NERVE TERMINI IN THE SKIN OF THE COMMON FROG.¹

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PART I.

With Plates IV and V.

The study here presented is a continuation of that which was begun last year under the direction of President Herrick and the results of which were published in Vol. VIII, Nos. 1 and 2 (combined) of this JOURNAL, under the caption, "The Somatic Equilibrium and the Nerve Endings in the Skin."² In this discussion we dealt with the skin of both Anura and Urodela. In the present study the frog only is considered; and the points further discussed here which were presented on pp. 37-51 of the volume referred to are: the isolated sensory cell or terminal cell, the ganglionic plexuses, and nerve termini on glands. Our belief with reference to these structures is supported by methods not used formerly, and other conditions related to them have come to our notice. The latter have to do chiefly with the musculature and innervation of certain glands.

The results of our earlier study of the nerve endings were obtained by the haematoxylin, picrocarmine and methylene blue stains, and by the use of chrom-acetic acid solutions as fixing agents. For fixing we have since used other modifications of the chrom-acetic acid preparations, and Flemming's solution in

¹ Thesis presented for the degree of Master of Science at the University of New Mexico.

² The present installment includes simply the author's results; an historical résumé may be expected with the second part which will be devoted to the embryogenesis of the skin.

addition; with application of Delafield's haematoxylin, Benda's Sulphate of Iron Haematoxylin, and Weigert's Haematoxylin. Further use has been made of methylene blue *intra vitam* preparations, and the silver method has also been employed.

The methods just mentioned show that free-ending nerve cells occur in large numbers in the skin on the head of the frog, that these are usually found in clusters of three or more, and that these clusters tend to be grouped to such an extent that they appear, in our best preparations, over a comparatively large field with varied frequency, while in an adjacent area they are entirely absent. For their distribution and general arrangement in the surrounding parts, the best results have been obtained from tissues hardened in the chrom-acetic acid and Flemming solutions and stained with Weigert's Haematoxylin. (Fig. 1.) In this preparation numerous clusters of fibers are seen to pass ectad through the corium and terminate in long rod-like cells in the Malpighian layer. The continuity of the terminal cell with the nerve is certain. In instances otherwise clear this point is made doubtful by the presence of pigment, but such evidence is now in hand that the continuity of cell with nerve must be accepted. This is shown also in the methylene blue preparations, in which case the deeply impregnated fiber has been seen to pass in a similar manner through the corium and, clearly differentiated from the neighboring pigment, to continue into the terminal cell, the wall of which is continuous with the sheath of the fiber. (Fig. 4.) The terminal cells are clearly differentiated from the neighboring epithelial cells. They are much longer than the latter, extending through nearly the entire height of the Malpighian layer, usually appearing to end just short of the horny surface of the skin. Their special cavity through the epithelial layer and opening on the surface which has been seen in other instances is not found in the later preparations. On the contrary, the terminal cells often diverge at the base of the Malpighian layer, the epithelium cells crowding in between them. This discrepancy in results may be due to the difference in the methods employed in the two instances, though it more likely indicates that the structure in question is

of a primitive and embryonic nature, becoming obscured in older tissue. The nucleus of the terminal cell, in accordance with earlier descriptions, is long and narrow, as are also those which appear in the nerve in its passage through the corium. Here the nerve is shown by both Flemming and chrom-acetic acid preparations stained with the Delafield, Benda and Weigert haematoxylins, and also by the methylene blue and silver impregnations. (Figs. 1-6.) The Weigert being primarily a sheath stain, the fibers are frequently seen with difficulty by that method, and to be seen at all require an exact decolorization. In special instances this process was observed under the microscope and the tissue removed at the proper stage of differentiation. The best results, however, for showing the structure of the nerve in its passage through the corium came from a teased methylene blue preparation (Fig. 3). In this special case the Malpighian layer was in part torn away, but the glands and other structures of the skin further entad were intact, so that the fiber could be traced with but one short interruption from immediately beneath the Malpighian layer to near the base of the corium. The sheath, also, for the greater part of this distance, was clearly differentiated from the fiber. Other similar fibers associated with it were tracable for a shorter distance.

The ental destination of these fibers from the terminal cells is difficult to determine with absolute certainty. However, in methylene blue and silver impregnations, they are traced for some distance beneath the corium and in a plane nearly parallel with it (Figs. 5 and 6), while with the Weigert method they have been seen to enter small bundles of fibers immediately beneath the skin. (Fig. 2.) This indicates that they are branches from the plexus of nerve fibers and bundles which is known to exist in this region. If they are such, the innervation of the terminal cells on the head, as has been demonstrated from the head of the tree frog for this study especially, must be by the frontal branch of the fifth nerve.

