom inflame; while those of the second which have an external communication commonly both inflame and suppurate." Modern surgery[3] has shown how right Hunter was. Would it not be well to designate by separate titles the parts of the inflammatory process due to the plasma-cell of the tissue, and to the migrated colorless corpuscle of the blood respectively? By

[1] See a discussion of this question, *Path. Soc. Trans.* Vol. xxxviii. pp. 423 and 424, 1887. Ballance and Shattock, "Cultivation experiments with Cancer."

[2] Quoted from Paget's *Surgical Pathology*, Ed. 4, p. 131.

[3] *Ibid.* p. 130.

such a terminology the destructive and suppurative processes of inflammation would be sundered from the constructive and reproductive. This would obviously be of advantage, if, as would appear likely, pathology is to teach that pus is an adjunct of the inflammatory process only when the irritation produced within the tissue is aggravated by the associated presence of a sufficient dose of bacterial virus.

REFERENCES TO PLATES.

Plate XXXI.

Fig. 1. Contents of experimental chamber that had remained 72 hours in the peritoneal cavity of the rabbit. Five large amoeboid plasma-cells, with altered red corpuscles and apparently dead leucocytes. Outlined with camera lucida. Apochromatic oil immersion and ocular No. 4. Zeiss. Prepared over osmic vapour.

Fig. 2. Contents of a chamber for 18 hours in the peritoneal cavity (rabbit); near the centre of the chamber. Fibrin filaments, leucocytes, red corpuscles, and an ill-defined granular mass forming a nodal point in the fibrinous network—the beginning of a "cell-islet." Outlined under camera. Similar method of preparation, and similar magnification to preceding.

Fig. 3. Fragment of inflammatory membrane formed within a chamber placed for three days in the subcutaneous tissue (guinea-pig). Islets and groups of islets scattered through the membrane. Zeiss, Obj. A, Oc. 2. Osmic acid solution, and Ehrlich's logwood.

Fig. 4. Contents of same chamber as in Fig 1. Close to the opening of the chamber. Five plasma-cells, one of them continuing a leucocyte within a large vacuole. Magnification and method of preparation as in Fig. 1.

Fig. 5. Contents of same chamber. Two plasma-cells and two red corpuscles; the plasma-cells are indistinguishably united with fine filaments of fibrin in their surrounding, some of which are given in the figure. Osmic acid vapour. Zeiss, apochr. system, oc. No. 2.

Plate XXXII.

Fig. 6. Giant cells from chamber 72 hours in the peritoneal cavity (rabbit). Zeiss apochr. system, ocul. 5. Osmic acid vapour.

Fig. 7. Plasma-cells from same preparation which furnished Fig. 6.

Fig. 8. "Cell islet" from inflammatory film obtained in a chamber left eight days in the subcutaneous tissue of the guinea-pig, At the margin it is united to outlying plasma-cells. Zeiss oil, oc. 4. Osmic acid vapour.

Fig. 9. Young cicatricial tissue of anastomosing branched cells, some of which are represented under the higher magnification in Fig. 17. From a thrombosed artery (syphilis) near the centre of the thrombus. Zeiss A, oc. 3. Logwood. Preparation kindly shown us by Dr Seymour Sharkey.

Fig. 10. "Cell islet" from inflammatory membrane obtained from chamber five days in the peritoneal cavity of the rabbit. Osmic acid solution. Zeiss oil imm. and oc. 2.

Fig. 11. Mass of blood-cells (? clot) surrounded by fibroblastic cells, and invaded by them at four places. Inflammatory membrane from chamber eight days in subcutaneous tissue. Magnification as in preceding, and prepared in similar manner.

Fig. 12. Fusiform plasma-cell (fibroblast) surrounded by a fibrillated material which forms a thread-like band of connective tissue. Zeiss oil and oc. 4. Osmic vapour. From chamber 10 days in subcutaneous tissue.

Fig. 13. Similar but larger and thicker fibrous band from same preparation. Similar preparation and magnification.

Plate XXXIII.

Fig. 14. From chamber five days in subcutaneous tissue. Plasma-cells adhering to a cotton-fibre. Osmic vapour and carmine. Zeiss apochr. oil and oc. 4.

Fig. 15. From chamber eight days in subcutaneous tissue. Plasma-cells adhering to a hair, which had accidentally been allowed to get into the wound. Zeiss obj. D, oc. 2. Osmic acid solution and haematoxylin.

