markable difference between the median and lateral buds figured by Gegenbaur in Siebold and Kölliker's 'Zeitschrift' for 1856; but knowing by experience the inaccuracy of his delineations, I would suggest that a link between Appendicularia and Doliolum seems to be indicated in the general resemblance of structure between the side buds of the latter, and the form of Appendicularia (fig. 5) described by me in the 17th volume of 'Linnean Transactions.'

On the Peripheral Distribution *of* Non-medullated Nerve-fibres. By E. Klein. (With Plates XIX, XX.)

After the terminations of non-medullated nerve-fibres had been discovered in the tactile corpuscles of the skin and in the Pacinian bodies; after Max Schultze had come forward with his surprising and stirring doctrine as to the special terminations of some of the nerves of the higher senses, and Krause had described what were named by him the "club-shaped extremities" in many nerves of common sensibility, as well as in those of the conjunctiva, oral mucous membrane, and genital organs; and after special terminal-apparatus had been found out in the striated muscles,—the doctrine of a terminal network of nerves in general seemed to be finally set aside, especially in the case of the nerves of common sensibility, which, according to it, break up into a network in the most superficial layers of the skin and mucous membranes, consisting sometimes of thin fibres partly medullated, partly non-medullated, or bending round to form a loop. Indeed, within the last ten years a great number of treatises have appeared on the modes of termination of the peripheral non-medullated nerves, showing in general that special terminal apparatus are to be found in the most various organs and in the most various nerves. Here I can call to mind only the termination of the nerves of common sensibility in the branching corpuscles lying between the epithelial cells of the *rete Malpighii* of the skin (1); the so-called *terminal corpuscles* of the genital nerves (2); the termination of nerves in the nucleolus of the smooth muscular fibre (3); the knob-shaped termination of the corneal nerves in the precorneal fluid (4); the ending of nerves in the corneal corpuscles (5); the nerve-termination in the nucleoli of the epithelium of the tail of the tadpole (6); and in the branched cells (7) of the same organ; the terminations of nerves in the pear-shaped structures of the

tissue of the epiglottis (8); in the nuclei of capillary blood-vessels (9); in special bodies in the mucous membrane of the urinary bladder (10); and in structures which lie between the epithelial cells of the mucous membrane of the stomach of the frog (11); the freely outrunning nerve-fibres in the tissue of certain serous membranes (12); the termination of nerve-fibres in the cells of the alveoli and of the ducts of the salivary glands (13); in the cells of the liver (14); and so on.

In regard to a number of these nerve-endings, on the one hand the very opposite has been demonstrated within the last few years; it has been shown, that is to say, that the finest nerves may be followed over and beyond these so-called special terminal structures; while, on the other hand, the existence of the same in some of the tissues has been called in question. Thus in the nerves of the conjunctiva, in the two last works (15) which have appeared on the subject, no mention is made of terminal corpuscles; on the contrary, it is shown that fine nerves enter into the epithelium, and ramify farther between its cells. In the same way it was shown that the nerves of common sensibility of the skin (16) may be followed farther towards the surface than the above-mentioned branched corpuscles are to be met with. In the case of the nerves of the tail of the tadpole, their connexion has been disproved both with the branched connective-tissue cells (17), and with the nucleoli of the epithelium (18).

Similarly the connection of the nerves of the cornea with its corpuscles has been denied (19), but again maintained (20). In regard to the finest nerves of smooth muscular fibres, it was further demonstrated (21) that they do not end in the nucleolus, but that these nucleoli are intercalated in the intra-muscular network. Finally, it was quite recently shown that the nerves of the mucous membrane of the vagina (22) and palate (23) are not furnished with terminal apparatus, but enter the epithelium to ramify farther within it, and to form networks.

