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UNIVERSITY OF NEW MEXICO.
No. 3.

THE SOMATIC EQUILIBRIUM AND THE NERVE ENDINGS IN THE SKIN.

By C. L. Herrick and G. E. Coghill.

PART ONE.
With Plates V—IX.

Few problems have proven more attractive or more illusory than the general question as to the nature of the nerve termini in the membranes, for it would seem that our concepts of the histogenesis and so of the real nature of the sense organs depend very largely upon the conclusion at which we arrive as to the relation between the various types of sensory epithelium. The senior writer suggested, in a series of papers on the brain of the lower vertebrates, reasons for believing that the first sense to come into the field of consciousness was that of smell, and a little later Edinger emphasized the same idea by his investigations of the olfactory tracts of the reptile brain. It may now be taken as fairly proven that, if the seat of consciousness is in the cerebrum, smell was the first of the special senses to find its way to recognition by it. It would then be natural that we should expect the peripheral organs of olfaction to retain a primitive character and so to afford us a clue to the early state of such organs. Then too the development of the accessory or non-nervous organs of sense has here hardly made any progress even in those most highly differentiated cases in which Jacobson's organ has assumed great proportions.

From studies of the development of the olfactory organs in reptiles, as reported briefly in earlier numbers of this Journal, the writer has been abundantly convinced of the truth of Beard's statement that the olfactory prota arise from the skin and, by a

proliferation, extend to the brain, there to enter into communication in the glomerules with the processes of the mitral cells of the tuber.

As studied in the embryos of snakes the process is as follows: The first indication of the change of the ordinary to the sensory epithelium is seen in the thickening of a portion of the superficial layer from the morphological front of the head (the region of the future infundibular recess) in relatively broad bands, one on either side of the head. As the head flexures increase, these areas are carried ventrad and come to occupy the roof of the mouth and adjacent parts of the buccal cavity. The development of the taste buds from this epithelium we have not traced in these subjects, though there is no reason to doubt that they are formed from this proton, as it is easy to see that the mucous part of the hypophysis is. At the time the first olfactory rudiments appear, the curvature is such that the hemispheres are protuberant in front and so come nearly in contact with the prota of the olfactory in the two bands of germinative epithelium above mentioned. Still there is no difficulty in seeing that the original proliferations take place in the skin and that the constant proliferation by division of the earlier cells spins the nerve fiber from the original source to the point where the tuber subsequently arises. In fact, the tuber, which has frequently been compared to the ganglion of origin of a cranial nerve, does not seem to afford origin for any centrifugal fibers whatever. In preparations by the silver method it is easy to see that the neurite of the moniliform chain of the olfactory nerve comes into relations in the glomerules with dendrites of the mitral cells. Though a considerable wealth of detail has been secured by study of Golgi preparations during the last few years, nothing has been brought to light to invalidate our original view.

For a long time during the development of the brain an obvious ganglionic mass lies below the skin at the base of the point of origin of the olfactory. The gradual elaboration of the cavities of the nares only serves to redistribute the protaq without materially disturbing the simplicity of the arrangement.

In a wide range of types it has been possible to make out the adult conditions which have often been correctly described. Merkel in his classical work gives a figure of sensory endings from a cirrus of Amphioxus that compares in every detail with the specific cells of the olfactory epithelium of a reptile or amphibian. (Plate III, figure 10.) Few if any of those who have studied the development of the olfactory will venture to deny that the "Stiftzelle" at the peripheral end of the olfactory nerve is a member of the nervous series having the same origin, though it is doubless conceivable that, through some strange fatality, every observer has failed to notice the intrusion of a foreign element at some stage of the process. (Fig. 31.) If, however, we take for granted that the fiber is continuous, we claim that there is an equal necessity for admitting the same for other clusters of nerve endings on the surface of the body.

Although there was for a long time considerable disagreement as to the actual connections of the olfactory nerve fibers, and the classical studies of Kölliker, Klein and Piana left the matter open, it seems as though the later studies of Ehrlich, Arnstein, Cajal, Gehucten, Retzius, Brunn and Lenhossék, who employed the silver and methylene blue methods, were sufficient to prove conclusively that the olfactory epithelium possesses rod cells whose proximal end is an actual continuity with the fiber of an olfactory nerve filament. The writer has frequently verified this in specimens of Amphibia double stained with hæmatoxylin and picrocarmine in which very unambiguous views can be secured. A few figures from these preparations were published by Mr. Bawden, then a student in the writer's laboratory (Jour. Comp. Neurol. IV). Our studies in the development of the olfactory nerve show that the proton of the nerve is formed in or under the epithelium of the nasal area and that the nerve grows by moniliform concrescence of cells which arise by mitosis from this proton. From this stand-point, then, it would be expected that the neurocytes of origin would be found in the epithelium. In all essential respects the relations in Jacobson's organ are the same as in the true nasal olfactory epithelium. The accompanying figure (Plate V, Fig. 10) from an article by Lenhossék (Anatom. Anzeiger, VII, 19-20.) illustrates these conditions and also the fact that other nerve fibers, apparently from the trigeminus, terminate in free arborizations between the epithelium cells. A very large following of the new school are prepared to claim that the conditions in the olfactory epithelium are peculiar to it alone and it is even attempted to correlate this with a supposed fundamental difference in origin and structure of the olfactory from all other nerves of the body. But we are able to show that in the epidermal sense buds of the tree frog and other amphibia the same continuity of nerve fiber and cell can be determined.

It has not been an altogether unnatural result of the remarkable complications of nervous structure revealed by the so-called specific methods that the results obtained by the old histological methods have been discredited and it has required some year's experience to teach us the danger of too explicit reliance on the former. Perhaps the greatest of these sources of ambiguity arises simply from the fact that has been regarded as the chief excellence of these methods, namely that the selection is so perfect that other tissues than those selected not shown at all or, even if the after-staining of sections succeeds, the conditions of impregnation are so unlike that the tracing of connections or definite relations is difficult or impossible. The absolutely contrary results of Dogiel and Cajal in the matter of the anastomoses in the retina illustrate the difficulty that exists even where the methods used are similar. The results of our own studies are rather to confirm many of the old observations and to show that there are two distinct classes of dermal endings. Of these the olfactory illustrates one and the most primitive one. In this case we have to deal with the remnants of nervous aggregates which were originally formed in or near the outer layer and in the phylogenetic development have not been diverted to a deeper level as is true in so many other instances.

In our laboratory in 1891 we made out the fact that in the oral region of the earth-worm there are cells in the skin which have a nervous nature and whose processes pass entad to the central system. Owing to a delay in the other aspects of the research the observation was not made public till the brilliant work of Gehuchten had afforded proof of the same thing, but the suggestion was of course inevitable that we have in the lower forms a permanent retention of cells in the skin which in higher types have tended to become concentrated in the central organs. What more natural, however, than that this concentration should be incomplete, especially where these cells have have acquired a specific sensory function. When the application of the Golgi and methylene blue methods revealed the fact that there is a most complicated set of free endings in the skin and that in many cases where a nervous continuity had been described there is simply a secondary apposition of a dendrite to preexisting non-nervous cells it was inevitable that the existence of cellular nerve endings should be discredited entirely. It is true that the greater part of the sensory prota are collected in the spinal and cranial ganglia and seem to proliferate thence to the periphery; but in various regions, particularly of the head, these ganglia never concentrate in a neural ridge but retain their original place in the neighborhood of pharyngeal clefts and the like and the possibility must be allowed that other cell-clusters elsewhere may have done the same. However, there is another possibility to be considered; namely, that the terminal portion of the peripherally proliferating nerve fiber may under certain circumstances develop a specialized terminal dendrite. When the nerve is in process of developing the subdivision of the distal member is repeated progressively until the definite terminus is reached and then the extreme element is charged with the function of adapting itself to the conditions there prevailing. In the case of the motor ending, even the careful researches of Huber and De Witt do not finally dispose of the question as to the origin of the end-structures. We may interpret them as follows: when the fiber reaches the muscle its terminal element, together with the nucleus, applies itself to the surface of the latter and prior to the formation of the muscle-sheath, proliferation goes on in a less regular way than during the development of the nerve itself, in this way is formed the "sole," which would, accordingly, be of a nervous nature. On the other hand, it is possible that the nerve on entering the

muscle comes in contact with a nucleus of the muscle which, under the stimulus afforded, begins to proliferate and the protoplasm of the cells so formed assumes an intermediary character and spreads out upon the surface of the muscular band as a means of applying the stimulation. To us the first is in the absence of direct evidence the more probable solution.

