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STRUCTURE AND DEVELOPMENT OF THE SENSE ORGANS OF THE LATERAL CANAL SYSTEM OF SELACHIANS (MUSTELUS CANIS AND SQUALUS ACANTHIAS)[1]

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EIGHTY-THREE FIGURES

CONTENTS

I. INTRODUCTION

For nearly 200 years after its discovery in selachians, in 1664, the canal system of fishes was supposed to be mucous secreting, and it was not until 1850 that Leydig produced histological evidence showing that the organs within the canal system of the head of bony fishes are sensory in nature.

[1] Contribution from the Zoological Laboratory of Northwestern University under the direction of William A. Locy.

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F. E. Schulze carried observations a step further and, in 1861, published sketches of histological preparations that embraced bony fishes and gilled amphibia and included in his observations the sense organs of the trunk as well as those of the head. Nevertheless, it was not till the publication of Leydig's elaborate paper of 1868 on organs of a sixth sense that the sensory nature of the canal organs was generally recognized. From that time onward the designations sense organs of the lateral line, or of the lateral canal system, came more and more into use.

Steno's observations, published in 1664 and 1667, were confined to pores of the head of a skate (1664) and of a shark (1667).

Lorenzini, a disciple of Redi, soon thereafter (1678) discovered another set of tubular organs on the head of selachians that had been unknown to Steno. These have long been designated the ampullae of Lorenzini.

Another variety of follicular organs, distributed on the lips and near the mouth of Torpedo, were described and figured in 1844 by Savi.

These three sets of organs have been somewhat confused in discussions of the sense organs of the lateral canal system. Accordingly it is essential to state at the outset that in this paper the ampullae of Lorenzini and the corpuscles of Savi are not taken under consideration.

As regards the sensory nature of these various organs, Jacobson, in 1813, maintained on the basis of reasoning that the ampullae of Lorenzini were sensory organs, probably of a highly elevated sense of touch, and Savi, in the comments on his original sketches of corpuscles of the torpedo, points out they are provided with more nerves than blood vessels and concludes that the also are sensory.

The main question, however, which concerns the canal organs, remained untouched, and it was reserved for Leydig and Schulze to take up the investigation on broader lines. Leydig on the basis of his morphological and histological observations ('50) concluded that the lateral line organs of the head are sensory in nature and not merely mucous secreting and Schulze ('61 and

'70) extended this to organs of the lateral line of the trunk of teleosts and amphibians. No earlier author had reached this conclusion, for the conclusions of Jacobson and of Savi applied only to particular organs of restricted range, while Leydig's and Schulze's were for the first time applied to the widely distributed lateral line organs common to fishes and to amphibians in their aquatic stages.

Since 1870 morphological studies of the lateral line system of fishes have taken two directions: (1) more extensive studies of topography including the anatomical pattern, the nerve supply, the comparative distribution and (2) studies of histology and embryology. It is in the second division named that investigation is most needed. While advances have been made in other directions, little progress has been made in reference to histological detail. The embryonic development is also imperfectly known except in its more general features. The method of growth of the sense organs, the formation of the canals, the position of the neuroblasts, the nature of the peripheral terminations of nerve fibers and the histological structure of the sense organs remain in doubt.

It was with the hope of adding something to this phase of the subject that the investigation reported below was undertaken. The observations cover the period from September, 1914, to June, 1916, and were carried on in the zoological laboratory of Northwestern University under the supervision of Prof. William A. Locy, to whom grateful acknowledgment is accorded for inspiration and pertinent criticism.

II. BRIEF COMMENTS ON THE LITERATURE

The literature on the canal system prior to 1850 is of little importance from the morphological point of view. The two papers of Steno[2] describing the distribution of supposed mucous pores on the head of the skate (1664) and of the shark (1667)

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[2] Regarding his name, Steno is the form commonly adopted at the present time, but the Latinized, Stenonis, was generally used by its owner rather than the Danish form, Steensen.

are merely of historical interest. The two rays dissected by Steno were probably Raia batis and the shark, which he designates as Canis carchariae, was identified by J. Mueller as Carcharodon rondeletii, Mueller and Henle. The latter paper was published in Florence in 1667, though the date usually given is that of the Amsterdam edition of 1669. Since the publication, in 1910, of the complete scientific works of Steno these papers are readily accessible to readers.

The illustrated memoir of Lorenzini (1678) is difficult to obtain and I have not had access to it in the original.

Savi's paper of 1844 was read, but it is relatively a side issue so far as organs of the sensory canal system are concerned. He figures and describes corpuscles that exist under the skin near the mouth and the lips of Torpedo. On account of the obvious size of the nerves of these corpuscles he regarded them as sensory structures.

We may take Leydig's paper of 1850 as the first step in the scientific demonstration that the organs of the canal system are sensory in nature. By histological studies of the knob-like organs in the canals of the head of a fresh water teleost (Kaulbarsch, Acerina cernua) he concludes, from their structure and from their connection with a nerve, that they are sense organs. He mentions those of the lateral line of the trunk but nearly all that he says applies to the knob-like organs of the canals of the head. He thinks at this date that the nerves come from the trigeminus and the vagus and their fibers seem to him to terminate between the long slender cells of the sensory knobs.

