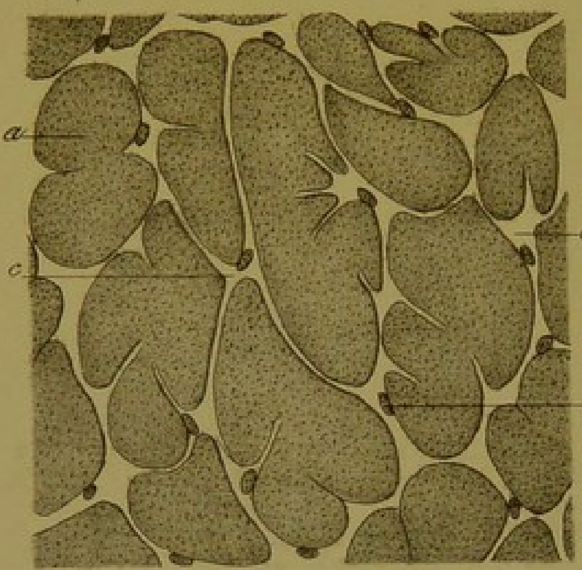

Fig. 1.

Fig. 2

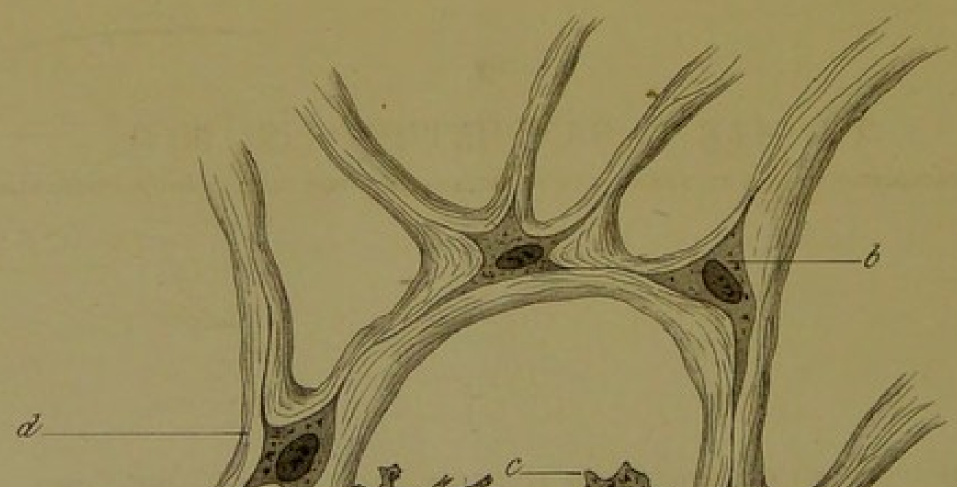

Fig. 4.

T. E. Satterthwaite, Del.

J. Maisonneuve, Lith.

ON THE

STRUCTURE AND DEVELOPMENT

OF

CONNECTIVE SUBSTANCES.

BY

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with the view of determining some of the more important facts that have been matters of controversy.

The name *connective substances* has been adopted because it is already in use by leading histologists, and because it is an extremely convenient word under which to group together a large number of animal substances that have a very close relation with one another. The name was first proposed by Reichert in 1845, and embraces *bone*, *cartilage*, *dentine*, and the delicate forms called *mucous tissue*, *adenoid tissue*, *neuroglia*, *fat-tissue*, *fibrillated connective tissue* (*fibrous tissue*), *intermuscular tissue*, *corneal tissue*, *tendon tissue*, and *elastic tissue*.

The general reasons for classifying these substances separately may be stated as follows: They are all said to be derived from the middle germinal layer of the embryo;[1] one form in one animal is often substituted for another form in another animal, so that fibrous tissue or cartilage in one animal will perform the same function as bone in another, and they have in this way come to be regarded as equivalents of one another in a morphological sense; again, it is quite apparent that one form often succeeds another form in the natural life of the body, fibrous tissue or cartilage of youth being transformed into bone in the adult. In the growths of tumors, these changes are frequently seen.

The word *connective tissue*, originally proposed by Johannes Müller, as distinguished from *connective substance*, has also sometimes been applied to one or more members of the same class, and, indeed, it is in this way that much confusion has been produced, for, while some observers have used the word in the broad sense of connective substance, others have limited it to some specific form, such as fibrous tissue (fibrillated connective tissue).

To avoid any such source of error, we shall call each form by its distinctive name, as mucous tissue, adenoid tissue, and the like, and then we shall find that, though there is a strong bond of relation between all the forms, they (many of them) show as distinctive differences as any other tissues in the body.

[1] The sustentacular tissue of the brain and cord is thought to be an exception to this. Frey's "Histology," p. 196.

The word *connective tissue* will accordingly be avoided entirely except where its character is specifically described, as when using the expression "fibrillated connective tissue," or "connective tissue of the kidney," etc. We may then expect to get more precise notions of the minute structure of each variety, and so of the peculiar relations they each hold to pathological change.

The three that stand at the head of the list, viz.: *bone*, *cartilage*, and *dentine*, are in many respects better understood than the others, chiefly because in gross appearance they show distinctive differences and because their anatomical elements have been more easy to isolate. The consideration of them, however, does not come within the range of the present work, and no further mention of them will therefore be made.

Our knowledge of connective substances dates from a comparatively recent period, for the first systematic efforts to determine their minute structure appear to have been made by Schwann in 1839. Since that time the doctrines in these matters have undergone important modifications, and it will be essential to consider the more important of them before we can get a clear conception of the views which are now entertained.

Schwann was the first to point out in these tissues certain bodies that he called spindle-shaped or caudate cells. The word "cell" is here used by Schwann in speaking of the variously-shaped fixed cells, as distinguished on the one hand from the wandering cells which are now called leucocytes or lymphoid corpuscles, and on the other from the intercellular substance. The word "cell" seems to have originated so much trouble that it would be desirable to avoid it entirely; but this is impossible from the very general use that is made of it. It will, however, be restricted in what follows to the fixed corpuscles of the parts. The difficulty in the use of the word "cell" has been, that observers have frequently, as we shall see, mistaken bundles of fibres for corpuscles, and because they are not agreed as to what properties belong to a cell. It has seemed better, therefore, to offer a description of appearances as they were noted during these studies, from which we may subsequently decide whether or not they are to be called "cells."

It is proper to state that, previous to Schwann's discovery, it was supposed that all connective tissues, by which were meant *connective substances* in general, were made up of fibres, though even this had been denied by Reichert, who insisted that there were no fibres at all, but the apparent fibres were simply foldings of the substance. A new impetus to these questions was given by Virchow ("Cellular Pathology," 1871, pp. 69–73 and 131), who at first opposed Reichert, maintaining that spindle-shaped or caudate cells did exist, and that in most cases the cells maintained their integrity, and consequently the connective tissue of early and late periods did not differ in general structure; the cells remained the same though they were not so easy to detect. He further stated that the connective tissues (connective substances) could not be distinguished by the character of their cells, for, in all connective tissue, round, angular, and long cells might occur; he also believed the cells were hollow and their processes hollow, constituting channels by which nutritive juices could be conveyed from place to place, being in fact like the lacunæ and the canaliculi of bone.

These views, however, he was obliged to modify at a later period.

Henle opposed Virchow's idea of connective-tissue corpuscles in certain particulars, especially in tendon tissue, and maintained that what seemed on cross-section to be cells, were merely spaces between the bundles, in which were nuclei and elastic fibres (*Müller's Archiv*, 1852, p. 92). This statement was based upon a method he had of injecting the interspaces.