Observations are at hand which tend to show that extensive nervous proliferation takes place below the corium of the skin at an early stage. In section of the skin of Amphibia these proliferating cells can be seen and this is probably the origin of the ganglion plexus of the skin. (Figs. 3, 5 and 6, Plate V.)

To pass then to the nerve endings in the skin, we may first note the isolated sensory cells. These may be seen in suitably prepared sections of the head in the tree frog and other Anura and also in the neighborhood of the eye in the axolotl and other tailed Amphibia. In the tree frog, where they most numerous, these cells are grouped in threes and fours in close clustres lying in a special cavity passing through the entire thickness of the epithelial layer. The terminal segment is a slender nucleated cell, the nucleus being very narrow. The peripheral part of the cell is a narrow rod which at the periphery bears a few rigid bristles. Entally from the nucleus the cell walls are very delicate but obvious and the nerve fiber within is easily distinguishable in the doubly stained specimens. The fiber is easily followed to the corium layer and in many cases through it. It seems too that more than one nucleus can be seen in the course of the fiber before the passage through the corium. The skin is at this point very thick and the presence of large glands serves to separate the corium from the epithelial layer, so that the course of these fibers is readily followed for a long distance. In the case of certain teased preparations it was possible to isolate these fibers and study them with oil immersions and there can be no doubt as to the relations here described. So far as could be told, these fibers do not connect with the subepithelial plexus as do the fibers of the free arborizations to be described later. (Figs. 2, 12, 13, 14.) The terminal segment seems to be entirely homologous with the segments of the nerve and its peripheral portion is perhaps simply a modified dendrite.

The endings above described must not be confused with the sense buds found elsewhere in the skin. In the latter there is a well-developed accessory apparatus in the form of the well-known beaker or "Stutz" cells, here there is simply a cavity or tube in the midst of unmodified epithelium cells. Yet it is not to be assumed without better evidence than is now at command that these two classes are of entirely distinct nature and origin. In the first place it is scarcely to be credited that two sets of sensory organs derived from the same proton and so similar in function as are the organs of smell and taste should be of an absolutely different type, and what may be said of the taste buds applies *mutatis mutandis* to the sensory buds of the skin.

The contrast between the results of different methods is nowhere better illustrated than in the different conclusions reached by Fusari and Panasci on the one hand (Arch. italiennes de Biol. XIV, p. 240) and those of Arnstein (Archiv f. mikroskop. Anat. XXXXI, 2). The former authors worked with the chrome-silver method and describe a direct communication of the nerve fiber with the axial (rod) cells of the taste buds. (This we are able to substantiate from personal observation.) Arnstein, on the other hand, denies such connection most emphatically and claims that teased preparations with methylene blue show with all possible clearness that there is no such connection, but instead that the varicose nerve fibers form a felting of fibers around the axial and outer cells of the bud and end free in the pore. Arnstein finds quite similar nerve endings in the filiform papillæ. He does not find forked cells, but inclines to the view that such cells result from the separation of the true nerve fiber from the peripheral end of the cell to which it is attached. The appearance of continuity between the cell and the nerve fiber is said to be illusory and is explained as due to the blackening of the cell as well as the fiber. Ehrlich (Deutsch. med. Wochenschrift, 1886, 4) described intensely colored cells in the mucous membrane of the olfactory region which pass without interruption into a nerve fiber, but these cases Arnstein also dismisses as illusory. Dr. Niemack has also reached similar conclusions by the use of different material (Anat. Heften, Merkel und Bonnet, Anat. Anzeiger, VIII, p. 20.)

Inasmuch as the epithelial layers of the mouth and tongue are morphologically only portions of the skin, it is necessary to examine these regions for light on the nerve endings as they may be modified under the special conditions here existing. In the frog, which has been the subject of the most elaborate invstigation, the sense of taste cannot be at all highly developed, for the animal is accustomed to swallow its food, chiefly horny coated insects, without mastication; and experiments (Bethe) prove a very sluggish response to chemical irritants. In the tongue of the frog, as well as in the palate, there are numerous scattered specific sense organs, those of the tongue being flat end-plates, while those of the palate are protuberant sensory papillae. Athough these organs were described by Leydig in 1858 they have frequently been the objects of special study since then and even now authors are not wholly in agreement as to the details of the structure. The cellular elements in these sense organs consist of the cylinder of flask cells forming the protection for the sensory rod cells, a subordinate variety of which has been termed forked cells by reason of the divided peripheral projection. Alate, or winged cells, around the cup or flask have also been noticed by some authors. Bethe, who has recently studied these buds by means of the modification of the methylene blue method which bears his name, finds two sorts of nervous termini in them: first, free termini lying between the cylinder cells and reaching the surface, second termini with bulb-like expansions on various cells. (Fig. 8.) One type of such endings is three-lobed and such endings are affixed to the sides of the cylinder cells; the other variety has simple circular end-plates and these endings are found on the rod cells, fork-cells and possibly also on cylinder cells. In no case did Bethe succeed in finding actual continuity between the rod-cells and the nerve. He in fact seems to find greater intimacy of connection between the cylinder cells, which are not supposed to have a nervous function, than with the rod-cells and in no case is there more than a contact with the cell wall. He explains the continuity detected by Arnstein and others as the result of faulty observation and imperfect methods. In the ordinary pavement epithelium of the palate Bethe finds termini on gland cells and ciliated cells, as well as deeper elements. It should be noted that the finding of the three-lobed end-plates on the cylinder cells was not a uniform occurrence but rather exceptional and the suggestion is near that this is the result of an accidental state of the fibers and not a natural or permanent organ.

Our own studies of the gustatory epithelium of the axolotle are in accord with the results of Bethe upon the frog so far as the diffuse endings are concerned, though the methylene blue does not give adequate insight into the connections between fibers and cells. The taste buds, on the other hand, afford similar results to those obtained from the sensory buds of the skin. The source of many of the erroneous conclusions reached is, as mentioned beyond, the fact that in successful methylene blue preparations it often happens that fibrous elements stain when the cells of origin for the same fibers do not.

Diffuse Peripheral Connections.—Various early writers have reported the existence of a dense net-work or felting of nervous material among the epithelial and even the corneum cells of the skin. This structure was first made out by the use of gold chloride and there was always left open the possibility that the appearance was due to the disposition of metallic salts in the interstices between the cells. Dogiel in his paper on the nerve endings of the genitalia figures a very extensive mesh-work of this kind with here and there a free knob-like termination and he traces the lower part of the reticulum to a direct communication with a set of nerve fibers passing perpendicular to the skin. (Fig. 1.) Strong in his paper on the cranial nerves of the frog figures a similarly minute meshwork which is revealed in this case by the use of the Golgi method. In all of the above cases there is the element of uncertainty growing out of the fact that the methods are impregnation rather than staining processes and are histologically uncertain. It would then be eminently desirable to supplement the evidence from these sources by other means. In the study of the skin of the Amphibia it is easily noted that there exists at the base or ental aspect of the layer of Malpighi a layer or stratum which is in a peculiarly nascent state. These cells are devoid of the thick and rigid walls chatacteristic of the superficial cells and are protoblasts rather than complete cells. In this layer we may find, at all stages, the evidences of mitotic division. In fact there is a permanent proliferating zone in this region. Comparison of this stratum with that of higher vertebrates shows that the latter form