Eleven years later, F. E. Schulze ('61), then a medical student working under the direction of Max Schultze at Rostock, gave an analysis of the histological structure of young stages of the perch (Perca fluviatilis) and of larvae of gilled amphibians (Triton). His sketches show the structure of the organs not only of the head region but also of the trunk. He found those of the tail region similar in structure but somewhat simpler than those of the head. His sketches of both perch and Triton show hair-like processes extending from the sensory cells into a hyaline tube and he maintains that the fibers terminate in

the bases of the 'hairs.' Although subsequent researches with the help of better histological methods have shown that Schulze was wrong in reference to the termination of the nerve fibers, still, we have foreshadowed in this paper the most important question regarding the sense organs—the existence of club-shaped sensory cells with hair-like processes and the relation or nerve fibers to these sensory cells.

Then followed, in 1868, Leydig's paper on organs of a sixth sense, in which he extended his observations to skin organs of amphibians and reptiles. He gives a comprehensive review of the early literature, including his own work of 1850 on the canal organs of teleost fishes, but in this paper he does not give sketches of the structural condition in fishes. The figures are limited to amphibia and certain skin organs of reptiles.

Schulze's paper of 1870 is fully illustrated for young teleosts and amphibia and made more clear and definite that, structurally, the chief characteristic of these sense organs is the presence of pear-shaped hair-cells, and that the nature of nerve terminations is the fundamental point of investigation.

By these researches the ground had been cleared, and the question outlined for neurological investigation was the histological structure of the sense organs and the nature of nerve terminations. But investigations along these lines were retarded for lack of proper histological technique and reliable methods of impregnation of nerve fibers.

All this, also, was before the demonstrations of His upon the origin of nerve fibers ('88) and the formulation of the neuron theory by Waldeyer ('91). Regarding the nerve terminations there arose a period of discussion as to whether there was continuity (Merkel '80) or merely contiguity between nerve fibers and sensory cells.

On account of the close generic resemblance between the sense organs of the lateral line and those of the ear it will not be out of place to mention in this connection two papers on terminations of the eighth nerve. Among others, Ayers ('90) represents the conception of continuity (his diagram, Plate XII) between ganglionic fibers and sensory hair-cells of the internal ear.

Morrill's demonstration ('98) of the nature of nerve termina-
tions among the hair-cells of the ampullae of the ear is equally
distinct on the side of contiguity. This paper with its excel-
lent results has been frequently overlooked even by investigators
of the nerve terminations of the eighth nerve.

Neurological investigations led finally to the recognition that
we have free nerve terminations among sensory cells and these
came to be designated as secondary sense cells. In the sensory
epithelium of the lateral canals we have a good illustration of
these secondary sense cells.

The literature in reference to topography and gross distri-
bution of the nerves is extensive and embraces widely known
standard contributions as those of Malbranch '76, Amphibians;
Ramsay Wright '84, Ameiurus; Garman '88, Selachians; Allis
'88, Amia, and others. Following these are the papers of Ewart
'93, Lemargus; Ewart and Mitchell '93, Raia; Cole '95, Chi-
maera; Collinge '94, Fishes; Clapp '98, Batrachus; Allis '02,
Mustelus; Norris '07, Amphibians; Ayers and Worthington '07,
Bdellostoma; Reese '10, Chimaera.

In reference to the acustico-lateralis system of nerves con-
tributions have been made by Herrick '99, '01, '05, '06; Johns-
ton '06; Landacre '14, ganglia, etc. A number of authors have
particularly emphasized the relationship between the sensory
canal system and the ear.

On the embryological side Balfour's classic observations on
elasmobranch fishes ('78) mark the beginning. Other embryo-
logical studies include those of Beard '83, metameric condi-
tion; Allis '88, connection between ear and nose; Mitrophanow
'93, surface views of development from a primordium embracing
the epithelium of the ear; Wilson and Mattocks '95, primordia
of ear, lateral line and branchial ganglia forming a furrow and
sac; Harrison '03, experimental observations of the growth of
lateral line organs of amphibia.

The publications that are more closely related to the subject
of this paper are those showing histology of the sense organs
and their nerve terminations excluding those that relate spe-
cifically to the ampullae of Lorenzini and the vesicles of Savi.

Positive observations in reference to histology and especially those showing nerve terminations are limited in number. But it has been repeatedly shown since Schulze's papers of 1861 and 1870 that pear-shaped and club-shaped sensory cells with cuticular hairs or bristles are characteristic of the sense organs of the canal system. The sensory cells of the sense-hillocks are central in position, do not extend to the basilar membrane and are intermingled and surrounded by long, usually slender, supporting elements extending from the basilar to the limiting membrane. Cells of this kind have been figured with more or less detail by Langerhans '73, Salamandra maculosa, good; Malbranch '76, several amphibians; Solger '80, a nerve plexus in teleosts; Bodenstein '82, Cottus gobio; Mauer '92, fishes and amphibians including Acanthias and Triton; Kingsbury '95, Diemyctylus, Nectorus; Heilig '12, Kaulbarsch, shows nerve terminations; Pfüller '14, Macruridae.