The figures that were regarded by Virchow as the stellate cells were, in reality, the angular spaces (Henle's spaces, Figure 1, *c*) between three or more bundles, and they contained either a cross-section of an elastic fibre, or more probably, perhaps, the profile view of a connective-tissue corpuscle (*b*). As tendons contain but little elastic tissue, and the cross-sections of such a fibre would be extremely small, the latter view is probably the correct one.

Henle at an earlier period had described as cells of this tissue, bodies that were like little plates, arranged in rows

(*Canstatt's Jahresbericht*, 1851, p. 23). He undoubtedly was one of the first to get a correct conception of the real nature of these bodies. Later, Rollett also expressed somewhat similar views (*Henle and Pfeufer's Zeitschr.*, 1859).

Subsequently, great advances were made in these studies, at first by the use of acetic acid which rendered the nuclei visible, and later, by the discovery of certain reagents which differentiated the elements even more strongly, and also by the application of certain fluids, such as Müller's fluid, which separated the bundles into their components, the fibrils.

Ranvier has really had most of the credit for directing the attention of histologists to the plate-like corpuscles, though, as we have seen, Henle had already mentioned them and Ranvier himself credits him with their discovery (*Archives de Phys.*, 1869, II., p. 471).

Billroth also described them in 1858. Ranvier, however, gave the most distinct statement that had been made of the relations the corpuscles bore to the fibres. He said that these plate-like bodies formed a sort of investing sheath about the bundles, and so constituted hollow cylinders, something like drain pipes, the plate-like bodies themselves being held together by a firm, cementing substance. In some cases, however, these plates were not firmly united together in rows, but had considerable spaces between them, forming open or incomplete tubes. He stated, in fine, that connective tissue (by which, however, it is not clear exactly what varieties he meant it to include) was formed essentially of bundles of fibres, of elastic tissue, and of cells, and the bundles were cylindrical.

There were only two kinds of cells, one kind flat, containing granular protoplasm and nuclei, and having irregular outlines and prolongations; the other round and having nuclei, and not to be distinguished from white blood-globules.

Among the comparatively recent studies are those of Lœwe. This author has thrown a great deal of light upon the subject of tendons, especially upon their sheaths, which he believes are lined with endothelial cells, and constitute passages for the flow of lymph. He states also, that the tendon bundles are covered with a continuous and closed sheath which is made up of the plate-like cells embedded in an amorphous elastic ground substance, and that the bundles present the same characters for great distances.

These corpuscles, "Ranvier's cells," are also covered by another layer which he calls the sub-endothelial layer, and which can be distinctly demonstrated by what is known as the silver method (*Medicinische Jahrbücher*, iii., and iv., 1874). The subject of these endothelial bodies is now attracting the attention of histologists, and promises valuable results. The views of later writers have so far agreed, that they have come to regard the fixed corpuscles of these substances not as spindle-shaped, but rather as thin, delicate, and plate-like. This view has, however, been attacked by Waldeyer as a generalization that has been carried too far. He believes that the corpuscles or so-called plate-like cells of tendon tissue and fibrous membranes are not simple, but complicated structures, and not single plates, but rather a number of plates meeting one another at different angles. The extremities of these plates terminate in fine processes that *often* anastomose with corresponding processes of other corpuscles; the nucleus is found on one of these plates. As for the corneal corpuscles, which have been so much discussed, he believes they are plate-like bodies, which are provided with distinct protoplasm about the nucleus, the amount diminishing toward the periphery, but in general characteristics do not differ much from the corpuscles of tendons and other fibrous tissues. The nuclei are difficult to make out, and are sometimes round, sometimes elongated like narrow rods, and sometimes are knobbed at each end, sometimes crescentic, and sometimes cruciform, though generally oval (*Archiv. f. mikrosk. Anatomie*, i., 1875, p. 176).

It may be regarded as a fair statement of the case, if we say that most histologists believe that these tissues, generally, though we shall except from them elastic tissue, consist of certain fixed corpuscles of a plate-like form superimposed upon bundles of fibrils of indefinite length. As further exceptions to this may be mentioned, mucous tissue proper, in which there are no bundles; perhaps also, adenoid tissue, for it is said by Klein to be made up of netted cells, without bundles or fibres; so, too, it does not appear that the statement has ever been made that the neuroglia contains these peculiar bodies. The intermuscular tissue of the frog's thigh is also regarded by many as having no fibrils excepting those of elastic tissue.

Thus we see that excepting only in the character of the corpuscles there is not much agreement among observers, and even on this point there is difference of opinion.

It has seemed impossible to get a clear idea of these matters in any other way than by a systematic study of each and every one of the forms, subjecting them as nearly as possible to the same method of examination. This accordingly has been done, and the main inquiries have been directed toward —1. The general character and dimensions of the corpuscles in each; 2. Their relations to one another; 3. The character of the intercellular substance. It is believed that the use of several new methods which do not appear to have been previously used in investigating the connective substances has helped to throw light upon these obscure subjects. The consideration of each tissue will be taken up in the order in which it has been tabulated. Some observations on *development of connective substances* will then follow.

1. *Mucous Tissue.*—It is well known that this substance is seen to great advantage in the umbilical cord of the embryo, and the following method has been found best suited to demonstrate it. Take a small piece of cord at about the third month and immerse it a few weeks in Müller's fluid; make a thin section through the very soft gelatinous part, then immerse it a few minutes in distilled water to which subsequently a few drops of acetic acid are to be added, so that the solution shall not contain more than one per cent. of acid, and then mount in glycerine. It will then be seen that the softest portion contains numbers of irregularly-shaped flattened plates, some containing an oval, flattened nucleus, others having none that are apparent (Figure 2). Some of these flattened bodies anastomose by these processes with those of other plates; others are quite free. The substance lying between the cells, the intercellular substance, is in the softest portions quite homogeneous or slightly granular, and has no

marks of fibrillation. In the neighborhood of the firmer tissue, lines of fibrillation occur, while at the same time these flattened bodies become smaller, though they are still flat.[1] The intercellular substance is distinguished by its chemical reaction, which distinguishes it from other albuminoid substances. It differs from albumen in not containing sulphur, from chondrin and gelatin in not being precipitated by boiling, tannin or the bichloride of mercury.

The corpuscles appear to consist of an oval, flattened central body, about which there is an extremely delicate and pale envelope, that may or may not be connected with other similar bodies. These delicate bodies are smaller the nearer they are found to the firmer or fibrillated tissue, while as they diminish in size there appear under them certain areas of intercellular substance having the form of elongated and flattened bands, which seen in profile give to the whole the appearance of a spindle cell of which the flattened body is the nucleus (Figure 3, *a*). That this is an illusion, however, may be judged from the fact that the flattened band will often be found to show the marks of fibrillation and the flattened body may be seen to be simply superimposed *on* the band and not *in* it, for, by carefully brushing these tissues with a camel's-hair brush after the prolonged use of Müller's fluid as above mentioned and the subsequent immersion in a solution of common salt (ten per cent.), the bodies may often be brushed away (*b*). Teasing of the tissue will often show isolated bands of more or less fibrillated tissue and having no central body that can be seen, even with the use of strong staining solutions; these evidences, therefore, seem to show pretty conclusively that such bands are not the bodies of the cell, as often stated, but rather portions of the intercellular substance in which fibrillation is commencing. About the flattened body will also be seen the remains of the envelope, either as a delicate film about it or in the form of irregular processes, projecting in various directions. According to this view of the case, therefore, the original flattened body or "nucleus" is at first surrounded by a delicate envelope, "the body of the cell;" the former undergoes compara-

[1] These points in the intercellular substance are not well shown in the lithographic plates.

tively little change while the latter may almost entirely disappear. The fibrillation, however, appears first in the intercellular substance, the flattened corpuscle apparently never taking any part in it. As the tissue becomes more fibrillated and consequently firmer, the little plates diminish in size and are further apart.