no exception, though it is not always easy to detect the protoblastic elements. A single theoretical consideration is sufficient to convince one that this is what should be expected, for it is of course recognized that every type of vertebrate has some provision for the constant or occasional removal of the skin. In some cases the process of removal of the corneum is intermittent, while in others it is gradual. In either case it is obvious that there must be a proton of undifferentiated material—of cells that have not passed beyond the plastic stage. In those parts of the skin where there is little differentiation between the various layers the difference between the corneum and deeper cells is not readily detected in preparations by the usual processes, but in the thicker portions where the so-called Leydig cells appear the basal protoblasts are crowded into the interspaces and pried apart. One effect of this process has been to stretch the connecting protoplasm into an excessively thin layer or film enveloping the Leydig cell either completely or as a coarse mesh-work of naked protoplasm. In all the preparations we have seen, even those in which the preservation has been as perfect as possible, without the least evidence of shrinkage, the appearance is that of a broad reticulum arising in the intercallary or basal protoblasts and enveloping the cell in such a way as to wrap it completely in the products of the adjacent protoblasts. The most perfect process of preservation for such structures is a combination of chrom-acetic and platinic chloride diluted in alcohol. The use of Merkel's solution also gave very good results, while the various osmic acid solutions invariably produce too great shrinkage of some parts, especially of the reticulum. In the first mentioned solution it appears that the natural tendencies of the alcohol and the chromic acid counteract each other while the fixing action of the platinic chloride is in no way interfered with. The avidity to all the usual stains after this treatment is also very great, while in the osmic preparations there is not only general diminution of the receptivity, but, what is worse, the effect is not uniform even in the same class of tissue in the same preparation. In properly prepared sections the reticular structure of the protoplasm of the Leydig cells is most beautful, but when osmic solutions are used the contents of the vesicles is blackened and the result is a granular appearance instead. The pericellular mesh-work is stained red by picrocarmine, as is all protoplasmic matter, while the nuclei are all selected by the hæmatoxylin. Nerve fibers stain red but their nuclei are purple. The nerve supply is abundant and the fibers can be traced without difficulty through the corium layer in all preparations. The sheaths seem to cease after passing the corium and the subsequent course is less easy to make out. In a considerale number of cases it has been possible to trace such fibers with all desirable clearness to actual connections with the bases of the lower protoblasts above mentioned. The fiber is red, as is the protoplasm, so that it remains possible that the exact nature of the union is not obvious, yet from the fact that two masses of naked protoplasm thus come in contact, the range for possible modes of union cannot be extensive. In any case the most careful examination under immersion lenses of well-stained specimens does not reveal any form of intermedation between the fiber and the protoplasm of the cell. Nor is this relation limited to the lowest layer of protoblasts alone, for it is possible to trace fibers to some of the higher members as well. The attempt has repeatedly been been made to count the number of fibers entering the given area and then to compare this number with the number of protoblasts in the same area, with the result that the fibers proved more numerous than the cells in the lower series, thus offering independent evidence to the effect that these fibers are destined to more than the single basal row of protoblasts.

The pericellular net-work has been described by a number of the earlier observers, but in each case the real nature of the structure has not been detected. Paulicki and Pfitzner both regarded it as a mesh-like thickening of the cell wall. The latter thinks these "ribs" serve for the point of attachment of the intercellular bridges. Part of Paulicki's description is given in full. "An einigen Leydig'schen Zellen wurde ich auf kleine kreisförmige, glänzende, dunkelconturirte Figuren aufmerksam, die in ziemlich regelmässigen Abständen von einander entfernt der äusseren Fläche der zellmembran aufsassen. Es stellte sich nun alsbald heraus, das dieser Befund bei allen Leidig'schen Zellen ein ganz constanter ist. Ueber die Deutung dieser Gebilde erhielt ich durch Zellen, wie deren mehrere abgebildet sind, Aufschuss. Hier fand sich ein doupltconturirtes Gitterwerk, welches uber die Protoplasmakörner hinwegging. Die Balken des Gitterwerks theilten sich öfters gabelformig und waren bald dünner, bald dicker. Es ist nun anzunehmen, dass das Gitterwerk hervorgebracht wird durch rippenartige partielle Verdickungen der Zellenmembran; und dass bei solchen Zellen, wo ein derartiges Gitterwerk zu sehen ist, der Schnitt die Zelle tangential getroffen hat, während bei den Zellen, die dieses Gitterwerk nicht zeigen, die dagegen in der Zellmembran von Strcek zu Streck kleine glänzenden Ringe besitzen, der Schnitt mitten durch die Zelle gegangen ist. Die kleinen Kreise, die der Zellenmenbran aufsitzen, stellen die Querschnitte der rippenartigen Verdickungen der Membran dar. Die rippenartigen Verdickungen der Zellenmembran Zeigen sich durch sämmtliche Farbemittel ebenso gefarbt, wie das Protoplasma, wesshalb sie leicht ubersehen werden könen." The author also notices that these bands are sometimes sharply stained by fuchsin, a fact that, in connection with the above, might well have suggested that these supposed ridges on the cell wall have a nature more in common with that of protoplasm. Still more suggestive was the additional observation that these ridges are not limited to any single cell, but often pass to neighboring cells without interruption. He says "Ich sah, dass die Balken von einer Leydig'scher Zelle continuirlich zusammenhingen mit den Balken benachbarter Leydig'scher Zellen, dass ein zusammenhängendes Balkenwerk sich uber mehere Leydig'sche Zellen ausdehnte. Ausserdam sah ich aber auch, dass ganz ähnlich gestaltete Balken sich auf die benachbarten Epithelzellen fortsetzen."

Our observations leave no doubt that this meshwork is not only of a protoplasmic nature but that the meshes are connected with the nuclei of the basal and intercallary series (Figs. 17-20). It is easy to trace the meshes into communication with the proplasm surrounding these protoblasts. It is more difficult, except in the case of perfectly preserved material, to follow the nerve fibers to the bases of the cells of the higher series, i. e., those about the sides and ectad of the Leydig cells. In good methylene blue specimens stained *intra vitam* (Figs. 21-23), the fibers can be traced for a considerable distance into the epithelial layer among the intercallary nuclei, but it is only in specimens stained with picrocarmine and hæmatoxylin that the actual connection with the cells can be made out. Even here the question (always left wholly undecided by the methylene blue method) as to the nature of the association is not entirely deprived of its ambiguity. When a fiber of naked nerve-plasm unites with a protoblast of naked cytoplasm, who shall say whether the connection is primary or secondary in the absence of the most intimate embryological evidence or regeneration experiments?

An important question in this connection is that as to the source of the nerve fibers. Do they arise in the prota of the skin or do they enter the skin from out-growths of the spinal ganglia? It would seem natural to conclude that the latter is the case, and yet it is not a little puzzling to see that nearly every cell in this series has its fiber. Then, too, the fact has been repeatedly observed that the protoblasts are continually dividing, even in rather large specimens of axolotl. (Fig. 20). It must be left to careful embryological studies to decide whether there are cells of origin in the skin for centripetal nerves or not. Another question must await either an embryological or pathological solution, and that is the detection of centrifugal fibers among those entering the skin. Such non-medullated fibers doubtless occur and we may think of the plexus immediately below the epithelium is the probable site.

We have sought to verify the results above described by the application of the methylene blue *intra vitam* method as well as the tissue methods used by Dogiel, Bethe and Huber. Making all due allowance for the ambiguity of these methods, it seems that the results are in harmony with those above mentioned. It is not difficult to secure impregnations in which every fiber is stained throughout its course through the corium,

but to our surprise they seemed to stop short in the vast majority of cases in the zone at the base of the layer of protoblasts, while only in comparatively few cases did we trace connections like those described by Bethe with cells of higher layers. In the chromatophore zone just ectad of the corium in many parts of the skin it was possible to trace fibers horizontally long distances and in some cases supposed communications with the chromatophores or similar bodies were noted. (Fig. 21). In most cases these cells were nearly destitute of pigment and pass by all gradations into undoubted ganglion cells.

In this connection mention should be made of the remarkable results reported by Dr. W. Pfitzner.[1] This writer claims to trace the fibers after their passage through the corium into the substance of the cells and to follow them to small knob-like endings free in the protoplasm of the cells. More than this, he traces to each cell, not only of the deeper layers but also of the stratum corneum, two independent fibers from quite distinct sources and founds upon this observation an elaborate hypothesis, which unfortunately is deprived of all standing-room by the evidence now at hand. Mr. Massie has pointed out that there is a stage in the young amphibian skin when a curious skein of a material staining deeply with some reagents is found in the cells. The senior writer, who made the preparations used by Mr. Massie, can vouch for the accuracy of this observation. It is not unlikely that the suggestion is waranted that this skein is an embryonic and transitory element in the development of gland cells, as it is not found in all the cells but in a certain class dispersed among narrower cells having a different reaction. This skein (Fig. 4) is as certainly intracellular as the nerve-fibers are extracellular in their course. Figures almost identical with those published by Pfitzner as the results of his observation can be secured by his methods, especially if the sections are taken a little oblique (Fig. 24.) The process serves to stain very distinctly the part of the nerve that is medullated, i. e. that part extending through the corium, but not that part which extends

[1] Nervenengungen im Epithel. *Morphol. Jahrbuch*, 1882, p. 726.

above the corium among the cells. Such fibers can be seen, it is true, but they are so different in appearance from the medullated part of these fibers that we are forced to conclude that what Dr. Pfitzner really saw is the intracellular skein of which mention has been made. It is a most natural mistake in the absence of more reliable methods and especially as the methylene blue process was not at his disposal. The finding of two nerve termini in each cell is apparently to be explained as a result of the fact that the base of the skein is hidden, as we found it to be in oblique or thick sections, so that the appearance figured by Pfitzner frequently recurs and if one had a preconception in favor of the the nervous structure of the element one might easily construe it as he has done. After the above we may be released from the obligation to consider the extensive and interesting theories based upon the supposed intracellular endings.