A number of observers from Leydig '50 onwards have published figures of the specific nerve of the sense organs traced to the basilar membrane without showing nerve terminations. Among the early ones are Allis '88, Maurer '92, etc. But the vexed question of the nerve terminations has proved elusive. Retzius '92 supplied good figures of nerve terminations of end bulbs of fishes and amphibians. Bunker '97 makes definite statements without illustrations of the nerve terminations in Amieurus nebulosus. Heilig '12, gives the best figures and the best summary for the Kaulbarsch (Acerina cernua) and Pfüller '14, shows ganglion cells and some fibers in the sense organs of the Macruridae.

The papers of Bunker, Heilig and Pfüller require separate mention.

Bunker '97, by using several methods including haematoxylin stains, Golgi and methylen-blue, worked out the histology of the nerve cells and of the nerve terminations. It is greatly to be regretted that his clear descriptions are not accompanied by sketches. He finds the usual pear-shaped hair-cells in the superficial half of the organ. The nerve at the base of the organ consists of 10 or 20 medullated fibers which lose the medullary

sheath and break up into a number of branches without anasto-
moses. These branches pierce the membrane in many places
and rise, still branching, to the bases of the sensory cells.
Around the bases some of the fibers intertwine in a kind of
basket-like network from which fibrillations rise still higher,
nearly to the free border of the organ.

Heilig '12 by publishing sketches of the histology of the sense
organs and the terminations of the nerve fibers took an advanced
step. His findings in reference to endings of the nerve fibers
are similar to those of Bunker. Heilig's sketches are confined
to teleosts and chiefly to the Kaulbarsch (Acerina cernua),
which was the form investigated by Leydig in 1850. The
title of his paper is more comprehensive, embracing the lateral
canal organs of fishes and amphibia, and his comments on the
literature are broad and comparative. Heilig shows that the
nerve fibers lose their medullary sheath just below the sense
organ, and piercing the basilar membrane, they spread out
tree-like ascending between the supporting cells to the region
of the sensory cells. The terminal branches form a sort of
cup around the bases of the hair-cells (secondary sense-cells)
which brings the cell bodies into close relation. The upward
extending fibers do not reach the limiting membrane. One
fiber can send its terminal twigs to more than one hair cell.
There is no anastomosis between the terminal fibrillae. These
points are shown in the sketches but the difficulties of impreg-
nation encountered by all observers and the difficulties of ob-
taining good pictures of the histology leave room for clearer
pictures.

Pfüller '14 published observations on the lateral canal organs
and on the nervous system of the head of the Macruridae—a
family of deep sea fishes. Only that part of his paper that
deals with the lateral line organs is pertinent to the subject
under consideration. He shows long slender supporting cells
and short thick hair cells, both elements containing rounded
nuclei near their bases. The sense-hillocks of the head are
richer in sensory cells than those in the lateral canal of the
trunk. His histological figures are lacking in details. In his

figures of Macrurus fastiatus and M. cavernosus are shown small ganglion cells at the base of the sense organ and also within the region of the epithelium. So far as I have been able to determine nothing of this kind exists in Mustelus and Squalus. The relatively small size of his 'ganglion cells' is in marked contrast with the large nerve fibers of the nervus lateralis and of the far removed ganglion cells of the selachians.

III. MATERIAL, METHODS AND TERMINOLOGY

The material available for this investigation consisted of a large number of Squalus acanthias embryos ranging from the open neural groove stage to small adults (garters), and Mustelus canis adults and pups, and a few rather poorly preserved embryos of this species between 26 mm. and 80 mm. in length. Squalus embryos between 36 mm. and 72 mm. were not plentiful and there were none between 72 mm. and 180 mm. The Squalus material had been fixed in the usual reagents and preserved in alcoholic and formalin solutions. Fresh Mustelus adults and pups were secured at the Marine Biological Laboratory, Woods Hole, Massachusetts.

Only a few words need be said concerning the methods employed. With preserved material iron haematoxylin gave the best results for sections. No success attended my efforts to secure silver impregnation of the peripheral nerve terminations of preserved specimens. The binocular dissecting microscope was found to be indispensable in dissecting and studying the distribution and nerve supply of the sensory thickenings in early embryos.

With fresh Mustelus material, good impregnations of the peripheral nerve terminations were obtained. Several modifications of the Ramón y Cajal method gave fairly good results, but the best preparations were obtained by a slight modification of the silver-pyridine method introduced by S. W. Ranson.