2. *Fibrous Tissue* (Figure 4).—This substance, which is also known as fibrillated connective tissue, occurs either in parallel bundles or in networks. The latter variety may be shown exceedingly well in the umbilical cord of an infant at birth. If the same method is pursued as in the former case, excepting that a cut be made through the spongy portion of the cord, the following appearances will be noticed.

It will be seen that the tissue is composed of bright, shining, branching bundles (*d*), superimposed upon which are a number of oval flattened plates (*a*) at intervals; about them is a delicate envelope (*b*), which appears to be highly elastic, so that it will stretch or relax according as the networks are compressed or dilated. By teasing with needles or immersion for a few days in a ten-per-cent. watery solution of common salt, these corpuscles can often be separated from the bundles, and then they will be seen to form a connected system. When entirely isolated from one another, they often appear spindle shaped. That this is not their character may be shown by *passing a current of fluid through the specimen*, which is done by the simple method of irrigation; that is, having affixed small strips of filter paper to the edges of the cover, and moistened one side with fluid, the excess will be absorbed by the other slip, causing a current by which the corpuscles may be made to roll over. We then learn that they are disks of an irregularly flattened form, having longer or shorter processes (*c c*)—variations in form which seem to depend in a great measure upon the tension to which they are exposed, and the position they occupy in the tissue. This explanation will serve to show why all measurements of such corpuscles are merely approximative, and have but little value. The nucleus may be regarded as an exception, for it seems, in fresh specimens, when the substance has been swollen by immersion in water, to be oval and flattened in whatever position it is placed. The bundles upon which these bodies are placed are cylindrical in form, branched, and composed of separate filaments, which can be separated by Müller's fluid, or a ten-per-cent. watery solution of common salt. Two other forms of corpuscles may also be noticed; the kind observed by Waldeyer (*loc. cit.*), and thought by him to be those that take up fat to make fat tissue, bodies four or five times the size of a lymphoid corpuscle, and rounded in form, containing a central body; and the ordinary lymphoid corpuscles seen at times in all tissues.

The form of fibrous tissue that occurs in parallel bundles is well shown in the mesentery of the frog, and in serous membranes generally. No great difficulty will be met with in preparing this tissue, for it is only necessary to remove it from the frog in the fresh state, acidulate it in a weak (one-per-cent.) watery solution of acetic acid, and mount it in glycerine.

It will be seen that these so-called spindle cells are really flattened plates when viewed flatwise, and generally of an irregularly quadrilateral form, though the form varies somewhat in each instance (Figure 5). [What relation these corpuscles bear to the interfascicular lymph-spaces described by Klein was not determined, as the silver method was not used. The bodies here described correspond very closely with those figured by this author, who regards them as standing in the radicles of the lymphatic system. "Anatomy of the Lymphatic System," II., p. 7.]

3. *Adenoid Tissue* (Figure 6).—Adenoid tissue is the name given to the delicate substance that forms the framework of the lymphatic glands. It consists of networks of fibres forming an intricate meshwork, that is filled with the rounded bodies commonly known as lymphoid cells. It is exceedingly difficult to analyze these tissues, owing to the fact that, with the exception of the lymphoid corpuscles, it is often hard to make out anything that conveys to the eye the idea of a cell body in the usual sense of the term. The best mode of procedure was found to be the following: Take a lymphatic gland that is in the early stage of inflammation, as an inguinal gland, for instance; harden it at first in Müller's fluid, and then in alcohol, and make sections through it.

On viewing such a section with the microscope, it will be seen that it is formed of a delicate meshwork containing numbers of lymphoid corpuscles (*a*). By taking such a thin section and agitating it in a test-tube with water for a considerable length of time, and then placing it upon a glass slide and brushing it with a camel's-hair brush, most of the lymphoid cells will be removed, and the delicate network will be more thoroughly exposed. It will be seen that, at certain parts in this meshwork, there are flattened bodies (*b*) of small size lying upon the larger parts of the meshes. It is held by Klein and other histologists that these are branching corpuscles; but it is by no means clear that this is always the case. In some instances this appearance is well seen in those portions of the glands that are regarded as the lymph passages, where the adenoid tissue forms the framework of the part. These fibres are extremely delicate, like fine silken cords, forming meshes which inclose vast numbers of lymphoid corpuscles, and appear to exhibit corpuscular bodies at the nodal points of the meshes. These delicate fibres, however, are often replaced by heavy cords (*c*), such as are seen in the drawing; and after continual inflammations the diameter of the cords may be found to be greater than that of the spaces. In these latter cases, it is often difficult to find any corpuscular elements that may not be separated from the fibres; and, indeed, large areas of these fibrous networks may, by diligent brushing with a camel's-hair brush, be swept clean of corpuscles. But neither this rough method, nor agitation in a test-tube, will always succeed in separating the corpuscles from the fibres, even after an immersion in common-salt solution for many weeks. I do not, therefore, feel quite satisfied in thinking that adenoid tissue does not consist of branched corpuscles; but it is quite clear that the so-called networks of cells are at times replaced by networks made up of branching bundles of fibres, and in which the corpuscles play a minor part. Whether in such cases the bundles are made by the splitting up of the corpuscles, or, on the other hand, they are formed about the corpuscles, I do not feel prepared to decide. In my individual opinion, I must incline to the latter view as more in accordance with the appearances that are seen in the growth of reticular tissue,[1] as I have had an opportunity of studying it in the umbilical cord. Where the fibrous networks have attained some thickness, there it seems that we find the ordinary flattened connective-tissue plates lying on the bundles, and surrounded by a delicate envelope in some cases.

It is not inconsistent with this theory that some, at least, of these lymphoid corpuscles may originate from the flattened corpuscles of the adenoid tissue, for it appears sometimes as if this production of the corpuscles could really be seen.

4. *Neuroglia* or *Bind-web* [Seguin], (Figure 7).—But a short time since, it was not known positively whether the delicate cementing substance of the nervous system, but more especially of the brain, was granular or fibrous. Even after Virchow claimed that this substance was like the other tissues known as connective, doubt was still thrown upon the matter, for the defining power of the objectives then used was often insufficient to make out these delicate objects. At the present time the actual existence of such a delicate network is hardly called in question, for it may be demonstrated with really good glasses, such as some of the immersion lenses (No. 10) of Hartnack's system. As to the question of the corpuscular elements there is, even now, some question, and it can hardly be regarded that their exact form and shape have been definitely agreed upon by histologists. We find, it is true, that, where there is considerable development of connective material along the central canal of the spinal cord, there we have the ordinary fibres and corpuscles already described, and so, too, near the surface of the convolutions. When, however, we examine the supporting substance of the white and gray masses, it is more doubtful as to the character of the delicate tissue we meet with. The real condition may be tolerably well seen by adopting the following plan. Place any portions of the brain or cord in a weak solution of bichromate of potash (five per cent.) or Müller's fluid for a few days, and then immerse it in alcohol until hard, and make thin sections, which stain in the following solution of hæmatoxylin for twenty-four hours:

Hæmatoxylini,	gr. lii.	
Aluminis,	℥j.	
Aquæ,	℥ viij.	M. and strain.