Fig. 16. Inflammatory membrane from chamber eight days in the abdominal cavity; taken from a tenuous portion of the membrane. Four fibroblasts, in a film which is composed of an extremely irregularly arranged network of filaments resembling fine fibrin threads. The processes from the cell-body are continuous apparently with the fibrils of the matrix. Osmic acid vapour and haematoxylin. Zeiss apochr. oil imm. and oc. 4. Outlined with camera lucida.

Fig. 17. Stellate fibroblasts and two leucocytes from same preparation as Fig. 9, more highly magnified. Zeiss apochr. oil and oc. 4. Outlined with camera lucida.

Fig. 18. The modified Ziegler chamber; the sketch shows the actual size employed.

Fig. 19. Portion of the chamber seen edgewise, showing the opening between the cover-glasses. Enlarged 12 times.

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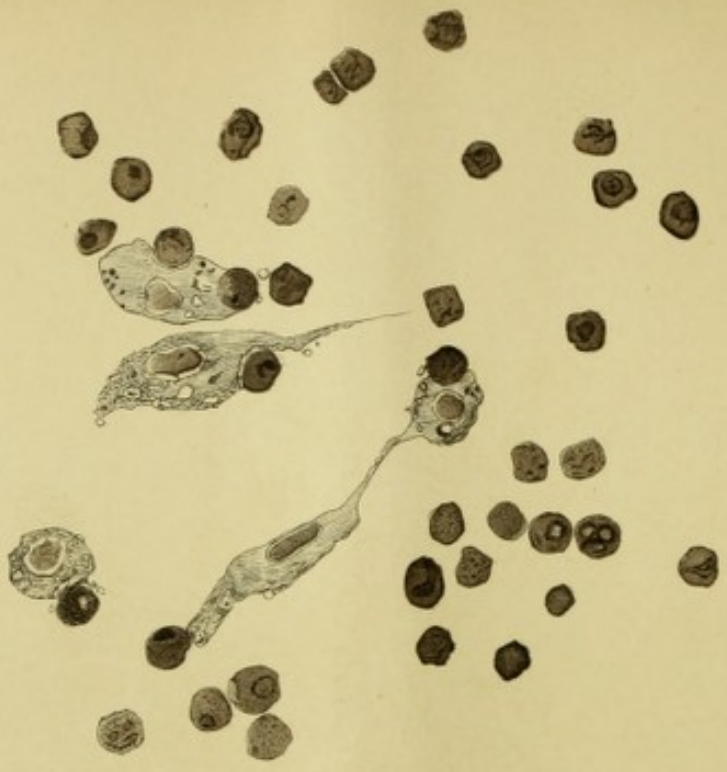

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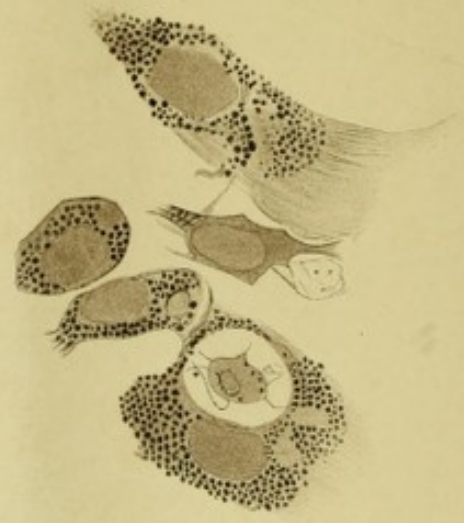