The modification I found most successful may be outlined as follows: Fresh specimens are decapitated, and narrow strips of integument containing the sensory canals desired are rapidly removed, cut into short sections, rinsed briefly in distilled water, drained on blotting paper, and put into a large volume of ab-

solute alcohol to which has been added 1 per cent of strong
ammonia. At the end of four hours the ammonia fixative is
poured off and replaced with fresh fluid. In this the tissue
should remain from two to four days. The tissue is then rinsed
in distilled water and put into a 7 per cent solution of nitric
acid for twelve hours.[3]

ABBREVIATIONS

Ac.O., accessory organ
Aud., auditory vesicle
Aud.Gn., auditory ganglion
Br.O., branchial sense organs of Beard
B.Cl., basal cell
Buc.VII., Ramus buccalis VII
Can., canal wall
Clm., columnar supporting cell
Der., dermis
Dt.En., ductus endolymphaticus or its pore
Dor.L., dorsal line of pit organs or their anlagen
Dor.O., dorsal line organ
Ect., ectoderm
Ep.Po., epidermal pocket or tunnel
Epd., epidermis
Ep.Fl., epidermal fold
Fbr., terminal fibrillae
Fb.Zn., longitudinal fiber zone
Grp., one group of 'hair-cells'
H., hair-like process of secondary sense cell
Hym., hyomandibular canal
I.Orb., infraorbital canal
I.Orb.Cd., infraorbital sensory cord
Lat.Nv., nervus lateralis
Lat.Cn., lateral canal or its pores
Lat. Gn., lateral ganglion
Medl., spinal medulla
Mn.Ex.VII, ramus mandibularis externus VII

Mnd., mandibular canal
Mnd.Pt.O., mandibular line of pit organs
M.Nf., medullated nerve fiber
Mus., muscle
N.M.Fb., non-medullated nerve fibers
Po., pore of tubule
Pt.O., isolated pit organs or their anlagen
R.Dor., ramus dorsalis of lateral nerve
R. Supt., ramus supratemporalis of lateral nerve
Rml., ramulus lateralis
Rt., root of lateral nerve
Sn.Cl., secondary sense cell
Sn.Col., sensory column
S.Orb., supraorbital canal
S.Orb.Cd., supraorbital sensory cord
Spn., spindle-shaped supporting cell
Sp.Gn., spinal ganglion
Sup.Oph.VII, ramus ophthalmicus superficialis VII
Supt., supratemporal commissure
Tub., tubule
Var., varicosity
Vas., vascular space or blood cell
V., fifth nerve, root, or ganglion
VII, seventh nerve, root, or ganglia
IX, glossopharyngeal, root, or ganglion
X., vagus, roots, or ganglia

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[3] After the manuscript for this paper had gone to The Wistar Institute, my
attention was drawn to the fact that a practically identical modification of
the pyridine silver method was employed in 1913 by G. C. Huber and S. R.
Guild, and is described in the authors' paper on the peripheral distribution of
the nervous terminalis in mammalia. The Anatomical Record, volume 7, page
253.
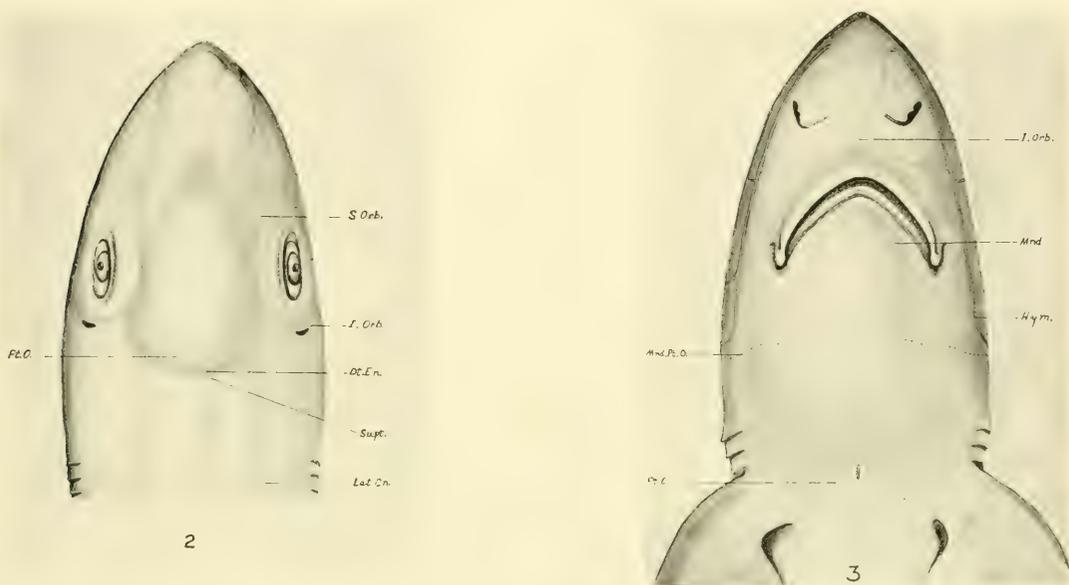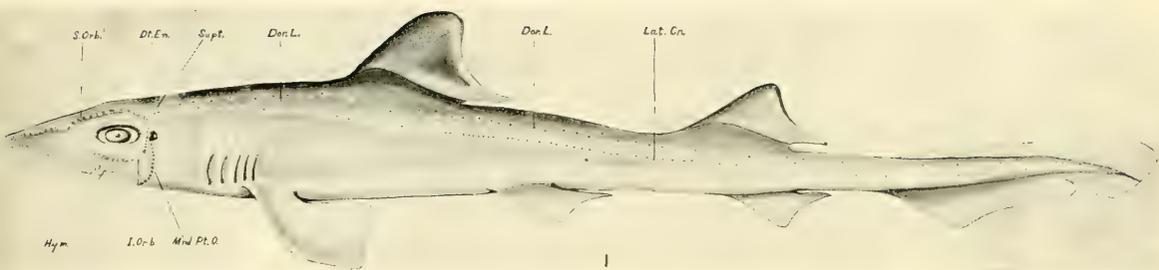
Through the courtesy of Prof. S. W. Ranson the artist of the Medical School, Mrs. Frain, finished in ink the author's outline sketches for figures 1 to 4, 9, 10, 15, 23, 26, 32, 37, 39, 44, 47, 48, 50 to 54, 64, 65, 70, 72 and 74.