The following treatise will be occupied, first, with the ramifications of the fine nerves of the cornea with relation to the anterior epithelium, the corpuscles, and the endothelium of the *Membrana Descemeti;* secondly, with the nerves of the nictitating membrane of the frog with relation to the epithelium, the glands, and the blood-vessels which it contains; thirdly, with the ramifications of the fine nerves in the canal to be found lined with ciliated epithelium in the tail of the rabbit; and finally, with the nerves of the mesentery in relation to the *propria* and blood-vessels of the same (see ' Cen-

tralblatt,' September, 1871). From the description of these we shall be able to deduce how far we are right in assuming the existence of free nerve-endings in the organs just mentioned. To obtain an easier view, we shall divide our subject into two parts. The first of these—the following paper—will treat of the nerves of the cornea, while in the second there will be discussed the nerves of the nictitating membrane of the frog, of the canal in the tail of the rabbit, and of the mesentery.

Part I.—*Nerves of the Cornea.*

After the well known and able description of the corneal nerves by Cohnheim (24), who has much enriched the knowledge acquired by his predecessors, His (25), Arnold (26), and Hoyer (27), it seems superfluous, if not ungrateful, to make these the subject of a treatise; for the description that has been given by Cohnheim of the nerves of the corneaof mammals, including every detail, was in fact, by the adoption of the chloride of gold method introduced by him, confirmed in most points by G. Kölliker (28) very soon after its appearance as a preliminary contribution. Nothing is indeed more easy than to convince oneself of the truth of the individual assertions of Cohnheim. In undertaking, therefore, to produce something upon the subject of the nerves of the cornea, I do so for two reasons: first, I believe, as my preparations obtained by a somewhat modified method teach me, that I shall be able to examine the finest nerves of the cornea in their ramifications more perfectly and richly, and thus to make some not altogether unreal additions to our knowledge of them; and secondly, I have also obtained more complete specimens than have yet been described of the nerves which run in the substance and posterior portions of the cornea, and am therefore in a position to be able to contribute, if not much, yet something, about these nerves as an amplification of our present knowledge.

First of all I might make some remarks upon the method which I have adopted. To every one who has occupied himself much with colouring with gold, and especially with the investigation of the nerves of the cornea by means of the chloride, this has been a source of trouble, viz., that one obtains at times the greatest variety of preparations under otherwise similar modes of treatment, one has to wait frequently for an exceedingly tiresome length of time until the necessary colouring has been effected, one is so much dependent upon the influencing light, and is at times entirely destitute of success; occasionally, also, the results are perfectly disproportionate to the time and pains bestowed upon the preparation. After

having sought for a long time in vain after means of guessing at these misfortunes and hindrances, the contribution of Henoicque came to my sight, according to which by placing the tissue treated with chloride of gold in a vessel filled with a concentrated solution of tartaric acid, and by putting the whole into hot water, a rapid separation of the gold-salt is brought about. Accordingly, I tried this method, and my endeavours were crowned with the best result, so that I obtained *always* and in every cornea without difference of light, and under all conditions the same preparations equally complete and equally pretty. I proceed in the following manner:—From a rabbit yet alive or just killed, I cut out the cornea with a border zone of the sclera of a few millimeters, and place it, after having removed with care the iris or ciliary body which was, in some cases, extracted with it, in a watch-glass with a pure half per cent. solution of chloride of gold, in such a manner that the convex surface of the cornea looks upwards, and the structure rests upon its scleral border. After three quarters of an hour or an hour, I remove it from the gold solution and transfer it, in the same position to a vessel containing distilled water. Here I allow it to remain from six to ten hours in the light. After this time the colour of the cornea, which was at first yellow from the action of the chloride of gold, is replaced by a light grey or steel grey. I then place the cornea so coloured in a small glass flask with a wide neck, in which is a small quantity (five to ten cub. cent.) of nearly concentrated filtered solution of tartaric acid. As soon as the cornea has imbibed this fluid and sunk to the bottom of the vessel accordingly, we remark that its colour has become much deeper, it has become more or less of a greyish violet. I now immerse the flask in a capsule into which has been poured as much water at 40° to 50° C., as, at least, corresponds to the surface of the tartaric acid solution. After a very short time, often in a few minutes, the preparation assumes an intense violet-red colour, which continually increases until at last the cornea, when the water has quite cooled, appears of a dirty dark brown-red colour, and with a shining velvety surface. I now lift it out and wash it for two hours or more either in common or in distilled water. From eight to twelve hours have now been required since the colouring with gold. I have prepared a great number of cornea of the rabbit in this manner. I cut out the cornea usually between eight and nine o'clock in the morning, subject it to the various processes enumerated, and between four and five p.m. the same is as darkly coloured as possible, and on being washed