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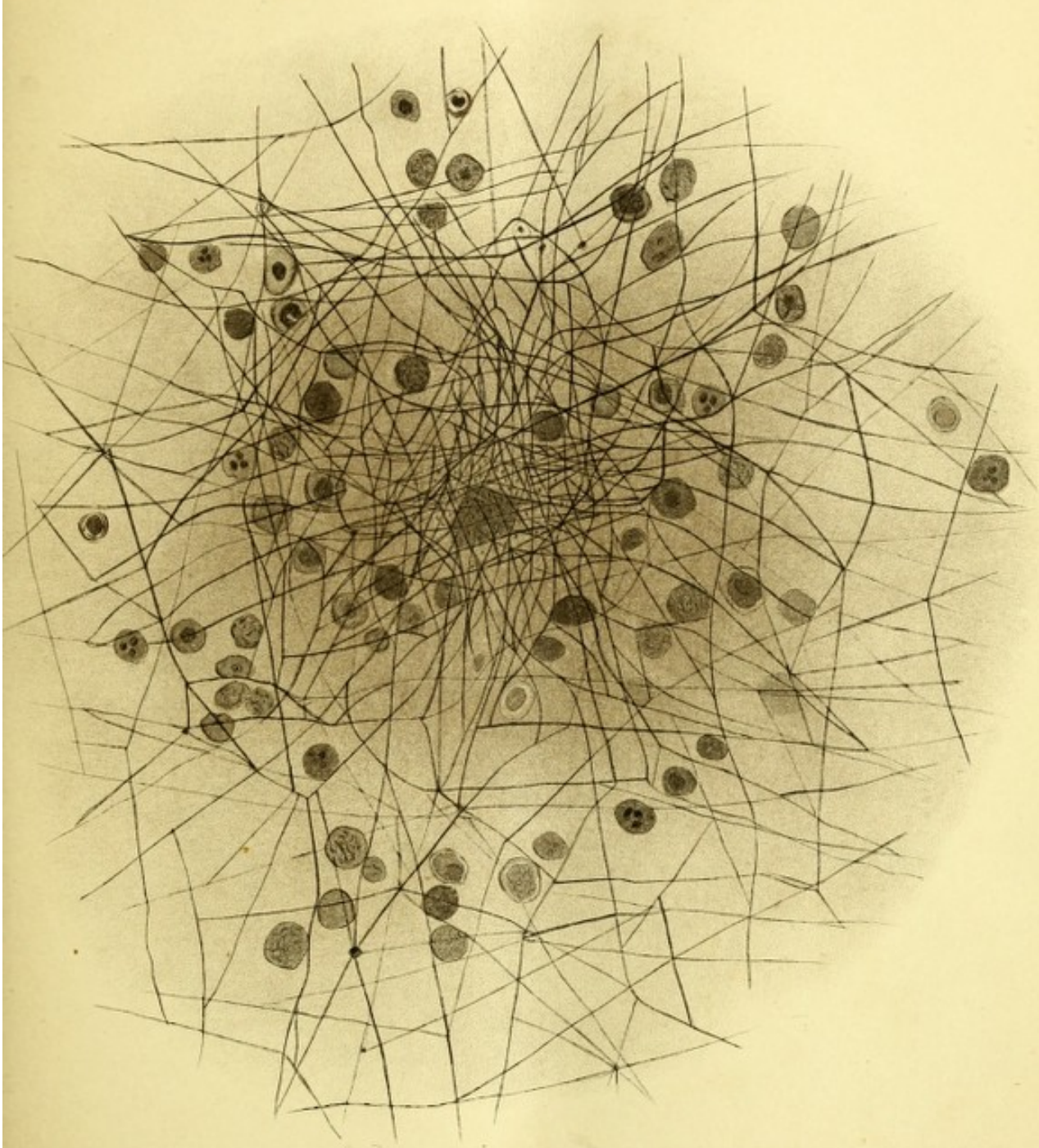

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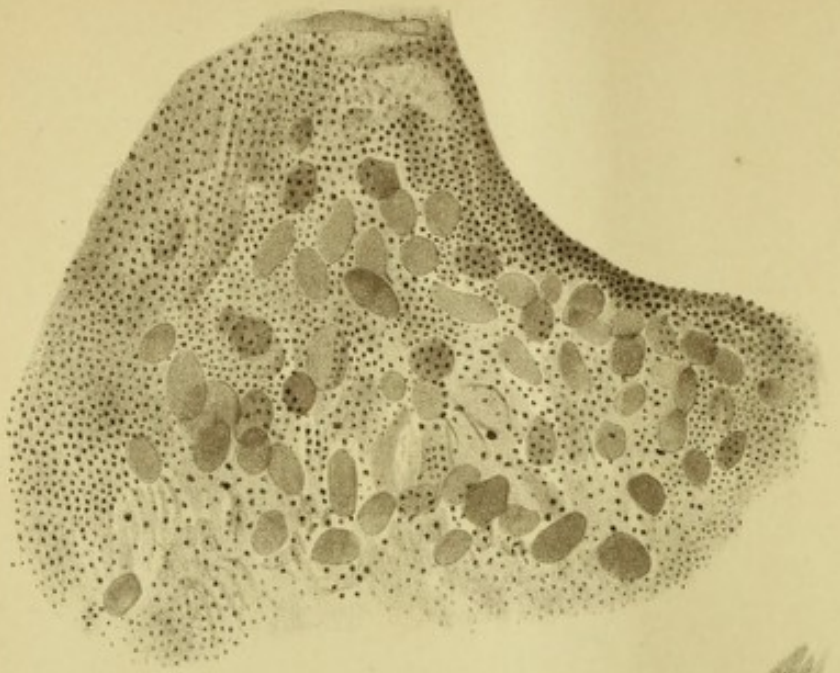