Fig. 1   Lateral view of young Mustelus canis a few days after escape from the uterus to show the distribution of the sensory canals and the pit organs.   The pores of the canals and the pit organs are drawn to a larger scale than the outline of the specimen, otherwise they could scarcely be seen in a specimen of this size. $\times \frac{1}{2}$.

Fig. 2   Dorsal view of the head of a larger specimen (garter). $\times \frac{3}{4}$.

Fig. 3   Ventral view of the same head.   Note the short line of pit organs on either side of the scar of the yolk stalk. $\times \frac{3}{4}$.

This treatment was found to be of great advantage as it facilitates sectioning the tough dog-fish integument and also prevents deposits of silver in the connective tissue elements, a drawback which was encountered in the use of other silver reduction methods.

The pieces of tissue are again rinsed in distilled water and then dehydrated in 80 per cent, 95 per cent, and absolute ammoniacal alcohol. From the absolute alcohol the pieces are put directly into pyridine. In this they remain for twenty-four hours, and are then washed for the same length of time in running water. The tissues are then washed for half an hour in distilled water and put into 2 per cent silver nitrate solution, at 37°C., for two to seven days. When the pieces of tissue in this solution have acquired the proper color they are rinsed briefly in distilled water, reduced in a 2 per cent solution of hydrochinon in 4 per cent formol, dehydrated rapidly, cleared in benzol, and imbedded in paraffin.

After treatment with the nitric acid solution it was often found advantageous to remove the sensory canal from its 'dermal tunnel.' This could be done quite readily with the aid of a pair of sharp needles and a dissecting microscope.

The greatest disadvantage of the method as outlined above is due to shrinkage of tissue and the failure to show cell boundaries in the sensory epithelium. The cell boundaries, however,

Fig. 4 Diagram illustrating the general relations of the lateral nerve and its ramuli to the lateral sensory canal with its surface tubules. Drawn from camera sketches except that the distance between serial ramuli and tubuli has been greatly foreshortened.

Fig. 5 Longitudinal section of the lateral canal of a Mustelus 'pup' at the level of the first dorsal fin. This semidiagrammatic figure is a composite, several preparations having been utilized in its construction. The nerve fibers and fibrillae were drawn from pyridine silver and the cellular parts from haematoxylin preparations. The sketch shows the nerve distribution and the general histological structure of the sensory epithelium. × 240.

Fig. 6 Transverse section of lateral canal at the site of a surface tubule. The tubule passes through the dermis in a ventro-lateral direction, opening on the surface near the base of the slightly modified scale. Mustelus canis pup. × 85.

Fig. 7 From the same specimen as the above, more highly magnified. × 260.

5

Lat.Cn.   Can   T.b   Spn.   Grp.   Rml.   Clm.   Sn.Cl.   Fbr.   Fb.Zn.