A discussion of the nerve termini on the glands requires first a reference to other structures related to them. The glands in the skin of the frog, as is well known, are of two classes,

distinguished here as the ental and the ectal series. The glands of the ectal series are distributed quite regularly throughout the skin of the head, and occur together with those of the ental series. In the latter case they are much smaller than the glands of the ental series, being forced peripherad into the interstices of the ental group which in places constitutes nearly the entire integument beneath the Malpighian layer.

Where the ental glands do not occur, those of the ectal series may become very large in some instances, extending through nearly the entire height of the corium and becoming very closely crowded together. But regardless of position or size, their structure is universally the same, and they are thus easily distinguished from those of the ental series. The peripheral cells of the ectal series are approximately cubical, becoming lengthened or depressed according to the shape of the gland and their position in it. The walls of these glands are made up of such cells, supported externally by fibers of connective tissue. There appear no differentiated muscular elements connected with them. On the glands of the ental series the peripheral structure is entirely different. As with the ectal series, there is a tendency for the connective tissue to fold about them, but within this tissue, lying compactly on the surface of the gland, is a complete tunic of non-striated muscle cells. In a section through the median plane of the gland this arrangement is not noticable, but in sections just denuding the periphery of the organ the form of these cells is plainly shown. They are broad in their middle part and taper to a more or less acute point at either end, being very much flattened throughout their entire length. Their nuclei are large, elongated and rounded at the ends. Each cell seems to be entirely separate from its neighbor, there being frequently a considerable space between them. This structure was first observed in tissues fixed in Flemming's solution and stained by the Weigert method. (Fig. 7). It has since been verified by sections of the same preparation stained by the Delafield and Benda methods; in similar preparations double stained with picrocarmine; also by the same stains applied to tissues fixed in the chrom-acetic acid solution,

and by the methylene blue method. The normal position of these cells seems to be with their axis approximately perpendicular to the surface of the skin, though in one instance a part of them seemed to lie obliquely. This single exception to the rule may, however, be interpreted as an artificial arrangement. It can be affirmed with certainty of these cells that their function is muscular. This position is sustained by the application of methylene blue which reveals a liberal nerve supply to them, and the typical form of muscular nerve endings on their periphery. (G. C. Huber and DeWitte, this JOURNAL, Vol. VII, 3-4, Fig. 25.) In one instance (Fig. 9), sketched under the oil immersion lense, the evidence by lower powers was sustained. Nodosities, stained a deep blue, are seen to be connected by a fine fiber, lying in a position corresponding with that of the tunic cells. At first there seemed to be a possibility that this appearance was due to a precipitation between the cells, and that it sustained no definite relation to them. But on closer examination it was discovered that there were a number of these cells sufficiently impregnated to show conclusively that the fibers with their varicosities lie, not between the cells, but upon them. In another instance, an individual cell teased from the gland showed the same arrangement. Two plainly differentiated nerve fibers with occasional nodules lie on the surface of the cell as if firmly attached to it. Furthermore, on three successive glands of this series non-medullated nerve fibers were seen to pass from the ental portion of the corium to the glands, and to distribute themselves there among the fibers and nodules on the cells, and in some cases the fiber itself assumed the varicose form and became very diminutive. These fibers going to the glands are smaller than those going to the terminal cells, and their nuclei are also smaller and shorter. This nerve connection is apparently sustained by the other methods employed, excepting only the Golgi, but the evidence from these methods is not such as to be of itself convincing.

As yet it has not been possible to trace these fibers to an entrance into a bundle or as a single fiber for any distance beneath the corium, as was done in the case of the terminal fibers.

The problem of their ental destination is probably involved with that of the ganglionic plexus, to be described.