[1] *See* Figure 4.

Wash in distilled water and mount in glycerine, tease with needles and examine with a high power; there will then be no difficulty in seeing that the delicate supporting substance of both gray and white matter consists of fibres. They may even be distinctly isolated, for the coloring matter darkens them somewhat, and they become hardened at the same time, so as to be somewhat stiff and unyielding. Then it will be seen that many fibrils are disposed in parallel rows which, perhaps, can hardly be called bundles, but rather thin laminæ; other similar fibrils cross them at various angles, giving to the whole, with a moderately high power, the appearance of a very delicate meshwork (*a*). It does not appear as if the fibrillæ anastomose with one another, though this point would be extremely difficult to settle. It must be stated that possibly some of these fibrils may be nerve elements, though this seems doubtful, because they do not even seem to be connected with the nerve fibres that are distinctly shown by this method of preparation. In the drawing they are not represented, to avoid confusion. Of course, granular appearances are always noted in the brain, but this must be the case when cross-sections are made of the delicate fibrillæ. There are three kinds of corpuscles met with in the brain and medulla. The first are the variously-shaped ganglionic corpuscles or cells (*b b b*), then the ordinary lymphoid cells (*c c*), which here are generally seen to have a pale envelope about them; lastly, smaller corpuscles (*d d*) of irregular shapes, and many of them undoubtedly flattened and appearing to have branching processes (*d*). They may be found in considerable numbers, and can be isolated, so that there is no doubt that they exist. The fibrillæ of the neuroglia do not differ substantially in size from the fibrillæ of fibrous tissues elsewhere.

5. *Tendon Tissue* (Figure 1).—Tendon tissue may be well studied in the gastrocnemius of the frog. It is prepared like the preceding. If, however, it is desirable to show the nuclei in adult tissue, it is well to use nitrate of silver. Cut a thin section of a fresh tendon and expose it for a few minutes in a one-half-per-cent. solution of nitrate of silver, until the section is turbid or milky, then place in the sunlight, and in a few minutes the turbid color will give place to dark brown or

black, owing to the deposit of silver, and the tissue may then be mounted in glycerine and examined. This method will show the corpuscular bodies to advantage. In some cases better results are obtained by the use of chloride of gold. The method is as follows: Freeze a thin portion of a tendon, then make the thinnest possible section, acidulate it slightly, and then immerse in a one-half-per-cent. solution of chloride of gold, until a straw-yellow color has been obtained, and then immerse in a one-fourth-per-cent. solution of dilute acetic acid, and expose to the sunlight until it is purple or reddish; this will take a variable time, and is not always successful, for reasons which are not easy to understand. This is the ordinary method now in general use. At considerable distances from one another there will be seen small dark bodies, which are the corpuscles already described. It is difficult to show that these corpuscles are connected together. To isolate them, take a small piece of young tendon tissue, immerse three or four days in a ten-per-cent. solution of common salt, and then tease. In this way the cells may be liberated, and they will be found to be irregularly-flattened plates. Silver or gold, the latter especially, is generally necessary to show the nuclei in old tendons. The same method shows the fibrillated tissue to advantage. These latter methods will also show that the tendon bundles are covered, more or less completely, with a delicate epithelium (endothelium). The tendon corpuscles do not by any means form a connected sheath for the bundles. In very young tendons the corpuscles are very near together, though even then they only form a partial investment for the bundles; but as the tendon grows older the corpuscles become smaller, withdraw from one another, and sometimes almost disappear.

6. *Fat-Tissue.*—This is a form of tissue that seems to be the ordinary fibrillated connective tissue in a changed condition. It becomes the deposit for oil which appears to fill the corpuscles, making them swell out enormously, as already stated. An excellent way of showing this tissue consists in making a section of a portion of fatty tissue that has been hardened in alcohol or Müller's fluid, or both. The appearances will, in this way, be well shown. After immersion in

Pl. II.

Fig. 3.

Fig. 5.

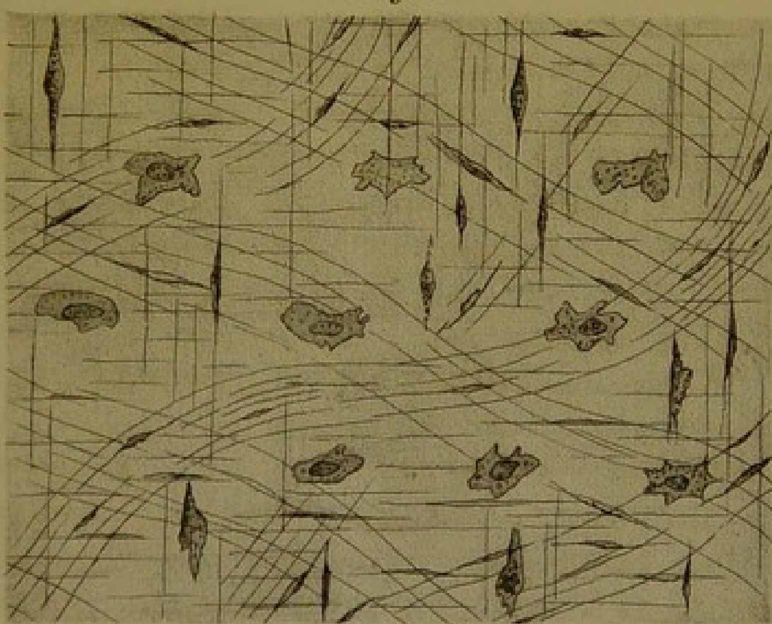
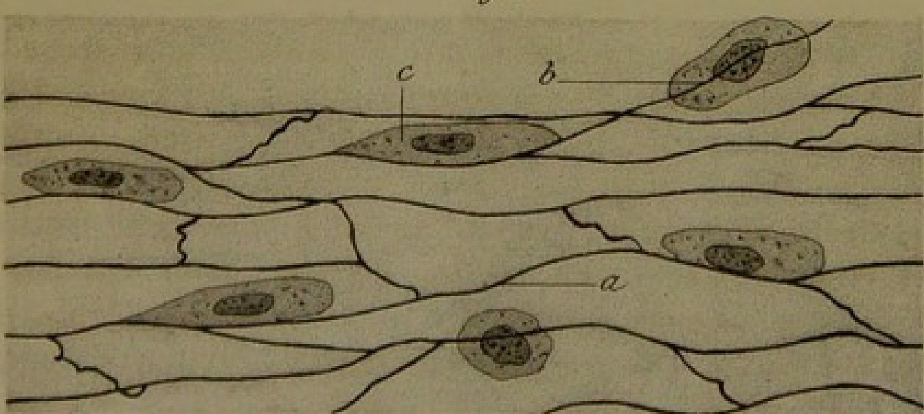


Fig. 8.



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an acid solution, it will be seen that the fatty acids crystallize in the centre of the sac.

The nature of the evidence that the fat-corpuscles are really the transformed corpuscles of the fibrous tissues is as follows: They occupy the same position, being in rows between the bundles and corresponding in position to the other corpuscles that we have mentioned; a few oil-drops at first appear, then others, until finally they coalesce into a single large drop which fills the envelope; if fat-tissue be pressed and the oil escapes, the walls of the fat-corpuscles collapse, and then the flattened plates (nuclei) may be observed on the side of the cavity.