6   Epd   Lnc   Pr   Tuo

7   Can.   Vas   H.   Sn.cl   Fb.Zn.

13

4   Lat.Hr.   Sn.Col.   Rml.   Fb.Zn.   Rml.   Lat.Hr.   Lat.Cn.   Sn.Col. —   Mus.   Tub.   Dcp.   Epd.   Pr.

14
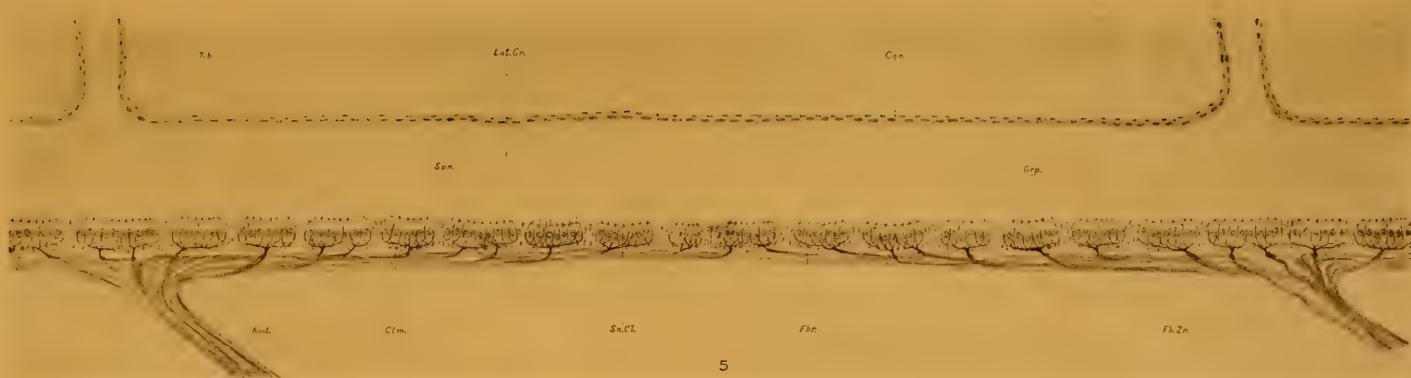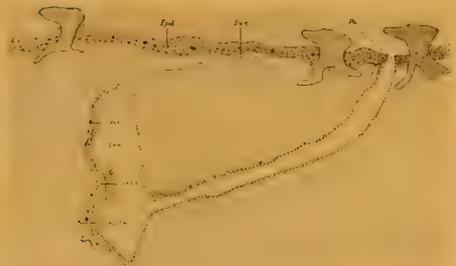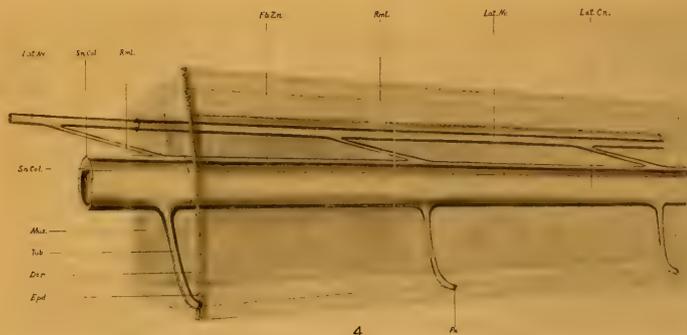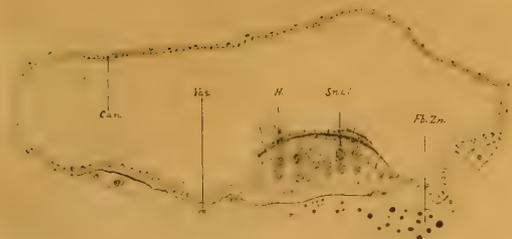
can often be brought out by counterstaining on the slide with Delafield's haematoxylin.

Sections were cut five to fifteen micra and mounted in the usual manner, avoiding, however, solutions not absolutely free from acid.

Terminology. The sense organs of the canal system are distributed in definite lines on the head and trunk of fishes and of amphibians in their aquatic stages. While usually enclosed in a canal, they are sometimes exposed on the surface or in shallow grooves. The lines may be multiple as is common in amphibia and they may reach the number of nine (Herrick).

The terminology employed in this paper is that in common use with the addition of a few terms that conduce to clearness.

The entire system of sense-organs often designated the lateral line system will be called the sensory canal system. This will include all of the canals of the head and the trunk and the surface or pit-organs which are genetically equivalent to the sensory canal organs. As before mentioned, no account is taken of the vesicles of Savi and the ampullae of Lorenzini.

The trunk canal will be called the lateral canal; the head canals will be designated supraorbital, infraorbital, and hyomandibular canals, respectively (figs. 1, 2, 3). The canal running over the temporal region and joining the two lateral canals will be called the supratemporal commissure (figs. 1, 2). Two short detached canals on the lower jaw will be called mandibular canals (fig. 3).

The term sensory canal will be used to denote any of the delicate epithelial tubes, one wall of which is developed into the line of sense-organs, sensory epithelium, or sensory column (figs. 4, 5). The numerous channels connecting the sensory canals with the exterior will be called tubules and their openings on the surface, pores (figs. 4, 6).

With reference to the nerves supplying the sensory canal system, the generally adopted nomenclature will be employed except in the case of the nerve supplying the lateral canal. This will be called simply the nervus lateralis instead of ramus lineae lateralis X. The branches of the N. lateralis which supply

the supratemporal commissure and the dorsal series of pit
organs will be designated supratemporal and dorsal rami of
the lateral nerve.

All nerves of the sensory canal system of Mustelus and
Squalus occupy a position considerably removed from the canals
which they supply. These nerves give off numerous minute
branches which in turn make their way through the muscles
and connective tissue to the sensory epithelium of the canals.
These little branches will be termed the ramuli of the respective
nerves (fig. 4).