Transitional Cells. In certain regions of the skin the epithelium layer is greatly thickened and the Leydig cells are reduced in number or carried to a higher (ectal) level. In such portions of the skin, as on the dorsal region, an interesting modification of the structure above described is found. Here the lower series of cells is elongated in a direction perpendicular to the surface forming a sort of palisade type of cells. A definite wall is often apparent in the lower portion proximad of the nucleus, while the peripheral part seems to fray out into a representative of the pericellular mesh-work. Where the Leydig cells are present there is every reason to believe that these cells participate in the formation of such of a pericellular network as has been described above, but somewhat modified by the changed conditions. In a large number of cases we have observed a nerve fiber after passing through the corium seeking the base of these cells and making an intimate connection with one of them. Here the opportunity to observe the union is much better than the other case and the connection is perfect. In a certain sense these cells are intermediate between the rod cells and those that supply the pericellular meshwork. (Fig. 25.)

Dogiel[1] has shown that in the eyelids of man, for example, where the number and complexity of the sense organs is extreme, the terminal bodies consist of a covering of several connective tissue layers separated by zones of flat epithelial cells enclosing the nerve net. The nerve net is described as lying free in the interior of the bulb, though a faintly stained material was noticed and regarded as coagulated lymph which may represent cellular elements not competent to be revealed by the methylene blue method. (Fig. 9.) The nerve fiber loses its sheath before it penetrates the bulb and at once divides into spirals or coils forming a loose mesh-work. Aside from these specific cells, there are extensive arborizations and nets of fibers diffusely scattered in the epithelium at large.

In some respects the fullest description of the highly differentiated sense organs of the skin of the genitalia has been given by Dogiel and his results are pertinent to our purpose, inasmuch as he finds that all the end-organs reduce to one type—a terminal reticulum. The so-called genital sense organs and the Krause's and Meissner's bodies all prove to consist of a capsule containing a reticulum of varicose fibers and, especially in the case of the genital corpuscles, those of the same order are frequently connected by lateral anastomoses. In addition to these special organs, Dogiel traces medullated fibers into an inter-cellular reticulum within the epithelium so fine and dense as to come apparently into relations with all the cells of the deeper parts of this layer. Occasionally a branch turns peripherally and ends in a knob at some distance below the surface. We seem, then, to have evidence that the typical form of nerve ending is a close pericellular network, though Dogiel's method is not such as to allow of determining the relation of the fibers to the cells. (Fig. 11.)

The most remarkable suggestion respecting the homologies of the sense organs of the skin in amphibians is that of Maurer who thinks that the hair of vertebrates can be traced back phy-

[1] A. S. DOGIEL. Die Nervenendigungen i. Lidrande, etc. Archiv f. Mik. Anatomie, XLIV, H. 1894.

logenetically to these sense organs. Leydig in Biolog. Centralblatt, XIII, scouts this idea and derives the hair from the so-called "Perlorgan" of certain fishes. The resemblance and affinity of the sense organs is rather with the auditory apparatus, as shown by Ayers and others.

The Sense Buds. It is interesting to observe the wide differences of opinion of competent observers as to the endings in the end buds. Lenhossek (Anat. Anzeiger, VIII, 4) denies absolutely Fusari and Panisci's statement that the proximal extremity of the sensory cells in the taste bud passes directly into a nerve fiber and states that the nerves always end free in the bud, or rather form a meshwork surrounding it, thus constituting a peri-gemmal reticulum. Nerve fibers pass in a horizontal course below the epithelium and give off collaterals from time to time which form a felting of free fibers among the general epithelium cells. Essentially similar conditions prevail in the sense buds of the mouth of fishes and the author concludes that the rod cells are to be considered as short apolar nerve-cells and that the class of nerve endings found in the earth-worm is found in vertebrates only in the olfactory organ. (Figs. 15 and 16.) Retzius takes the same view, but finds that the nerve fibers are not perigemmal but intragemmal, thus illustrating the difficulties growing out of a reliance on the Golgi and methylene blue methods alone.

A. Geberg in a brief article in the Anat. Anzeiger, VIII, 1, claims to be able to demonstrate the endings of the auditory nerve in the cochlea by the methylene method, but, inasmuch as the tissues were not stained, it seems that his conclusion, that the fibers attach themselves to the hair cells without communicating with the latter, must be considered as non-conclusive.

Having reinvestigated the nerve endings in the sensory buds of the skin of the axolotl with material leaving little to be desired as to the fixation and hardening, and which had been double stained successfully, we are able to assert with great confidence that, in this case, there is a special cellular nerve terminus having a direct basal connection with a nerve fiber. The nucleus of these cells (which cannot be termed appropriately rod cells or "Stiftzelle") is narrower and more deeply stained than the supporting cells and occupies the entire width of the cell. The peripheral part of these cells has not been correctly described as yet. In reality it consists of a projection of the cell walls to form a narrow tube. These walls are delicate and very thin but easily seen because of the contrast with the protoplasmic fiber contained in it. The latter structure is delicate but stains a deep red with the picrocarmine, while the walls are not stained by that reagent. (Figs. 26-30.) This axial fiber differs not at all from that seen in the clusters found in the scattered sense organs on the head of the tree frog and the frog. (Fig. 32.) The proximal portion of the cell is not as easy to trace, for the corium and often the chromatophores obscure the connections to a degree. Yet it now and then happens that the direct communication with a nerve fiber rising through the corium can be made out. Of course it may be insisted that this connection is only a secondary one, but nothing but evidence from embryology or degeneration experiments will substantiate or refute the claim. So far as the evidence now goes, the scattered cells above mentioned and those in the buds stand or fall together, and for the former the evidence of direct continuity between cell and nerve is unimpeachable.

The Plexus Beneath the Corium.—In portions of the skin stained *intra vitam* by the methylene blue method and examined at once in glycerine very perfect views of the marvelously elaborate plexus beneath the corium can be gained. The fibers are of two sorts, the larger being connected with the fibers from the nerve bundles from the central system, while a part at least of the fibers of smaller calibre have a local origin in certain ganglion cells of this region. These cells were first detected in preparations double-stained with hæmatoxylin and picrocarmine and were seen in section in a plane parallel to the surface. In the methylene blue preparations they are very conspicuous and surprisingly numerous. The nuclei are large, while the protoplasm of the cell does not stain or only slightly with the blue. It is an interesting and most instructive fact that the cell body remains transparent, while its own neurite or axis cylinder process is mostly intensely stained through its entire length. The hiatus between the fiber and its cell is slight but sufficient to cast a doubt on the fact of communication were the conditions not absolutely favorable. With a high power it is possible to see the sheath and the faintly tinged protoplasm so that no doubt is in this case possible.

It may be noted also that other methods seem to show that it is entirely possible for the protoplasm of a cell to react differently from that of the axis cylinder derived from it. Thus may be explained many of the ambiguous and conflicting results of the applications of the methylene blue process. Fig. 33 illustrates the appearance of a section stained with hæmatoxylin and picrocarmine, while Fig. 23 is from a methylene blue preparation. Figs. 33-37 are from surface views of the plexus, showing the ganglion cells. Figs. 38 and 39 are from the same region, showing connections with vessels and chromatophores (Fig. 3.)

It will be seen that the fibers of this plexus below the corium are of two sorts. The fine fibers arise, in part at least, in the local ganglion cells and can be traced to the nerve bundles, which they enter and then mingle with the fibers of the larger sort. In the perpendicular sections it is easy to see that a certain number of fibers from the general "mixed" nerves pass without interruption into the skin and so do not participate in the formation of the plexus. Others, on the other hand, divide dichotomously in the level of the plexus and the branches give off "collaterals" that pass through the corium and so reach the epithelial layer. It is not possible to state positively that fibers from the ganglion cells of the plexus give off fibers to the skin, though such certainly is the appearance. After passing through the corium, the fibers do not all at once seek out their definite termini in the cells of the epithelial layer, but they often turn sharply at right angles at the ectal surface of the corium and pass long distances parallel to the surface. This tendency is more marked in some regions than in others. This fact greatly complicates the study of the endings. In the case of taste buds and the organs of the lateral line this is one of the most serious difficulties in the way of a correct interpretation of the appearances presented by sections.

A discussion of the theoretical bearings of these facts and further details must be deferred to the second part of this paper.