Waldeyer (*loc. cit.*) believes that there is a peculiar corpuscle, three to five times the size of a lymphoid corpuscle, and roundish, which is especially prone to take up fat, and be converted into a fat-corpuscle.

These corpuscles have recently been noticed in most of the connective substances, but it seems uncertain whether they are undergoing fatty change as a physiological or pathological act. They are said to occur constantly in the skin disease known as zanthelasma, and I have found them frequently in diphtheritic membranes, where they appeared to represent the corpuscles of the imperfectly-developed tissue of the membrane, on the third or fourth day of the disease.

7. *Intermuscular Tissue.*—It has been claimed by some that there is a form of spindle cell in the intermuscular tissue in the thigh of the frog. This, however, is apparent rather than real. We find broad plates, in which are oval flattened bodies placed at certain distances apart (Figure 8). These seen in profile appear spindle shaped. There is something peculiar about these bodies, for they seem to bear a close relationship to the elastic networks (*a*), so that in some cases it appears as if the flattened central bodies were directly connected with the elastic fibres as stated by Boll (*Arch. für mikrosk. Anat.*, 1870). In many instances these elastic fibres lie upon the plates (*b*). The broad plates rest in a homogeneous, intermediate, and apparently structureless substance. In this tissue, therefore, it has not as yet appeared that there are fibrils in the intercellular substance. On the

contrary, this substance is soft and homogeneous and highly elastic, and gives the appearance of a tissue that has retained to a great extent its embryonic form.

Connective Tissue of the Kidney (Figure 9).—Here also the plate-like corpuscles may be seen (*a*) as distinguished from the lymphoid corpuscles (*b*), though the exact nature of the intercellular substance, whether fibrillary or not, is difficult to determine with satisfaction. A normal kidney thoroughly injected through both vein and artery was employed in order to differentiate completely the vessels, with the corpuscles in their coats, from the sustentacular tissue or supporting substance proper.

8. *Corneal Tissue.*—There has been a great deal of discussion within the past few years as to the structure of the cornea, and the views of observers have differed according as they have confined their attention to the interlamellar spaces or to the bodies in them. The term *corneal corpuscles*, strange as it may appear, is even now used of the spaces by some of the best-known writers, and it seems evident that there is still doubt as to whether any real corpuscles exist or not. Recently, this subject has been restudied by Waldeyer, and we have been able to verify his conclusions in a very great measure, both as to the character of the corpuscles and the spaces in which they lie. These bodies appear, as stated by Waldeyer, to be in general flat, having a considerable amount of protoplasmic material about their nuclei (Figure 10), though in the direction of the periphery they gradually taper off into thin expansions, which are nearly homogeneous, and extending from them are distinct processes which in part unite with those of other corpuscles and in part end blindly. In structure these corpuscles are not materially different from those of tendon tissue and the other varieties already mentioned. In them is the same flattened oval body, which seen on the side is rod-shaped (*b*), and is surrounded by an irregular envelope that assumes almost any shape. Thus the corpuscles are not always flat, though they are usually so. Their shape depends upon many different causes, such as the method of preparing the tissue, the amount of laceration to which it is subjected, etc. The best method of examining the cornea

consists in preparing it by the gold method, already described.

After the tissue has been properly stained, which is known when it has taken a mauve or violet tint, the specimen may be allowed to stay in the sun; then thin lamellæ are to be torn off with the forceps, and mounted in dammar varnish or Canada balsam, after the tissue has been made thoroughly transparent by soaking in oil of cloves. It will then be seen that there are bodies within certain well-defined areas—the corneal spaces as they are called by Recklinghausen and others. These bodies are disposed at pretty regular intervals throughout the cornea and are generally flat, with rounded contours, though often they have processes extending from them in various directions. In the accompanying drawing the spaces may be distinctly seen as well as the variously-shaped corneal corpuscles; one (*c*) is crowded into the prolongation of a corneal space, while another (*b*) is connected by its processes with a neighboring corpuscle. One corneal space (*a*) is entirely empty. These differing conditions are in a measure due, probably, to the laceration of the tissue in preparing it, some of the bodies having been torn out and others forced to the side of the corneal space. There seems to be a pretty general agreement that the intercellular substance may be separated into independent fibrils, but upon this point I have seen no decisive proof. Dr. Thin, of London, has called attention to certain peculiar corpuscles which he has observed in the cornea, and which were different from the corpuscles already mentioned, and the lymphoid corpuscles also met with there (*Lancet*, February 14, 1873). Mr. Priestley, in a recent number of the *Journal of Anatomy and Physiology* (October, 1875), has stated his experiences in looking for these corpuscles, but decides that they were probably epithelium from the anterior layer of the cornea. Dr. Thin seems to have suspected this at one time, but he tells us that he satisfied himself that he had not committed this error.

9. *Elastic Tissue* (Figure 8).—We have thus far omitted the discussion of elastic tissue for the reason that it is different from the other tissues already described, both micro-

scopically and chemically, though often combined with them. It is also convenient to class it by itself for other reasons, chief of which are that its corpuscular elements have not yet been definitely shown in adult tissue. Virchow, some years ago, stated that this tissue, as well as other connective substances, was composed of networks, the substance of the fibres containing certain markings, and he inferred that these might be the corpuscles of the tissue. Elastic fibres were, however, according to him and others, nothing but the ordinary fibrous tissue condensed. Each fibre was hollow and capable of conveying the nutritive juices.

Henle in his earlier writings regarded the elastic fibres as originating from the nuclei, of which in fact they were prolongations. Subsequently, he seems to have believed that the fibres originated in the basis substance (*op. cit.*).

Reichert could not trace the connection between the nuclei and the elastic fibres, and, when the latter had formed, the former had disappeared.

Boll, however (*op. cit.*), distinctly states that the elastic fibres, each one constituting an "elastic cord," arise from the plate-like cells.

Ranvier (*op. cit.*) examined tendon tissue, as mentioned before, but he was only able to find the elastic fibres after boiling the tissue for from eight to ten hours. It is proper, however, to remark here, that elastic fibres are very uncommon in tendon tissue, at least they have not often been observed.

The fibres of the elastic substance are pretty readily recognized by the fact that they are not colored by carmine or hæmatoxylin, and do not swell with acetic acid; they branch dichotomously, these branches forming, with similar branches of other elastic fibres, networks; this is the general form of adult elastic tissue, and it is probable that exceptions to this rule are, at the most, extremely rare; this form prevails in the ligamentum nuchæ of the ox, in the elastic coat of large arteries and veins, in the serous membranes generally, and in the subcutaneous connective tissue of the skin, as well as in the delicate intermuscular tissue already described. It will generally be found that where this tissue occurs in bundles it is not because there are no meshes, but rather because the meshes are compressed laterally, so that they are not apparent unless most carefully teased apart. When such fibres are broken off, their extremities curl up; further, the fibres are unaffected by boiling solutions of strong acids and alkalies, such as thirty-five-per-cent. solutions of caustic potash or nitric acid (standard preparations in common use in laboratories), unless the action is prolonged for a considerable time. These networks are beautifully shown by taking the mesentery of the frog in contraction and immersing it in acetic acid. The fibrillated connective tissue will swell up and become invisible, while the elastic fibres will be unaffected. The ligamentum nuchæ also affords an excellent opportunity for studying this tissue by itself. To render this study more easy the tissue may be allowed to remain a few days in a ten-per-cent. watery solution of common salt, and it may then be more easily teased. In the subcutaneous connective tissue of the skin the elastic fibres are well shown by hæmatoxylin preparations. Being unaffected by this staining solution they appear as bright, silk-like cords, which lie in close apposition with the wavy bundles, and the branches arch over the bundles to anastomose with corresponding branches of other bundles, so that in this way meshes are formed. Some writers have spoken of little knobs at the nodal points of the meshes, but these appearances appear to have been illusory.