In the skin from the head of the frog treated with the silver method, there is found at the base of the corium, a relatively thick stratum containing a most intricate plexus of ganglion-like cells with their fibers. This plexus is to be distinguished from that which was mentioned in connection with the ental course of the fibers from the terminal cells. The latter lies farther entad, and is composed wholly of nerve bundles with their ramifications. The ganglionic plexus occupies the base of the corium. In some instances the elements of the two may mingle, but their structures are wholly different. In many instances the fibers of the ganglionic plexus are so numerous that the central portion of the stratum becomes perfectly opaque with the stain, while the ental and ectal edges show a purely fibrous arrangement. In other localities of the stratum there are blackened areas showing the structure just described but united with each other by numerous plainly differentiated fibers. In still other instances there are large areas in which the structure is purely fibrous. (Fig. 11 and 12.) At first sight there seemed to be a possibility that this appearance might be due to the presence of extensive pigmentation; but examination of sections of the skin from the same region fixed in the chrom-acetic acid and Flemming solutions and stained as previously mentioned showed no pigmentation whatever in this part of the skin, while sections treated in methylene blue showed that there might be occasionally a granular pigmentation in this region and that typical chromatophores occur very seldom. By reference again to the sections treated with the Golgi method, it was found that the stratum impregnated universally had a fibrous structure in part, and that, in relatively large areas where there was the clearest differentiation of the stain, the structure was purely fibrous. In the latter instance, if the appearance in question were due to the impregnation of chromatophore elements, it would be expected that the stain would first select the body of the chromatophore and not bring to light its phenominally long ramifications, if the fibers could

possibly be interpreted as such. Furthermore, an occasional chromatophore was found in the immediate vicinity of these elements, which differed widely, both in structure and in reaction to the stain, from the cells and fibers under consideration (Fig. 14). Frequently a number of these cells are matted together in such a way as to form an irregularly shaped body, from which numerous fibers of variable length ramify in all directions. Often the fibers from different bundles are so closely woven together that they appear to be continuous from one cluster to the other, as indeed they may be. This type of cell, however, is not confined to the stratum at the base of the corium. It tends to wander peripherad. In such cases fibers from one end of the cell have been traced for long distances till they became lost in a complex of fibers, the destination of which is in the stratum just described. (Fig. 13.) From the other end of the cell, shorter fibers ramify among the surrounding tissue. In some instances they seem to attach themselves to the periphery of the glands of the ectal series.

The function of the fibers arising from the lower stratum, however, seems to be more intimately related to the glands of ental series, for these fibers pass ectad in marvelous numbers and embrace the entire surface of the glands. This condition was demonstrated by the projection upon a median plane of drawings made with the camera lucida from consecutive sections. (Fig. 8, A-E).

These fibers do not seem to be identical with those described as innervating the tunic cells of the ental gland. They differ from these in their distribution and relative positions. They correspond, however, with the structures described by President C. L. Herrick, in this JOURNAL, Vol. VIII, p. 51 (Fig. 40, reproduced as Fig. 15, Plate V, of this article). In this instance it was found that a net-work of non-medullated fibers crossing at slightly different levels appeared on glands in the toad, and that these fibers apparently were connected with the ganglionic plexus at the base of the corium, though the latter point was not certain. This relation, however, is now satisfactorily demonstrated. It seems, there-

fore, that there are two groups of nerves passing to the glands of the ental series; the one attaching by the typical endings to the enveloping muscle cells, the other ramifying promiscuously over the surface of the gland.

It should be noted further that nerve bundles enter the ganglionic stratum on its ental aspect, and that fibers, either singly or in clusters, pass out of them and distribute themselves among the fibers and cells of the plexus. The exact destination of these fibers cannot be determined, though it seems plausible that a part of them at least terminate in the ganglion cells, since the cells in the more peripheral parts of the skin send long fibers into the plexus. Furthermore, in the skin of the Axolotl it is known that such a relation exists between similar cells and the nerve bundle.

In submitting the above results, the writer gratefully acknowledges the aid given him by President Herrick, under whose supervision the investigations were made.

METHODS.

1. CHROM-ACETIC ACID. The skin was removed from the top of the head of the frog on one side the median line and fixed seven hours in the following solution: chrom-acetic acid (Fol's formula), two parts; water, one part; 10% solution of platinic chloride, ten drops to about two ounces of the preparation.

2. FLEMMING'S STRONGER SOLUTION. The skin from the top of the head of the same specimen as in No. 1, on the other side of the median line, was fixed seven hours in Flemming's stronger solution.

For tissues fixed in both these solutions the usual methods of paraffin embedding were employed, and the sections were cut serially. The stains used were as follows:

(a). Delafield's alum haematoxylin and picrocarmine. On a number of slides, the haematoxylin alone was applied.

(b). Benda's sulphate of iron haematoxylin. With this stain the best results were obtained by mordanting about one

hour in the solution usually employed, and destaining with a thirty per cent. solution of acetic acid.

(c). Weigert's haematoxylin. The tissues were mordanted three hours in a one-half saturate solution of copper acetate, stained three three to four hours in Weigert's haematoxylin, and differentiated in Weigert's decolorizer. Three hours in the stain for Amphibian tissue has proved sufficient, and no doubt less time would give good results.