is perfectly fit for preparation. If the cornea is cut out fresh, coloured in good gold solution, and carefully treated afterwards, success is certain. The flake-preparation which I employ for the study of the fine nerves lying surperficially, I make by pulling off the epithelium along with quite a thin layer of the corneal substance by means of a pointed forceps beginning at the scleral border—a proceeding which after some practice is accomplished without difficulty. Vertical as well as longitudinal sections are employed with good results. On such a flake preparation just mounted there appear not only the plexus of the more deeply-lying nerves, but also those lying in the most superficial layers of the *propria* of the cornea. Besides these we see many of the fibres which form the subepithelial network, and several of those which run in the epithelium. What, however, comes into view with greater clearness is, that the epithelium is quite homogeneous over large tracts, and with moderate powers we can distinguish none of its elements. We recognise only the upper and under surfaces, respectively the anterior and posterior bordering surfaces of the same. The former is recognisable by means of the precipitate lying irregularly dispersed upon it, the latter by an extraordinarily pale mosaic, in some places more, in other places less indistinct. With stronger powers (450 diam.) we may even recognise just a slight indication of nuclei in the flat cells lying superficially. Further on we shall see of how great advantage this condition of the epithelium is in tracing the nerves within it. It is a matter of no consequence whether we preserve such preparations in the light or in the dark. After one or two days they become decidedly darker and the nerve-fibres are perfectly visible to their finest ramifications. Since the preparations thus become darker, it is very important that their thickness should not exceed a certain degree. If we have taken care that they contain only the epithelium and the most superficially lying layers of the corneal tissue, so, indeed, that the preparations may be penetrated through its whole depth by No. 8 Hartnack, we may be sure that they will be quite useful for a long time to come. I have in my possession some preparations which were made more than six months ago; and yet if the examination of these be only undertaken with good light, the relations of the finest nerves are to be seen with all the perfection to be desired.

It is by no means part of the plan of this treatise to describe minutely the distribution of the more deeply placed plexus of broader nerves, nor the subepithelial network of the fine nerve-fibres. This is all the more unnecessary, since I could

not produce much which could amplify or modify the complete statement of Cohnheim on this subject. I shall only allow myself to make some remarks upon the structural conditions. These remarks apply both to the plexus of the broad nerve-fibres, and to the subepithelial networks of the fine ones.

If the nerves are followed from their entry into the cornea uncut where they form a plexus in the tissue by the successive division and anastomosis of their branches, it is found on the one hand that the neurolemma, with its oblong nuclei, is prolonged from the trunk on to the branches, and on the other that the individual nerve fibrils of the trunk at the same time lose the medullary sheaths, but do not do so at corresponding points in their course. The branches, moreover, of the same trunk do not lose their sheaths at corresponding points in their course. In the non-medullated parts it may be recognised that the colourless or pale reddish neurolemma contains only bundles of delicate filaments, with varicosities at more or less regular intervals, which filaments may run more or less parallel to each other, or twine round one another in spirals. At the points at which a bundle of this kind divides into smaller bundles, or at which fibres branch off laterally, there exist enlargements of triangular form, the neurolemma being prolonged from the trunk on to all the branches.

The filaments of the branches of this plexus, which are to be met with in all parts of the *cornea propria*, are consequently to be regarded as bundles of fine fibrils, which were separated so long as they formed a series of single axis-cylinders.

In addition to the points already mentioned as to the arrangement of the non-medullated fibrils, another peculiarity is to be noticed. The fibrils form within the neurolemma, particularly at the points of enlargement, a mesh-work of extraordinary closeness, which is produced by the bifurcation and coalescence of neighbouring fibrils. The interspaces are rhomboid or oblong.