Recklinghausen seems to have believed ("Cellular Pathology," 1871) with Virchow, that the elastic fibres contain peculiar nuclei of their own, which in adult tissue become extremely small, and are represented by the dark markings seen in such tissues. Thin, of London, claims (*loc. cit.*) that they originate in branching corpuscles, which by their coalescence form the network, and the remains of the nucleus may be shown by hæmatoxylin. These markings may, it is true, be seen in the ligamentum nuchæ of the ox, but it is doubtful whether they are nuclei or mere clefts in the tissue. Examination with such high powers as Gundlach's No. 15 immersion and Wales's $\frac{1}{10}$th fails to clear up this point. We may now review these substances as a whole, and decide as to the characters they have in common. Elastic tissue, having a wholly different significance from the others, will be treated separately.

1. The most constant form that is met with in all these tissues is a somewhat flattened ovate or rhomboidal body that assumes the coloring matter deeply. It is found in each tissue we have enumerated. In some, as in the fibrillated connective tissue, it is often larger and flatter than in others, as the tendon tissue, but this difference appears to depend upon certain conditions which modify the original form. For example, when the tissue is young or inflamed, these corpuscles are larger, more vesicular, and closer together, and have better-defined edges; when, on the other hand, the tissue is older or in a state of comparative inactivity, as in ordinary health, these bodies are shrunken and irregular, and conform more to the precise locality in which they are placed; they are also further apart, and often are hard to discover at all—facts which serve to explain the statements of Waldeyer, who says that these corpuscles are often paddle-wheel shaped, and instead of consisting of *single* plates, as Ranvier says, they are made up of *many* plates, the paddles, which radiate from a centre. In examining these tissues with a lens of high power, such as the No. 8 of Hartnack's system, there will be no difficulty in detecting them in every instance, and there can be no doubt that the use of hæmatoxylin in these examinations has afforded us the best means of demonstrating many points which have previously been obscure. The particular reason of the advantage obtained by the use of hæmatoxylin lies in the fact that very often it obviates the use of alcohol and acetic acid, both of which distort the parts, and very often give rise to false appearances. It also chiefly affects the plate-like bodies and only slightly tinges the delicate envelope, while it wholly avoids the rest of the intercellular substance. In this way we are able to employ a solution that differentiates the elements most excellently, in fact far better than carmine, and it is dissolved in water and not in acids or alkalies, which have peculiar actions upon all tissues. During the latter portion of this work use was often made of Hoffmann's violet, which was used as a substitute for the *violet de Paris* or methylaniline (Poirier) recommended by Cornil for showing waxy degeneration. It was dissolved in water in about the proportion of two grains to the ounce. In half an

hour the nuclei were beautifully stained of a delicate violet, while the cell body and fibres were unaffected. The reaction was much the same as hæmatoxylin in regard to the tissue affected.

It is with the use of these two reagents that we may get the best ideas of the structure of connective substances.

2. Most of these plate-like cells which have often been called nuclei are invested by a delicate envelope, the body of the cell. This is mentioned by almost all recent observers, and was even spoken of by Henle long ago. This substance is seen to advantage in the reticular form of tissue (fibrous tissue) already mentioned, and is not so deeply stained by hæmatoxylin. When the tissue is expanded, this sheath or envelope is capable of being drawn out to great length, while, when it is separated from its connections, it shrinks, assuming the most irregular forms. When the plate-like bodies with their envelopes are attached together, they give the appearance of "spindle-shaped cells," a name often given to them. It may readily be imagined that the changes in form which such delicate envelopes assume may be manifold. In moist tissues they may swell, and in dry ones they may shrivel. It is the processes of such envelopes that often communicate, and the term "netted cells" has often been applied to them.

The theory developed by Heitzmann ("Über das Verhältniss zwischen Protoplasma und Grundsubstanz im Thierkörper," *Sitz. d. Wiener Akad. V. und Hs. Jahresbericht*, 1873), that the connective tissue of the umbilical cord, periosteum, and tendons, etc., demonstrates a continuous connection between the processes of the corpuscles, as he observed in cartilage, and is commonly seen in bone, could only be substantiated in a few instances. We have noted that, both in the cornea and in the mucous tissue of the umbilical cord and reticular tissue, there is such a connection to be sometimes seen, but at other times it is not seen at all, and this is the more difficult to understand, if the theory of a constant connection between these processes is tenable, for the method of preparing the cornea (tearing off the lamellæ) appears to give us a view of the corpuscles, or some of them at least in their proper connections, and the gold method defines them clearly.

As we have seen, they are, however, only occasionally united. In the older forms of tissue, as in tendons, there was no such connection noted; indeed, in most of the tissues enumerated, the corpuscles with their delicate sheaths appeared to be quite separate from one another.

In fat-tissue it seems, as we have already stated, that the delicate envelope takes up the oil, at first in minute globules, which by their union form larger ones and so finally completely fill up the sac. The flattened corpuscle, or "nucleus," that belongs to the tissue is unchanged, however, but takes its position in the side of the sac.

The second form of corpuscle that is frequently met with in all situations in the tissues is the round corpuscle already mentioned, and known as the lymphoid cell or corpuscle. It has often a pale, fleecy investment about it, which does not color with hæmatoxylin, or Hoffman's violet, or only slightly. Very similar bodies are often seen in close connection with the plate-like corpuscles, from which it often appears as if they originated from the latter. The third corpuscle similar to the one mentioned by Waldeyer and by Klein is also sometimes seen. It is large, about four or five times the size of a lymphoid corpuscle, and pretty globular in shape, and contains coarsely refracting bodies. These appear to be minute oil-drops, but whether these are the result of physiological or pathological change is uncertain. The adult intercellular tissue is made up of bundles of indefinite length. As for the bundles, each one is made up of separate fibrils which do not anastomose but run a parallel course. The fibrillar connective tissue, adenoid tissue, neuroglia and tendon tissue have this character clearly, while in the other forms it is not so certain that this is the case. The fibrils are held together by a firm cementing substance which can be dissolved by long immersion in Müller's fluid, or for a few days in a ten-per-cent. watery solution of common salt.

3. We can now study the relations of these parts to one another. It may be stated as a fact that is indisputable, that in all the adult tissues, excepting, perhaps, the supporting tissue of the kidneys and, of course, elastic tissue, and of the cornea, there are two principal substances met with; more or

less flattened corpuscles; and an intermediate fibrillar substance. The exact relation of the fibres to the cells has never been thoroughly stated of all these tissues, but it seems proper to conclude from the foregoing statements that in each instance the plate-like cells are superimposed on the fibres and form in this way, often, a partially investing sheath. The envelope which is the investing substance of the plate-like cells forms a bed upon the bundles to which they adhere very strongly. By their processes these envelopes anastomose with other adjacent bodies of like structures; they do not always anastomose, however, or do not appear to, and may be quite free. In the cornea they often unite and often do not, but, in so doing, they extend their processes through the channels which are supposed to convey lymph from the corneal spaces. Klein's view that netted cells form the network in adenoid tissue is untrue in many cases, judging from the observations that were made, for it was found possible to brush off many of the bodies, showing that they were on the tissue and not in it. These so-called cells were also seen by sufficiently high powers to be fibrillated, and therefore not "netted cells" but bundles of larger or smaller size.