3. METHYLENE BLUE. The solutions used in this method were those employed by Prof. Huber, viz., one-tenth per cent. solution of methylene blue in normal salt, fixed either in a saturated solution of ammonium picrate or in a solution of ammonium molybdate as follows: distilled water, 10 cc.; ammonium molybdate, 1 gram; hydrochloric acid, 1 drop. For sectioning, the latter was used; for examining *in toto*, the former. The stain was injected beneath the skin of the head by means of a hypodermic syringe. About six hours after the injection the frog was killed and the skin removed. After exposure to the air till the nerves were well differentiated, the tissues were placed for one day in the fixatives mentioned. Those in ammonium picrate were then changed to glycerine. Those in the ammonium molybdate solution, were treated in the following manner: one hour in 95% alcohol, one hour in absolute alcohol, one hour in warm turpentine, and embedded in paraffin in an oven at a temperature of 70° C. This method of dehydrating and embedding involves some decolorization. Nevertheless, it gives good results if the process be hastened as much as is permissible.

4. GOLGI. Strong's modification of the silver method was followed in the main. Small fragments of skin were placed for 48 hours or more in a solution of 3½% potassium bichromate four volumes and 1% osmic acid one volume. After four days in a 1% solution of nitrate of silver, they were washed two hours in 95% alcohol, one half hour in equal parts absolute alcohol and ether, and one hour in warm turpentine. For embedding, paraffin was used as with the methylene blue method.

DESCRIPTION OF PLATES IV AND V.

PLATE IV.

Fig. 1. Section of skin fixed in the chrom-acetic acid solution and stained by the Weigert method, showing the terminal cells (a) in continuity with fibers (b) passing through the corium.

Fig. 2. Section of the same showing terminal fibers (a) connecting with a bundle (b) beneath the corium, also ental gland (c) showing partially the tunic of muscle cells.

Fig. 3. Teased preparation of skin stained *intra vitam* with methylene blue, showing structure of the nerves passing to the terminal cells. Fiber at a.

Fig. 4. Section through skin stained with methylene blue, showing terminal cells (a) with fibers through corium. Other fibers appear in the middle portion of the corium.

Fig. 5. Section of same, showing course of fiber beneath the corium. The fiber and its sheath are plainly distinguished throughout this extent.

Fig. 6. Section stained by the silver method, showing nerve (a) passing ectad through the corium and becoming obscured at the ental portion of the Malpighian layer by a chromatophore (b). The ental part of the nerve (c) by a less perfect impregnation is shown to extend beneath the corium for some distance.

Fig. 7. Section fixed in Flemming's solution and stained by the Weigert method, showing the periphery of a gland of the ental series with its envelope of muscle cells (a); b, gland of the ectal series; also terminal fibers at c.

Fig. 8. A-E. Five consecutive sections stained by the Golgi method, showing glands of the ental series (a) with enveloping fibers (b) from the plexus (c) at the base of the corium. Ectal glands at d.

PLATE V.

Fig. 9. Section stained by the methylene blue method, showing tunic cell at a, and nerve endings at b.

Fig. 10. Similar section, showing nerves (a) passing to ental glands, with typical endings at b; c, blood vessel; d, nerve bundle.

Fig. 11. Section stained by the silver method, showing the structure of the ganglionic plexus at the base of the corium; a, opaque central portion with fibrous arrangement at the ental and ectal edges; b, nerve bundles entering the ental aspect of the plexus.

Fig. 12. Section stained by the silver method, showing purely fibrous structure of the plexus at the base of the corium; a, ganglion cell; b, nerve bundle passing into the plexus and becoming obscured by a more densely stained portion.

Fig. 13. Section stained by the silver method, showing ganglion cells located in the ectal portion of the corium; a, several cells matted together; b,

fibers passing from the cell into the plexus; *d*, a typical ganglion cell; *e*, group of fibers entering the plexus; *f*, ental gland; *g*, ectal gland.

Fig. 14. Section stained by the silver method, showing a typical chromatophore in the ganglionic stratum at the base of the corium; *a*, chromatophore; *b*, single ganglionic cell; *c*, a number of ganglion cells matted together.

Fig. 15. "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-medullated 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. 16. "Intra vitam methylene blue preparation of skin of axolotl, showing cell 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*, *c1*, *c2* and *c3*, ganglion cells of the plexus beneath the corium."

NOTE: Figures 15 and 16 were drawn by Prof. C. L. Herrick, and are reproduced with their descriptions from Vol. VIII, Plate IX, of this JOURNAL.

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