IV. OBSERVATIONS

A. MUSTELUS CANIS

1. *Distribution of the canals and their gross innervation*

The supraorbital canal of the adult may be said to begin a
little above the posterior angle of the eye where it is joined by
the infraorbital canal (fig. 2). It passes forward above the
orbit and a little in front of the latter it descends by a sharp
bend to a more ventral position. The canal then passes directly
to within a few millimeters of the tip of the snout, where it
curves sharply ventralwards and backwards to merge with the
infraorbital canal posterior to the nostril (fig. 1). At its an-
terior extremity the supraorbital canal forms a union with the
anterior end of the infraorbital and, as indicated above, a second
anastomosis between these two canals is formed posterior to
the nostril (fig. 1).

The supraorbital canal has more than a hundred tubules.
The tubules vary in length from 0.2 mm. to 1 mm. or more,
and the majority of them take a postero-medial course to the
surface, where they open in simple pores. An exception to
this condition is found about half way between the orbit and
the snout. Here the tubules are double and for a short dis-
tance many of them branch and anastomose in a complex man-
ner. The significance of this condition is not apparent. It
may indicate the fusion of two originally separate canals, or it
may represent a modification foreshadowing the complex sys-

tems of branched tubules (dendritic systems) found in some of
the more recent fishes. The former assumption seems improb-
able in view of the fact that the anlage of the canal is a single
cord of thickened extoderm.

The supraorbital canal is innervated by the N. ophthalmicus
superficialis VII. This division of the VII nerve, as well as
the other nerves which innervate sensory canals, has an in-
dividual ganglion near its root and, like these other nerves, it
terminates in the same internal nucleus in the tuberculum acus-
ticum (Herrick), or better the area acustico-lateralis.

The infraorbital canal. For descriptive purposes, and on
account of its mode of development in Squalus (fig. 53), we
may consider the infraorbital as beginning where it unites with
the supraorbital. Allis says that in embryos of Mustelus the
nerve distribution of buccalis and otic fibers goes back to the
supratemporal commissure and on that basis the infraorbital
would extend to the supratemporal commissure. Commencing
with its point of union with the lateral and supraorbital canals
the infraorbital passes ventralwards between the eye and the
spiracle, crosses below the eye, and passes forward as far as the
anterior margin of the orbit. Here it bends ventrad and slightly
caudad, anastomosing with the inferior limb of the supraorbital
immediately posterior to the nostril. Slightly anterior to this
anastomosis a prebuccal limb of the canal passes antero-medially
to the median line on the ventral surface of the head, where it
fuses with its fellow of the opposite side. The median canal
represented by this union passes forward a short distance and
forks into two canals which extend forward and join the supra-
orbital near the tip of the snout, as mentioned above.

Between the eye and the spiracle the infraorbital canal lies
deeper than any of the other canals, having separated completely
from the inner surface of the dermis.

The tubules of the infraorbital canal were not all counted, but
in number there are considerably over one hundred. On the
lateral region of the head the tubules are simple and lead directly
to the surface. On the ventral surface of the head, however,
most of the tubules are branched or double and open on both

sides of the canal. The infraorbital receives its nerve supply from the buccalis and oticus facialis.

The hyomandibular canal begins at the ventral angle of the bend of the infraorbital below the eye (figs. 1, 3). The nearly straight canal passes backward to a point beyond the posterior margin of the spiracle. Here it terminates in a slight upward curve. Thirty or more tubules open from the canal to the exterior. Its innervation is from the hyoid division of the R. mandibularis externus VII.

Mandibular canals. On each side of the lower jaw is a short mandibular canal (fig. 3). The two opposite canals approach each other anteriorly near the median line, but do not communicate. These canals are innervated by branches of the mandibular division of the mandibularis externus.

The lateral canal (fig. 1) starts from its point of junction with the supratemporal commissure and passes backward in practically a straight line, inclining slightly ventralwards, posteriorly. Over the anal fin there is always a slight but characteristic elevation of the canal for a short distance. Posteriorly the lumen of the canal gradually decreases in diameter, and the tubules become shorter. The lateral canal has approximately 140 tubules and they open to the surface in a line of pores which lies considerably ventrad to the actual position of the canal except near the posterior end, where the tubules are very short and open directly to the surface opposite the canal.

The lateral canal is innervated by the main division of a large nerve, the N. lateralis, which is attached to the side of the medulla oblongata anterior and superior to the first vagus root. It should be noted in reference to figure 1 that the pores and the pit organs are represented as if magnified while the outlines of the fish are reduced.

Supratemporal commissure (fig. 2). A short canal crosses the supratemporal region immediately posterior to the openings of the endolymphatic ducts. This is the supratemporal commissure and it communicates at each end with the lateral canal of that side. In three different specimens 19, 22, and 23 tubules were counted. They average about 1 mm. in length and open

posteriorly. Innervation is from the supratemporal ramus of the lateral nerve.

Surface organs. In addition to the organs which are enclosed in canals, three lines of pit-organs deserve mention: the dorsal and mandibular series of the adult, and a short line of temporary pit-organs which are found in the pup stages on each side of the scar left by the yolk-stalk.