Elastic Tissue.—1. There are no corpuscles that have been found in adult elastic tissue that can be positively shown to belong to it exclusively. In young tissue they certainly occur, according to excellent authorities, but they do not maintain their integrity long. Possibly higher powers than those in use may discover the bodies mentioned by Recklinghausen and Thin, but at present they elude our observation.

2. The fibres are cylindrical and branch dichotomously, and they exhibit an indifference to micro-chemical reagents that is not shared by others of the connective-substance group. They do not appear to be made up of fibrillæ, but each elastic cord is the ultimate element. They have no necessary connection with the other connective substances, and that they are not always present may be shown by boiling adenoid tissue and tendon tissue in the solutions of caustic potash before mentioned, when it will be found that these substances will dissolve entirely, which would not be the case if elastic fibres were present, for they resist the action of boiling acids and

alkalies, as we have seen. We have stated that in the intermuscular tissue of the frog's thigh, which is extremely rich in elastic networks, there are numerous large and flattened corpuscles, which rest, apparently, upon broad and flattened plates of a fibrillated character, and here it often appeared as if the flattened bodies were continuous with the firm branches of the network. In other cases the elastic fibres pass directly over the plate-like body. Whether, however, there is a connection between them as Adickes (*Archiv der Heilkunde*, iv., 1872) claims, is difficult to determine.

The Development of Connective Substances.—Opportunities for studying this portion of the subject were given by the examination of different portions of the *umbilical cord* of an embryo of three months, also *cicatricial tissue* which had been removed from the face on the third day, from the *fibrous alveoli* of a cancer of the breast, and a *fibrous thickening of the scalp*, which developed rapidly from a bony tumor of the calvarium on which it lay (Figure 11). In the cicatrix removed on the third day, plate-like cells were found measuring from $\frac{1}{1200}$ to $\frac{1}{2000}$ inch in diameter, and surrounded by a nearly hyaline membrane.

The central body was about the size of a lymph corpuscle, and whenever fibres occurred they were arranged in parallel rows, and upon them were the flattened corpuscles surrounded by a hyaline substance. In one instance the central body was dividing. In examining the fibroma of the scalp, the bodies were seen to be plates (*b*), though ammonia-carmine did not show them, and it was not until acetic acid was used that they became granular and in this way apparent. The intercellular substance was seen to consist of flattened ribbon-like laminæ tapering off at each end. It seemed as if fibres ran over these corpuscles and were continuous with the elastic fibres, but this appearance was by no means constant, and when gold was used these plates appeared, at the end of twelve hours, to be irregularly rhomboidal (Figure 11, *a*). In the alveoli of a growing carcinoma, the plate-like cells were also seen, but the intercellular substance did not give the uniform appearance of fibrillation, owing perhaps to its early age.

These specimens also afforded instructive testimony of the

Pl. III.

Fig. 6.

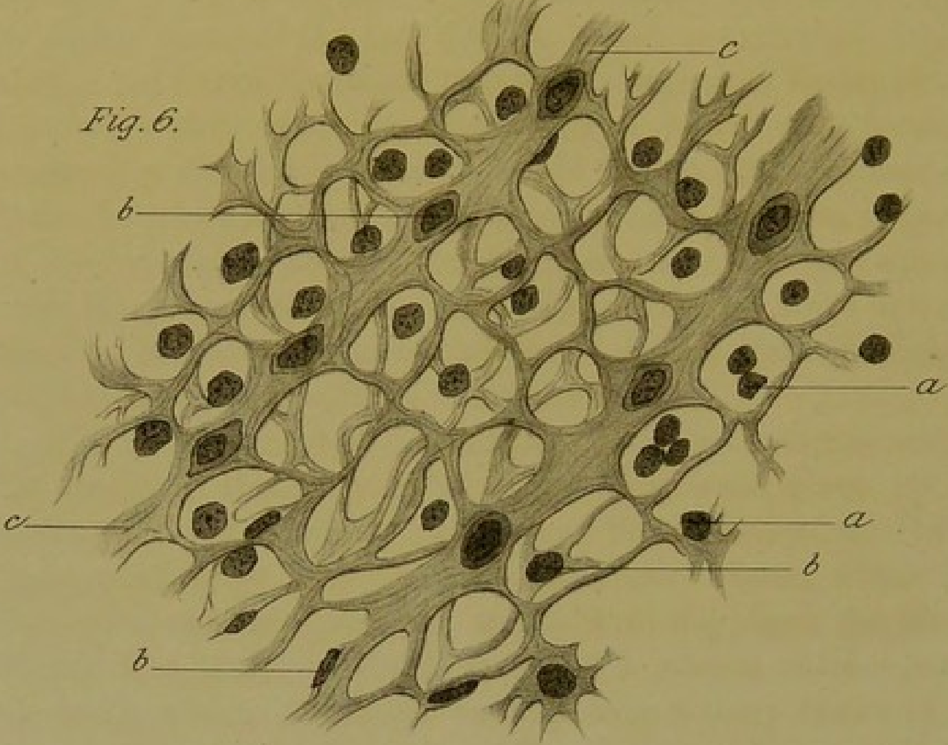


Fig. 11.

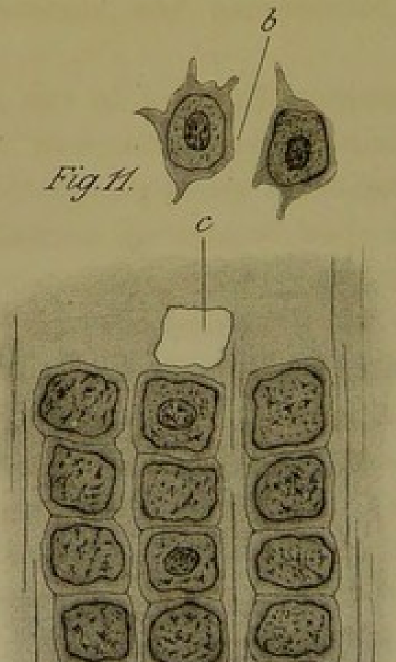
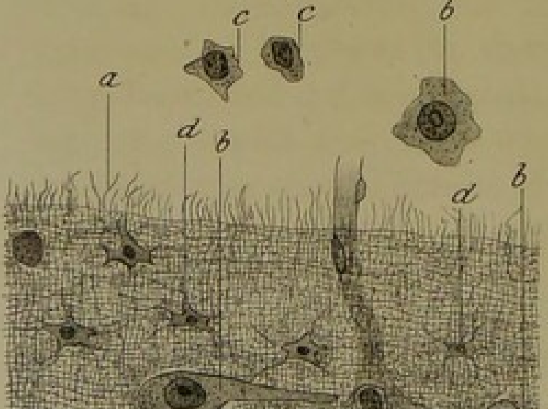


Fig. 7.



T. E. Satterthwaite, Del.

J. Maisonneuve, Lith.

Fig. 9.