Since writing the above we have been able to settle several points previously in doubt. None of our preparations of the skin of amphibians gave unambiguous results for the glands of the skin. We have at last succeeded in securing excellent *intra vitam* impregnations in the toad (Bufo sp.) in which it is easy to trace the non-medulated fibers from the plexus ectad of the corium, and also from that entad of it, into the most intimate connection with the superficial walls of the glands, which in this species are very large and highly functional. The fibers are of small caliber but are excessively numerous and envelop the whole gland in what at first looks like a closely woven reticulum, but a close study shows that the appearance of a reticulum is due to the repeated dichotomous branching of a large number of distinct nerve fibers. These fibers cross at slightly different levels and there is no doubt in most cases of the complete distinctness of the fibers as they cross. Upon these fibers are frequent varicosities which may be due to imperfections of the process or may be the points of attachment of the fibers upon the cells of the gland. Of course this method does not admit of determining the exact relation of the nodosities to the several cells, but there can be no doubt of the existence of a very intimate and necessary connection. One is forcibly struck by the close resemblance of this periglandular felting to the perigemmular reticulum described by many authors in the case of the sense buds. The latter is, as we have before insisted, entirely distinct from and totally unlike the intragemmular endings in distinct cells which may be demonstiated by a wide range of independent methods.

The same preparations used in the earlier parts of this paper have also afforded to a more extended study a number of satisfactory views of the connection of the ganglion cells of the reticulum below the corium with fibers—not only with such as pass directly into the nerve bundles but, as we now find, with with non-medullated fibers which pass through the corium and end in relation with the cells of the epithelium layer. We also find that these and other fibers, after passing through the corium, turn and pass for long distances parallel to the surface to their final destination in the upper layer. This seems to be particularly true of the fibers of the perigemmular series of the sense buds. In some cases well defined bundles of nerves in a common sheath pass through the corium, while in those cases where the nerve sheath is present it is soon lost after passing the corium. It seems natural to conclude that the non-medullated fibers of the epithelium are essentially similar to the fibers of the same structure that supply the glands. If so, we may add that these are in both cases centrifugal and we have a suggestisn at least toward the solution of the puzzle as to the respective functions of the several classes of fibers. That the general cells of the skin have more or less power of absorption and excretion, as well as secretion, can hardly be doubted and, if so, why may not these fibers from the disperse ganglia of the peripheral sympathetic system be the neural sponsors for these functions? The methylene blue method reveals the same sensory endings in the skin that we have described fully from histological preparations, but curiously enough they appear as fibers simply because the nuclei are not stained and this fact explains the discrepancy in the two methods.

It is interesting to compare the intercellular net-work described above with the similar so-called connective tissue network described by Bruyne (Arch. de Biol., XII, 1892) surrounding the muscle fibers. The figure given in the article by the same author in Anat. Anzeiger, X, 18, is so remarkably similar to the apearance we have called attention to that one may be pardoned for suspecting similarity of nature. It may be that more than one instance of intercellular bridges rests on the misinterpretation of similar structures. The relation of the space so kept open between the cells to the circulatory fluid is a question of greater interest than seems to have been suspected.

NOTE ON THE METHYLENE BLUE PROCESS. It appears that we have had in one respect the usual experience with the methylene blue *intra vitam* impregnation process. It is not difficult to secure excellent impregnations of the nerves of the skin of the Amphibia in which the nerve fibers are deeply and quite selectively stained, yet it appears that there is a strong tendency for the stain to be extracted or rendered diffuse by the process of imbedding so that tissues which were very perfectly stained in the glycerine are quite unsatisfactory in thin section. It appears that the difficulty is in the action of the alcohol, which is required in both the paraffin and the celloidin methods of imbedding. To obviate this difficulty we have resorted with good results to the use of a mixture of gum arabic and glycerine. The fragment is placed in glycerine or may be placed at once in the gum-glycerine. After an impregnation of a day or two in a closed bottle the specimen is mounted in a paper tray with the mixture and the latter is allowed to evaporate till a consistency is reached which will permit it being placed in the microtome and sectioned. In this way sections are secured thin enough to serve the pupose desired and these may be mounted in gum-glycerine or may then be dissolved out of the gum and treated in any way desired.

EXPLANATION OF FIGURES.

PLATE V.

Fig. 1. Diagram of the skin of the sexual organs, after Dogiel.

Fig. 2. End-organs in the skin of the tree frog, original. Teased preparation.

Fig. 3. Sense bud of young salamander. Original.

Fig. 4. Skin of tadpole with nerve endings and the transitory skeins interpreted as nerve endings by Pfitzner.

Fig. 5. Skin of very young tadpole. Original.

Fig. 6. Skin of tadpole, near angle of mouth, Original.

Fig. 7. Sense bud of Amblystoma. Original.

Fig. 8. Nerve endings in the epithelium of the frog, according to Bethe.

A.—"Gabelzelle," from sensory papillæ of tongue.

B.—Cylinder cells.

C.—Isolated rod cell.

D.—Upper part of papilla.

E.—Ciliate cell of palate.

Fig. 9. Nerve ending in the human conjunctiva. Dogiel.

Fig. 10. Nerve endings in Jacobson's organ. Lenhossèk.

Fig. 11. Nerve endings in the taste buds. Arnstein.

PLATE VI.

Fig. 12. Section from the skin of the head of a tree-toad. *a*, nerve bundle and endings; *b*, gland; *c*, corium; *d*, small gland; *e*, chromatophore.

Fig. 13. Skin of head of leopard frog showing cellular nerve endings in groups penetrating the skin.

Fig. 14. Similar endings from the tree frog.

Figs. 15, 16. See Plate VIII.

Fig. 17. Part of the skin of the axolotl showing the nerve bundle on its way to the skin and the pericellular net-work.

Fig. 18. Skin of axolotl showing pericellular net-work and the nerve-fibers entering from below.

Fig. 19. Similar section fixed in Flemming's solution.

Fig. 20. A section of portion of axolotl skin where the Leydig cells (*L. c.*) are two-layered. Proliferating cells (*k*) in lower series of protoblasts; *c*, corium; *B. V.*, capillary; nerve fibers entering from below.

Figs. 21-23. See Plate VIII.

Fig. 24. Skin of tadpole as figured by Pfitzner.

PLATE VII.

Fig. 25. Section from a different part of the skin with cellular nerve termini. This is probably to be explained as the result of the elongation of the basal series of the epithelial cells.

Fig. 26. Sensory bud from skin of axolotl, showing the tubular peripheral ending of sensory cells with fine thread of protoplasm extending to periphery and the basal connective with nerves.

Fig. 27. Sensory bud from another part of skin.

Fig. 28. Similar bud in which the peripheral portion of the sensory element seems divided. Explained as due to the shrinkage and "fraying out" of the wall.

Fig. 29. See Plate VIII.

Fig. 30. Isolated supporting cells from specimens similar to Fig. 28, stained with hæmatoxylin, picro-carmine and methylene blue. Are the blue fibers nerves, or are they lines of precipitation in folds of the cell wall due to shrinkage? Compare Fig. 11.

Fig. 31. Cells from nasal cavity of leopard frog.

Fig. 32. Nerve endings from skin of same to illustrate similarity to the last.

Fig. 33. Skin of gills of axolotl to show ganglion cells beneath the corium.

PLATE VIII.

Fig. 15. Pericellular nerve fibers from sensory bud of conger eel.

Fig. 16. Intrabulbar endings in *Barbus*. (Both 15 and 16 from Lenhossèk.)

Fig. 21. Skin of the axolotl showing nerve endings in or near the chromatophores and in the skin of the axolotl. Methylene blue.

Fig. 22. Similar to Fig. 21, showing endings in layer of protoblasts.

Fig. 23. Perpendicular section through skin of axolotl stained *intra vitam* with methylene blue and cleared in glycerine. The plexus beneath the corium is clearly visible.

Fig. 29. Cells similar to to Fig. 28, stained with methylene blue.

Fig. 34. Surface view of methylene blue preparation, similar to Fig. 33, showing connection of ganglion cells with nerve bundles.

Fig. 35. Same as Fig. 34.

Figs. 36, 37. Ganglion cells of large ramose form from same layer as above.