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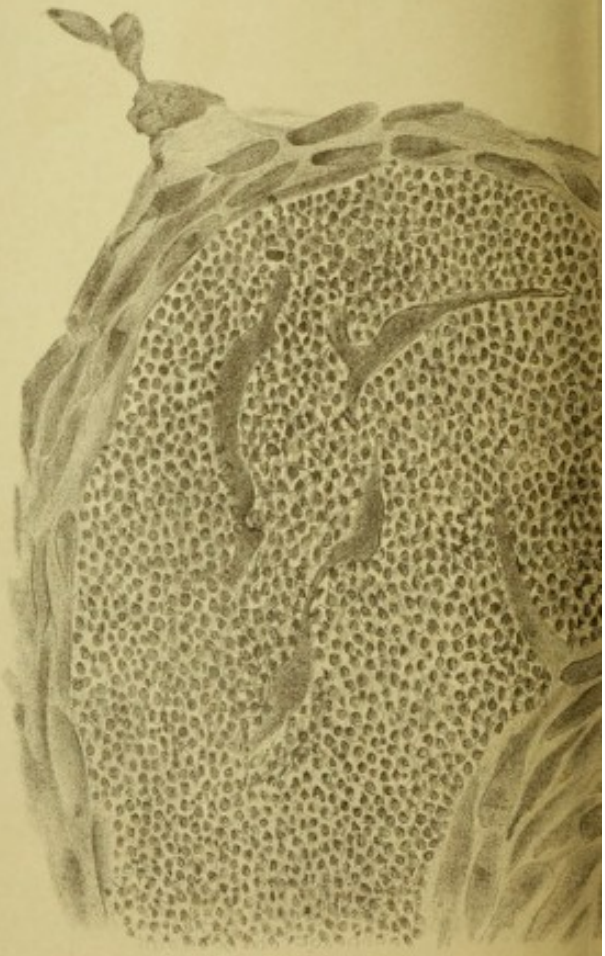

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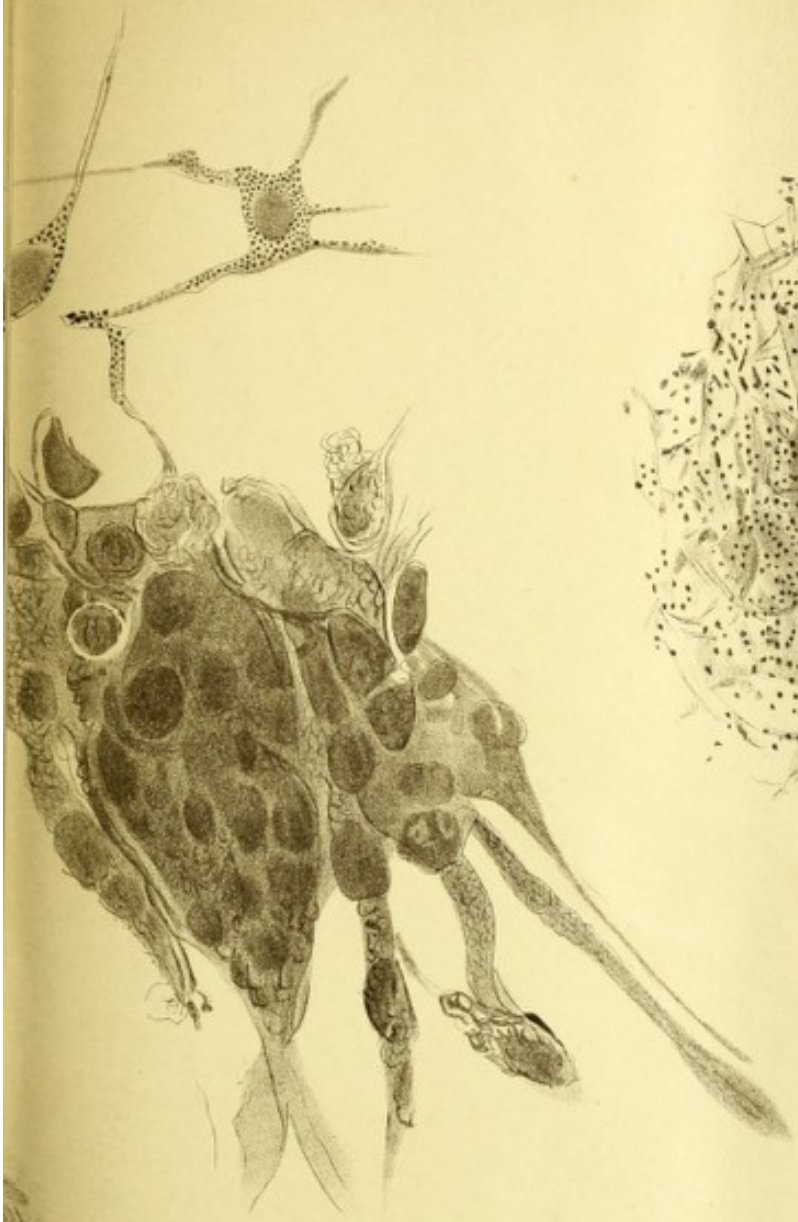