In the anterior superficial layers of the corneal tissue, minute bundles of fibrils spring from the plexus, which lose themselves in the subepithelial network. Cohnheim has explained in the most complete manner the differences which this network presents in the central, peripheral, and middle parts of the cornea. Cohnheim's research is so well known, that it is scarcely necessary to give quotations. I will content myself with adding to his description, that as well on the central as on the intermediate zone, between centre

and periphery, finer bundles of fibrils spring from the plexus of thicker fibrils, which either immediately after their origin spread out into individual fibrils anastomosing with each other, or give off fibrils in succession in a manner resembling the mode of branching of a weeping willow; these, like others, reticulating with each other.[1] It is at all events certain that the subepithelial network, whether its arrangement is that of a trellice-work or a network, consists of extremely fine fibrils, which are marked by the possession of varicose swellings. It is maintained by Cohnheim that fibrils of extreme tenacity spring from the subepithelian network in a direction almost vertical to the surface, finding their way between the deepest cells of the epithelium; and that after giving off connecting fibres in the middle layers, they can be traced to the surface where, although some of them terminate among the superficial layer of cells, they mostly end in filaments with terminal knobshaped swellings (Endknöpfschen), which float free in the præcorneal fluid. As regards this distribution in the epithelium my preparations show the following:

The fibrils which rise among the pallisade-shaped epithelium, give off very numerous fibrils, often only distinguishable as linear series of granules, which take a horizontal course on the ends of the columnar cells next the surface. These horizontal filaments wind among the epithelial elements in zigzag lines, and are connected together both directly and by lateral branchlets, and thus form a network which is not much inferior to the subepithelial network in density. After giving off the horizontal branches, the vertical fibrils wind in a convoluted manner towards the surface. In this part of their course filaments spring from them which bifurcate, and either anastomose together, or accompany them towards the surface. The nerve fibrils which are to be found in the superficial layers, exhibit here and there in their course, in addition to the minute varicosities above described, swellings of relatively much larger dimensions. Separated from the surface by one, or at most two, layers of elements, they form a very dense network of fibres, the meshes of which are smaller than those of the deeper network, and the fibres (which have

[1] While this is going through the press, I have made out in preparations from which only the anterior epithelium had been removed, that the network which is so well drawn by Cohnheim, and which is seen in my figure 1, Pl. XIX, by no means contains the finest and most numerous fibrils. With an immersion 10, I have discovered an immense number of the very finest fibrils given off from each of those seen in fig. 1; all of these run nearly parallel with one another, join with one another with similar fine fibrils, and are, without exception, marked by regularly placed bead-like enlargements. A drawing of these fibres will be given in the next part of this essay.

a more or less winding course among the epithelial elements) somewhat thicker.

Finally, fibrils spring from this superficial intra-epithelial network which, after attaining the free surface, divide into two branches. These at first run horizontally in opposite directions, and soon take a course towards the depth of the cornea, and lose themselves in the superficial intra-epithelial network above described.

No one will be disposed to dispute that it is a matter of great difficulty to determine in a preparation in which the epithelium-elements are not distinctly seen, whether a nerve filament attains the under or the upper surface of the most superficial layer. I cannot, however, regard this difficulty as insurmountable, on the following grounds:—It is in the first place to be borne in mind that by comparison of surface-preparations with vertical sections of the same part of the cornea, it is always possible in any given preparation to determine whether or not the whole of the epithelium is present. Further, we have a certain guide for the recognition of the true surface in the precipitate which adheres to it. Secondly, I have observed when I have exercised the greatest caution in cutting the cornea and in preparing it, that the more perfect the preparation was, *i. e.* the more completely the intra-epithelial network could be distinguished, the more rarely did it happen that filaments could be seen either on the surface or between the superficial cells which could not be shown to be connected on either side with others. It follows, then, from this that the terminal knobs of Cohnheim are only intercalated swellings occurring in the course of fibres, and that the appearances he has described depend on imperfections in the preparations.