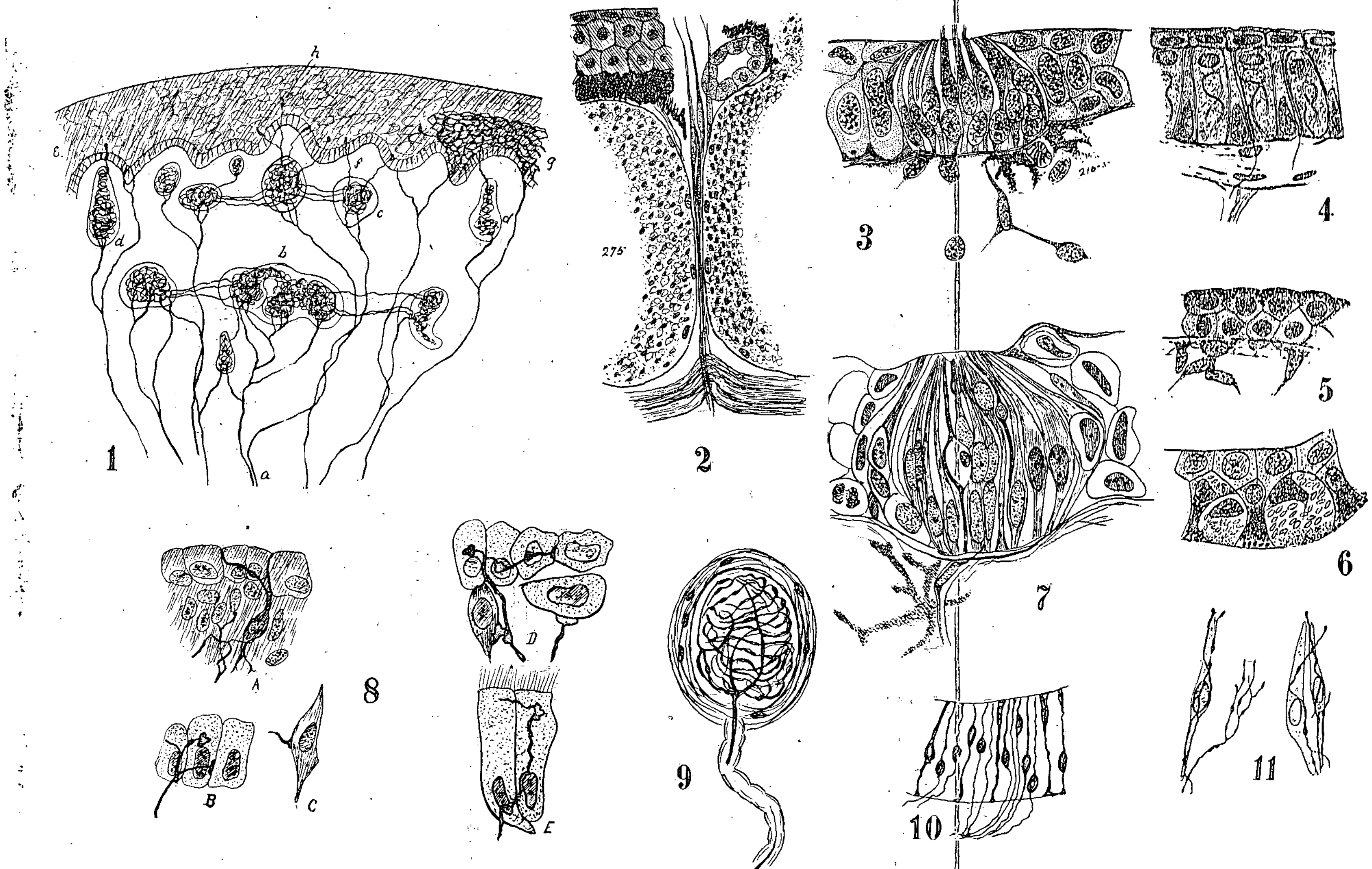
Fig. 38. Relation of nervous reticulum below the corium to the capillaries.

Fig. 39. Chromatophore-like ganglion cells.

PLATE IX.

Fig. 40. Section of the skin of the head of a toad (Bufo) after *intra vitam* injection with methylene blue and fixation with Bethe's solution of molybdate of ammonia. Examined in glycerine. The section is somewhat oblique so that the duct and part of the body of the gland is removed. The delicate non-medulated fibers are seen generously distributed over the uncut surface of the gland. Coarser fibers are also seen in the lower and upper plexuses, also a bundle of sensory rods at the left.

Fig. 41. Intra vitam methylene blue preparation of skin of axolotl, showing connection of cells of the ganglionic meshwork beneath the corium with the epidermis. *a*, fiber passing to cells of the intracellular reticulum; *b*, non-medullated fibers from a nerve piercing the corium; *c*, c^1, c^2 and c^3, ganglion cells of the plexus beneath the corium.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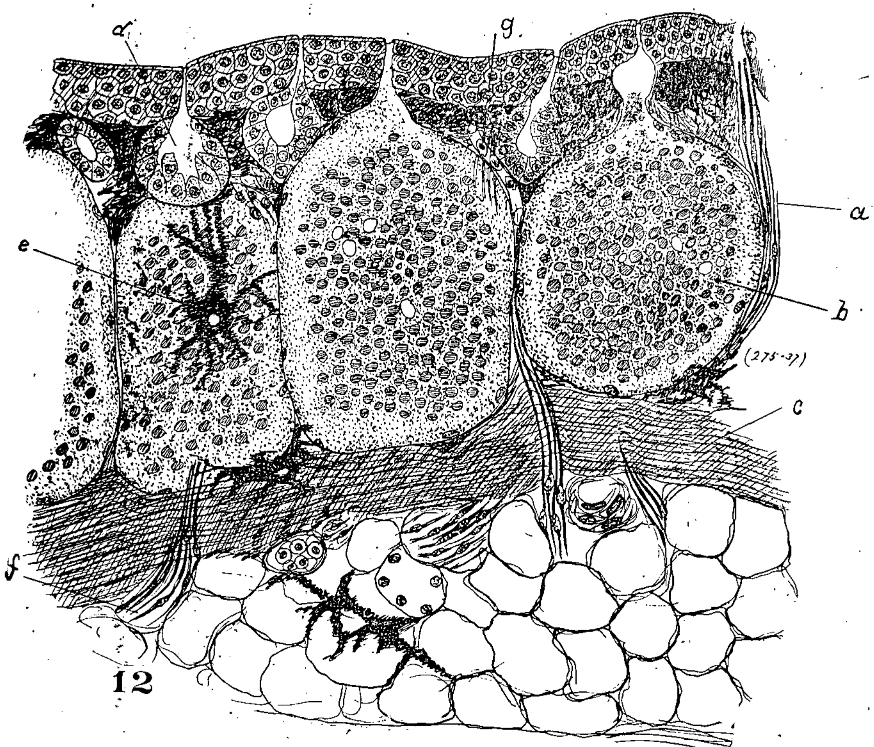

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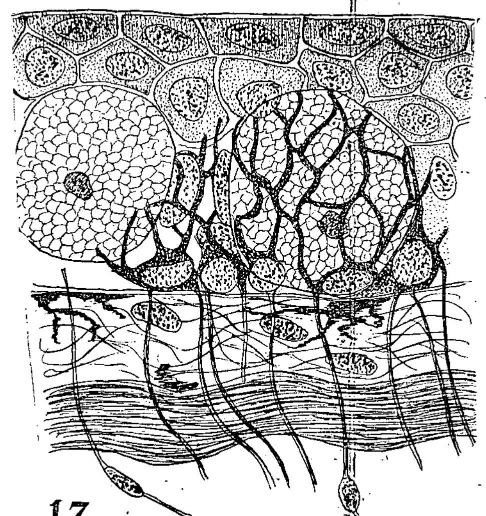

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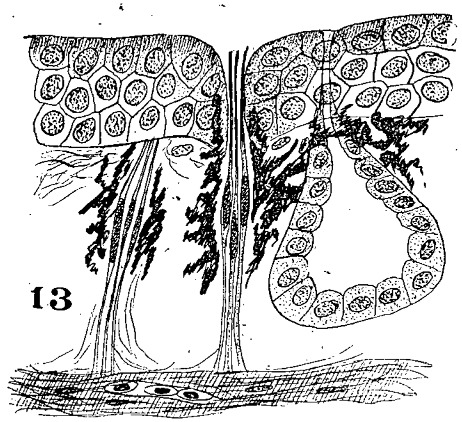

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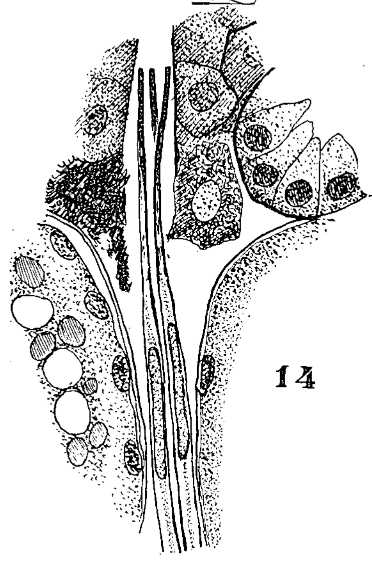