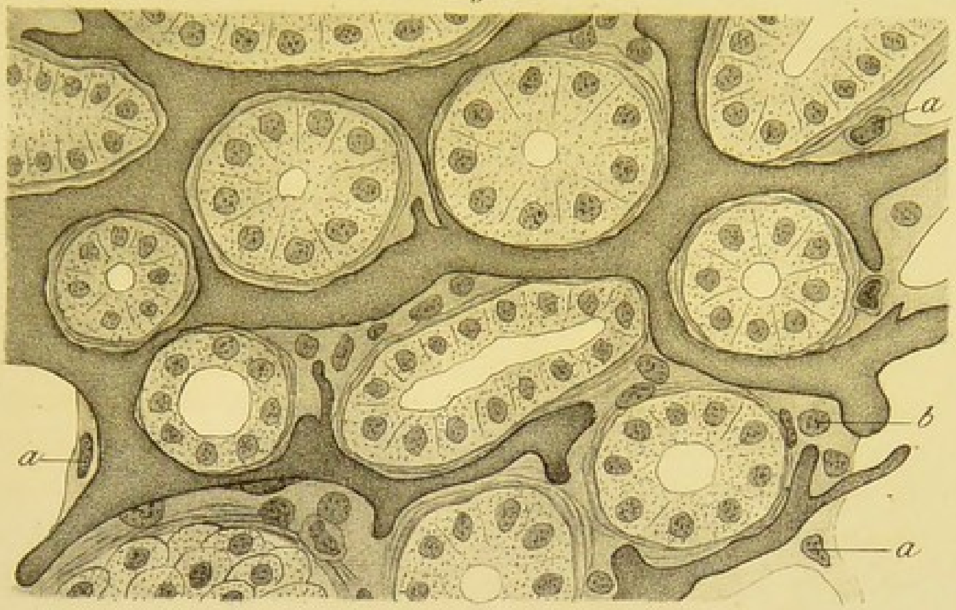
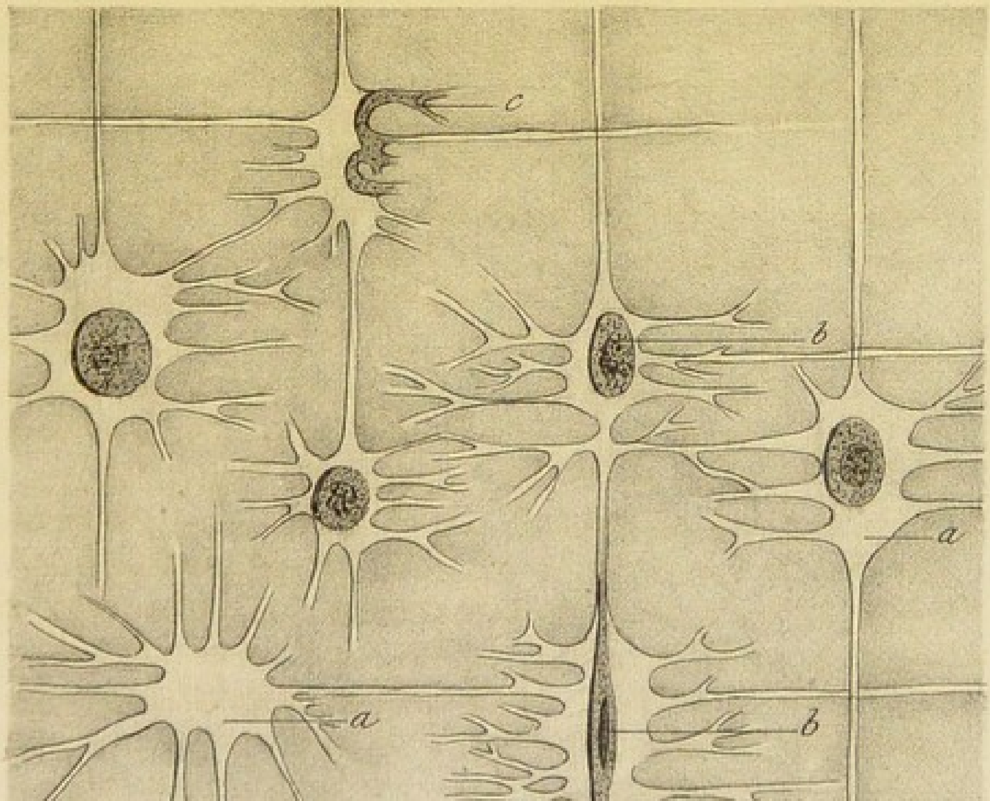


Fig. 10.



T. E. Satterthwaite, Del. J. Maisonneuve, Lith.

fact that there is apt to be great confusion in the use of the word "cell," for it may often be shown that the so-called spindle cells—by which are here meant some of the larger figures embraced under that name—are often not such at all. A good lens shows that the so-called spindle cell may be a thin ribbon-like portion of the intercellular substance that we have learned to know, upon which is the flattened corpuscle, itself surrounded by a more or less delicate investment, generally of a hyaline material. The gold method brings out these points in great perfection. Further proof in support of this statement may be gained by pressing such a spindle cell between the cover and the slide in the way already mentioned. The "nucleus," or flattened corpuscle, may then be made to drop off. The valuable results that follow from recognizing these points are, that they at once give us an insight into the structure of the so-called spindle-celled sarcomata or fibro-recurrent tumors which are formed of a tissue very similar to some of the connective substances already mentioned, for in describing them the very same error has often been committed as in calling the plate-like cells spindle-shaped. Any one who examines such a tumor in the way just described will have no great difficulty in demonstrating to himself that the so-called nuclei of the spindle cells are, in reality, very similar both in form and size to the plate-like corpuscles already described in the umbilical cord. These facts were elicited from the study of such a sarcoma, whose character was definitely established by its microscopical character and its frequent recurrence (five times). The younger portions were known to be such by the description given of them by the patient, for, the tumors being nodular, the age of each nodule was pretty nearly established. In the young portions the same plate-like bodies as are found in the umbilical cord, or bodies at least in general very similar, were found, but they were imbedded at intervals in a homogeneous material in which as yet there was no fibrillation. This portion was actually a young growth. Older portions were then examined, i. e., those known clinically to be older. It was then found that the intercellular substance had a fibrillated appearance and by suitable reagents it could be broken up into thin ribbons or bands, as by Müller's fluid or by a ten-per-cent. solution of common salt; numerous long spindle-shaped figures having a flattened body at their centre were then found; and there were also numerous similar spindle cells without any central body or "nucleus." Where, however, such appearances are observed it is easy to introduce a current, roll these bodies over, and then it may be seen that they are long, flattened, and of irregular size, appearing on profile view to be spindle-shaped, and yet we may often press off the "nuclei" by pressing the cover upon the slide, showing conclusively that such spindle cells are really the intercellular substance at an early stage of fibrillation, and almost precisely what may be seen in certain parts of the umbilical cord of young embryos as already described. It does not, however, follow from this that all of the spindle-celled sarcomata are of these varieties, indeed we sometimes see them where they appear to be composed of real spindle-shaped bodies, closely packed together, and where each body contains within it a smaller flattened body. From a study of these gradual changes it seems likely that, in growth and repair, the corpuscles at first *round* soon become *flattened*, and have a broad envelope (*b*). About this envelope there is a further delicate and lightly attached investment, which, uniting with the investments of other similar bodies, is the commencement of the intercellular substance. At first the plate-like bodies lie in niches, as it were, in the intercellular substances, and if one is brushed out it leaves a socket behind it (*c*). As the intercellular substance increases, the corpuscles are arranged in rows and they become smaller, while immediately under them thin laminæ are formed from the effused fibrin—the commencement of fibrillation—while the corpuscles are unchanged except that they become smaller, their envelope shrinking, and they recede from one another. In advanced life these corpuscles are generally more or less flattened, but their form is also considerably modified by the age of the tissues, and various mechanical alterations to which they are subjected, according to the particular locality in which they occur.