To sum up what has been stated as to the intra-epithelial network of the cornea of the rabbit, we have to distinguish two nervous networks, the one at a level corresponding to the ends of the pallisade-epithelium, the other separated from the surface only by one or two layers of cells. The former we propose to designate, the *deep intra-epithelial network;* the latter *the superficial intra-epithelial network.* The latter is distinguished from the former in the greater density of the network, in the greater thickness of the fibres, and in the existence (in addition to the smaller varicosities which also exist in the deep network) of yet larger swellings above described, which are merely found at the junctions of two filaments. From this network filaments spring similar in character to those of which it is formed,

which reach the surface; they do not end there, but return towards the depth of the cornea.

We come now to the second division of this part of our undertaking, that is, the nerves of the substance of the frog's cornea. The results to which I am led by the investigation of the same were obtained by the following methods. I must, however, first state that although very fine nerves of the frog's cornea have been minutely described in fresh preparations, though chiefly in such as had been treated with gold chloride, they are not always and not so completely to be made out by the ordinary method of gold preparation as in that now to be described. I pass a silk thread through the centre of the cornea of a healthy middle-sized or large specimen of *Rana esculenta*, and bringing it out again at the sclerotic ring, tie in it a loose knot to hold it fast; in short, I proceed in the same way as one does in inflammation studies. After the thread has remained from five to eight hours in the cornea, I cut out the latter with the greatest care, allow it to remain from a quarter to half an hour in pure half per cent. solution of gold chloride, and place it then in distilled water so long as the action of the light lasts, that is, until it has obtained the well-known dark violet-red or red-brown colour, a space of time which varies according to the season from one to three days. Then I tear off from this the epithelium, together with a very thin layer of the corneal tissue, and enclose the remaining portion in glycerine. We must assume that the reader is too well acquainted with the characteristics of the corneal corpuscles, both the still normal, beautifully branched ones, as well as those exhibiting already some slight change, and the wander-cells present in some places sparingly, in other places abundantly, than that we need go into greater detail here concerning them.[1]

[1] The controversy concerning the cells and plasmatic channels (Saftkanälchen) of the cornea substance, which has been going on during the last few years, will, as well as the physiological and pathological characters of the lymph system and the cellular elements of connective tissue, be treated at length on another occasion. Only one remark will here be made. Those who maintain that the sharply-marked, clear, branched figures, embedded in a yellowish-brown ground substance, which come out in a corneal tissue after treatment with silver, and which correspond to the well known beautiful branched flat cells with oblong flattened nucleus, produced by gold treatment, do not represent the cellular elements of the cornea, but are occasioned by coagulation, fissures, or the like, in an interfibrillar albuminoid substance, and who take their stand upon this—that no one has yet succeeded, nor ever will succeed, in demonstrating in the silver figures the branched cellular elements,—these persons, I say, I would advise of the following facts. When the cornea of a living rabbit is rubbed with lunar caustic so long that a great portion of the interior epithelium is

We have to deal here only with the nerves. The nerve-trunks form, as is well known, in the corneal substance by division and anastomosis a rich plexus. What we have said with regard to the plexus of the rabbit's cornea holds true equally of this in the frog. Here also we find the nucleus-bearing sheath of the same to be a prolongation of the neurilemma of the trunk, and so also the fibrillæ possessing granular enlargements and embedded in the sheath, exhibit the same relations to one another as those of the rabbit's cornea. We find them here also running side by side, stretched or undulated, or spirally entwined, or, finally, in many places by dichotomous division, forming a network within the sheath.

We may distinguish these branches of the plexus as *nerves* or *bundles of the first order*. From them branch forth smaller bundles, which for a short distance have a serpentine or rectilinear course. They possess no sheath of Schwann, and hang together by a few anastomoses to form a not very dense plexus. These we will call *nerves* or *bundles of the second order*. They give off after a longer or shorter course numerous lateral fine fibres, or terminate in several such fine fibres arising at one point. These we will call *nerve-fibres of the third order*. They are distinguished by the following characters:—(*a*) Apart from their size, varying only within small limits, they possess more or less regularly placed varicosities stained dark by the gold. The clearer portions