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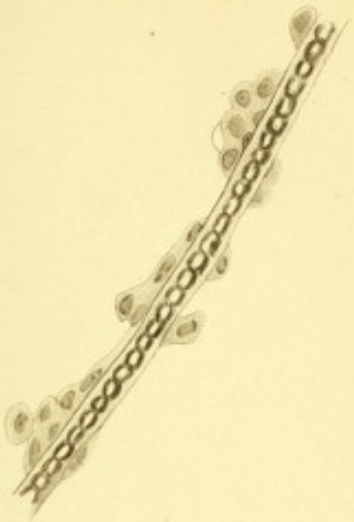

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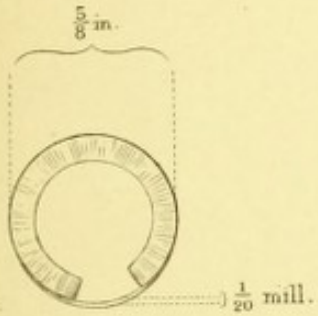
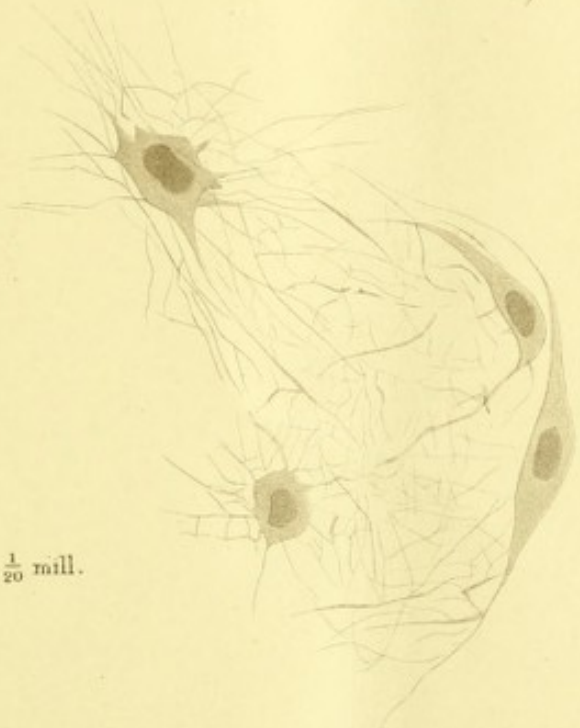
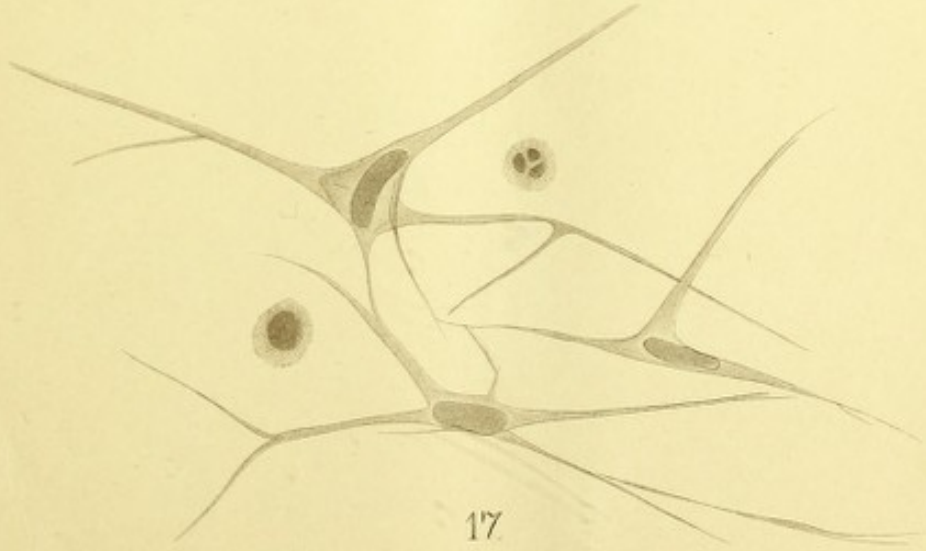


18



16.

19



17.