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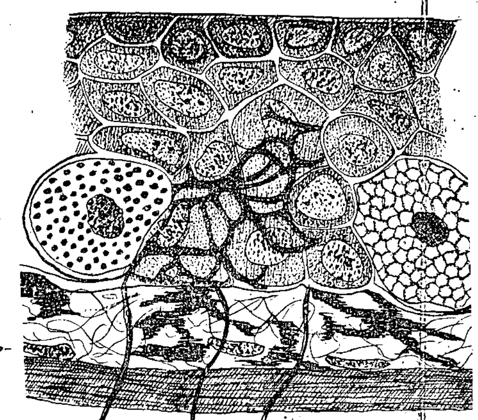

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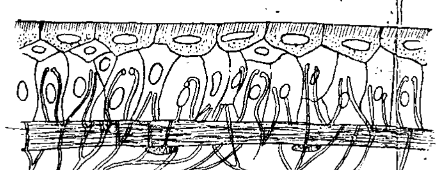

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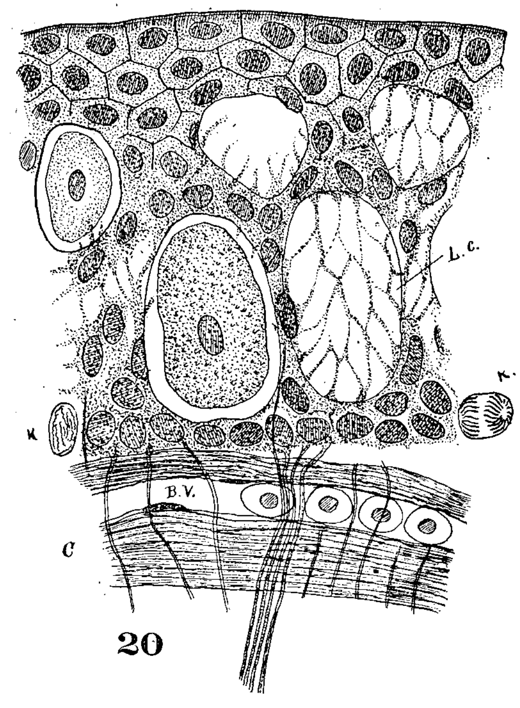

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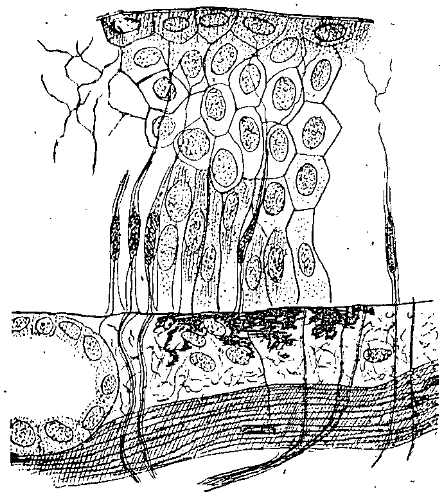

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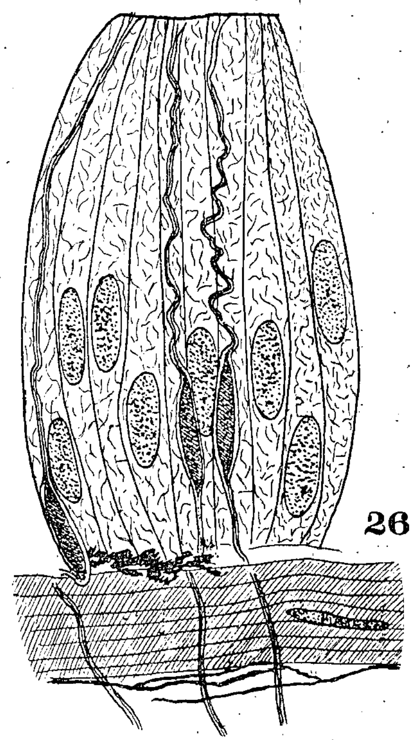

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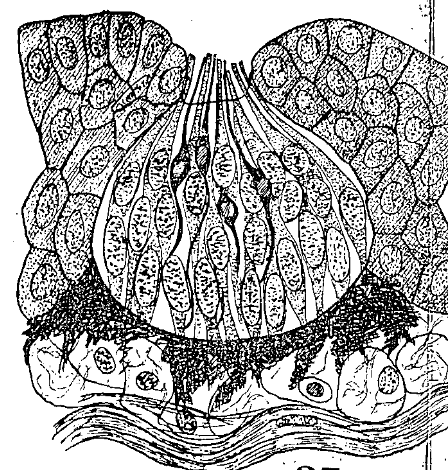

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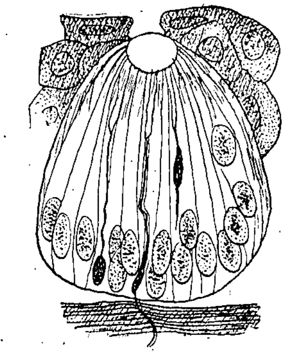

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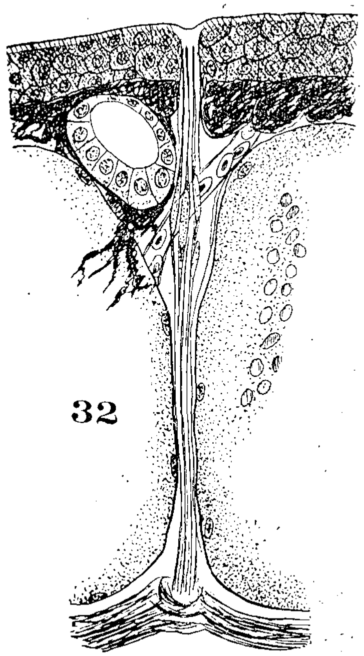

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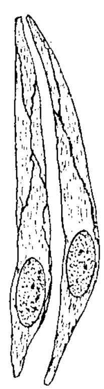

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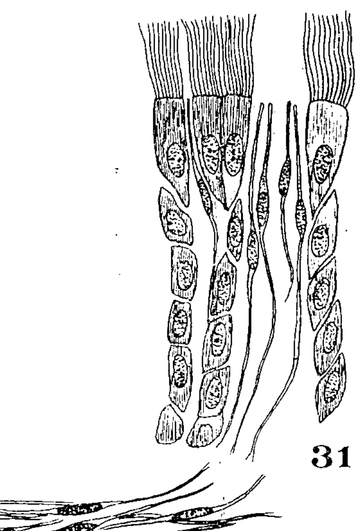

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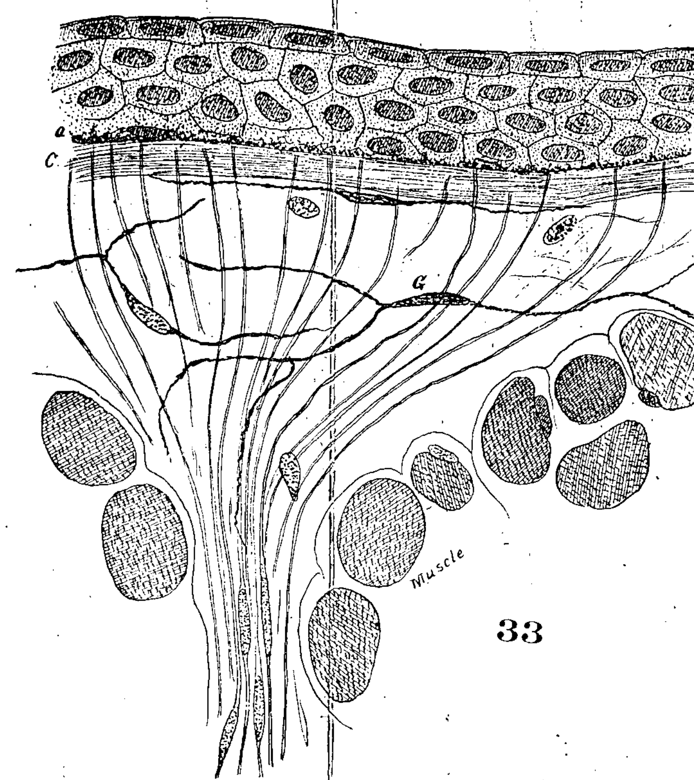

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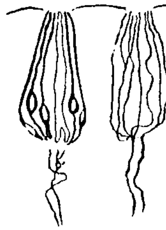