removed in the form of a cauterized membrane, and then after an interval of a quarter to half an hour, a few drops of a twenty per cent. solution of gold chloride are allowed to fall on the ash-coloured cornea, and then, after fifteen to twenty minutes the cornea is sliced off with a razor, and for four and twenty hours is placed in the light in very slightly acidified water, and is examined by means of horizontal sections, or by thin layers torn off, we find remarkable appearances. More or less darkly grey-coloured areæ are separated from circumscribed violet-red-coloured areæ by darkly red-coloured intermediate areæ. In the first of these the branched spaces are seen embedded in a yellow-brown ground substance, in the second the same spaces are embedded in a violet-red ground substance, and in the third we find only sharply-outlined, uniformly-granulated, branched, dark-violet-red corneal corpuscles. The nearer one approaches the central region, the more clearly do the characteristic nuclei of the corneal corpuscles make their appearance in the spaces; and, secondly, a substance at first of a pale blue, then violet, then dark violet, is more and more to be seen filling up the spaces. It is possible, without much trouble, to find places where branched clear spaces and branched dark and violet coloured cells, sharply defined in all their parts, are so situated that the processes of the coloured cells on the one side project into the channels of the clear spaces, and on the other side are in connection with the processes of other red stained cells. I cannot conceive of appearances more significant than those which the method just described afford. Should, however, any one still maintain in the face of these facts, that after all these very figures stained by the gold are not in reality pre-existing formations, then I cannot help him further.

of such fibres lying between the varicosities appear, with a high magnifying power (immersion 10), striated, as if made up of fibrillæ. (*b*) They possess a nearly completely direct rectilinear course, and bend after a longer or shorter course into a direction which is at right angles to the former one. (*c*) They remain for long distances unbranched. It is not rare to find a field of view with the microscope, where, out of the enormous number of fine fibres crossing one another in all directions, relatively few give off a lateral branch at right angles, which connects itself at a similar angle with another distant fibre. This is found to be true of all the fibres of this order when they are followed out—*i. e.* that they are connected one with another by cross fibres running at right angles to them, and in this way a rectangular trellice-work is formed. In a successful gold cornea from which only the outer layer of epithelium has been removed, it is quite impossible to say precisely, on account of the great richness of the network of the finest nerves, and of the network of the processes of the corneal corpuscles, whether the fibres of the one come into anatomical connection with the processes of the other. It is otherwise, however, when one splits up the cornea into as fine lamellæ as possible, and makes use of these for the investigation. In such a preparation where the corneal corpuscles are present only in one layer in some places, in others in two layers, we see finer fibres passing off at right angles from the fibres of the nerve trellice-work, which finer fibres are also beset with globular enlargements. Two or more of these finer fibres pass directly on to a corneal corpuscle, on the surface of which they divide themselves into short branches, and join together by these branches reticularly.

I could not convince myself of a penetration of these fibres (which may be called *fibres of the fourth order*) within the substance of the corpuscles, nor of a connection with the nucleoli of the nuclei of the corpuscles. Where I find these connected fibres in my preparations forming a terminal network—and as such I will venture to designate them—I observe them always to lie on that surface of the corneal corpuscle which is directed to the outer surface of the cornea.

In preparations, which consisted of the hindermost layers of the cornea, indifferently whether they were mounted with the endothelium (28) of Descemeti directed upwards or downwards, I found fibres of the fourth order, which I was able exactly to follow into the layer of the endothelium. This is the easier, since one has no trouble from the interference of

the lines of the cement substance of the endothelial cells in this preparation. We find the *Membrana Descemeti* in this case covered with a more or less granulated violet coloured substance, in which clear nuclei, some ovoid, some constricted, are inlaid at nearly regular intervals. Only here and there can we see between the nuclei pale contours, by means of which areæ of more or less polyhedral shape are delimited. Corresponding to these contours run our dark, varicose, finest nerve-fibres. In some of them dichotomous division can be seen. They withdraw themselves, however, completely after a short course from our view. I may once again mention that we have here to do with fibres which can be traced to the fibres of the third order lying beneath (that is, deeper in the substance of the cornea than) the *Membrana Descemeti*.