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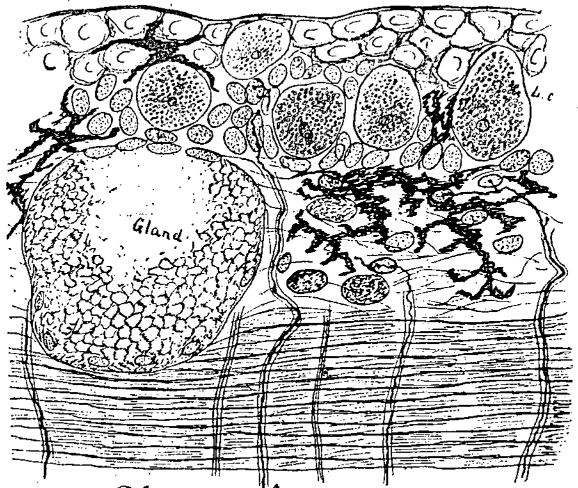

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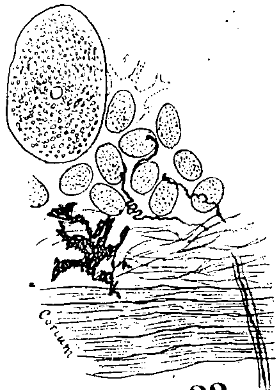

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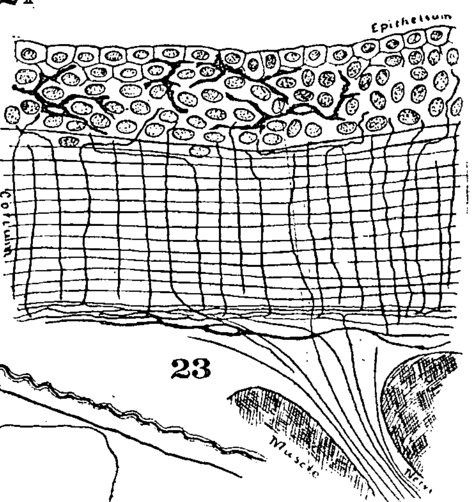

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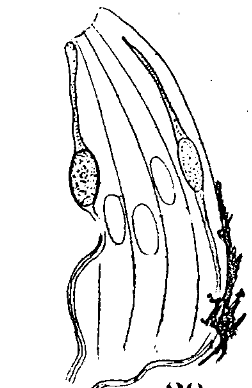

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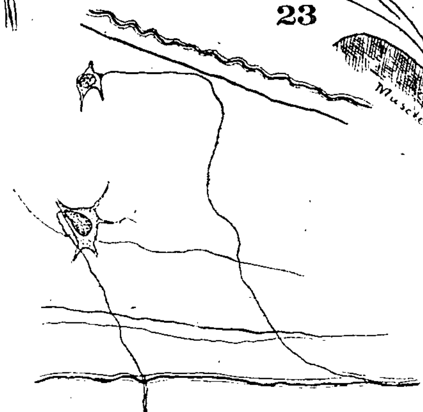

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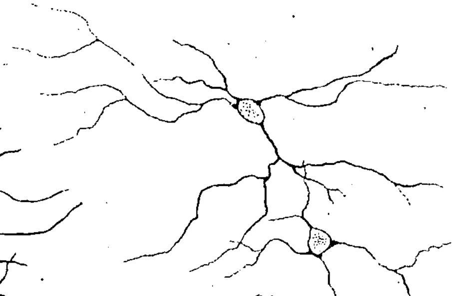

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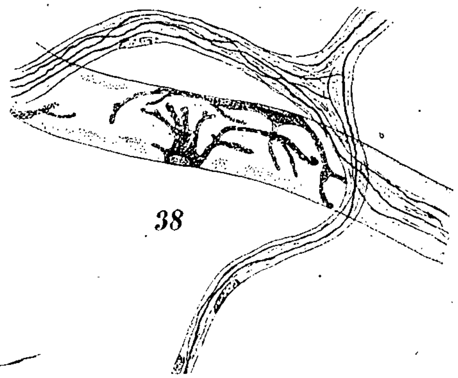

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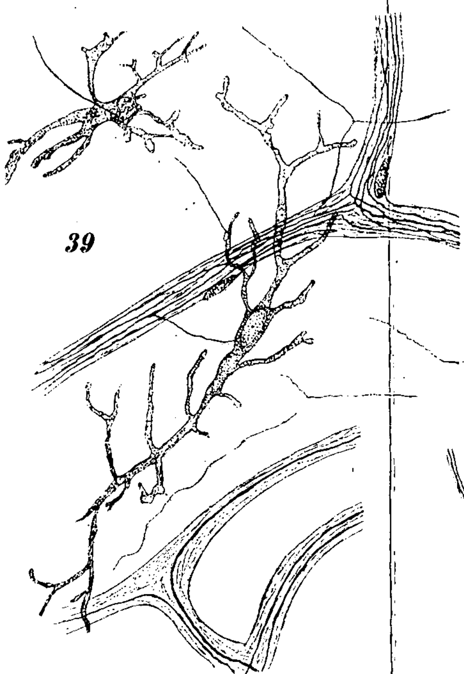

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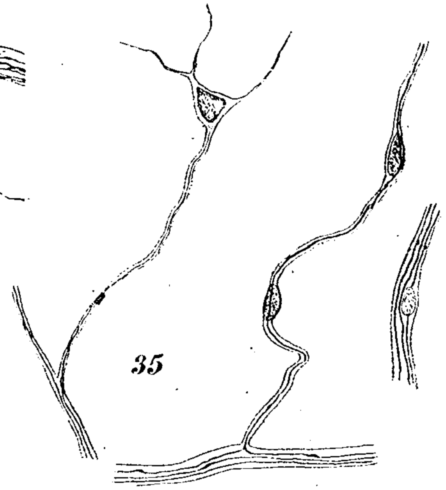

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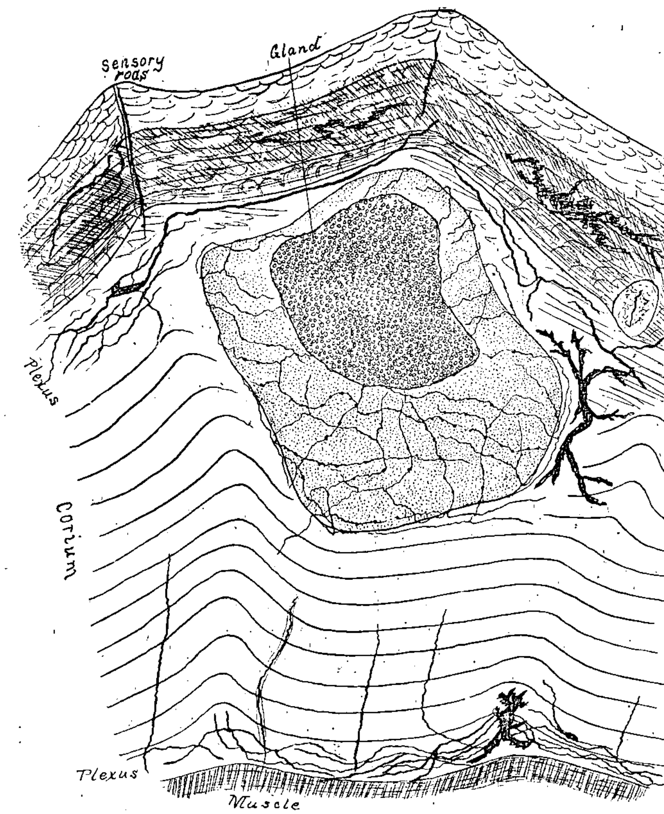

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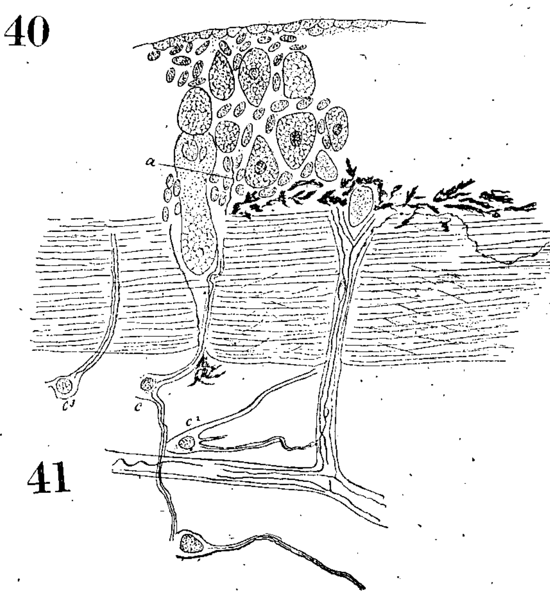

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