The dorsal series consists of approximately 80 pit-organs running dorsal to the pores of the lateral line in a somewhat irregular course. In Mustelus (fig. 1) this set or organs extends from a point a little posterior to the eye throughout the length of the body. In Squalus the corresponding set of pit-organs extends only to the region of the first dorsal fin. The nerve supply of these organs comes from a slender nerve which arises from the lateralis ganglion (figs. 45 and 50) and runs nearly parallel to the lateralis nerve.

The mandibular series of the adult contains approximately 45 sense organs. They begin at the posterior margin of the spiracle and then curve backward around the end of the hyomandibular canal and forward reaching nearly to the median plane (fig. 3). The innervation of this line of organs has not been determined.

The two short lines of surface organs situated on opposite sides of the yolk-stalk appear to be very poorly developed (fig. 3). Each line contains 9 or 10 organs. These organs have not been observed in the adult and their innervation is unknown.

2. Structure of the sensory canals

a. General anatomy. The delicate tubes of the sensory canals in Mustelus have walls consisting of two layers of epithelial cells, except on the dorsal side, where the canal wall is greatly thickened into a ridge of sensory epithelium (figs. 4, 6, 7). The diameter of the canals varies in different parts of the body as well as in specimens of different size. The average diameter of the head canals is about 0.3 mm. in a specimen two feet long, while in the same specimen the trunk canal varies from about 0.2 mm. at its anterior end to 0.08 mm. or less posterior to the

caudal peduncle. Even in large adults the lateral canals terminate in open grooves on the sides of the caudal fin.

Figure 4 is a diagram to show the relations of sensory canal, surface tubules, lateral nerve and its ramuli. The ramuli of the nerve as well as the tubules have been much foreshortened in order to bring them within the limits of the diagram.

The sensory epithelium of the lateral canal of Mustelus forms a column that is substantially continuous throughout the length of the canal (figs. 5, 6, 7, 8). It is not divided into segments corresponding to the metameres of the trunk. This sensory column occupies the dorsal wall of the canal. It is two or three cell layers in thickness and it is in this sensory column that we find the development of sensory cells that are called the sense organs of the lateral line. Figure 5 is a semi-diagrammatic sketch of the sensory column between two tubules. There are 16 clusters of cells (outlined with the camera) between the two tubules. The ramuli of the nerves correspond to the tubule and the nerve fibers overlap.

The thickness of the lateral sensory column is not constant throughout its length, but becomes gradually thinner posteriorly (figs. 9, 10, 11). It is also thinner as a rule midway between two ramuli of the lateral nerve. This may indicate a tendency for the sensory column to assume a segmental character, but in no instance in Mustelus has the sensory column been observed to be completely divided into separate 'buds' or segments, as appears to be the condition in Amia and several other forms.

On each side of the lateral sensory column are large blood spaces and the adjacent columnar epithelium of the canal wall is considerably thickened (fig. 8). Little tubules lead from the canals to the surface of the integument at approximately regular intervals. Their walls are composed of two layers of epithelial cells which are continuous with those of the inferior wall of the canal (fig. 6). The tubules coincide in number with the ramuli of the lateral nerve and they do not increase in number after the canals have closed. A hundred and thirty-six tubules were counted in the lateral canal of a specimen 55 mm. long, while

a specimen 300 mm. long had a hundred and thirty-eight. In the adult the tubules do not lead in a direct path to the surface, but pass postero-ventrally for the distance of 1 mm. or less and then bend laterally, passing through the epidermis and opening to the exterior near the base of a slightly modified scale (fig. 6). In the posterior region of the body the tubules are much shorter and take a more direct course to the surface.

The sensory canals may be said to occupy 'dermal tunnels' which, on the trunk, lie within the dermis, but on the head most of the canals sink to a somewhat deeper level.

b. Histology of the sensory epithelium. The sensory epithelium of the lateral canal is limited to a narrow ridge or column which forms the greater part of the dorsal wall of the canal. As mentioned before, this column of sensory epithelium is practically unbroken throughout the length of the canal except for a great many minute transverse ridges. These ridges are usually a little less than 0.1 mm. apart (in a 2 foot specimen) and mark the spaces between the groups of hair-cells. The sensory column varies considerably in thickness from the anterior to the posterior end of the canal, and usually presents a marked depression between consecutive ramuli of the lateral nerve. This is not invariably the condition, however, for in many preparations the sensory epithelium is as thick between the ramuli as it is elsewhere (fig. 5).

Four different kinds of cells have been distinguished in the sensory column: (1) hair-cells; (2) basal cells; (3) spindle-shaped supporting cells; (4) columnar supporting cells.

The hair-cells are arranged in clusters and are considerably larger than the other cells. They are somewhat club-shaped, their proximal ends being greater in diameter than their distal ends. The nuclei are large, round, and centrally located as a rule. In suitable preparations hair-like processes always appear at the distal ends of these cells (figs. 7, 8). Whether there is a single process on each cell, or whether there are several clumped together by reagents so that they appear as one could not be determined, but the evidence is in favor of the view that there is a single 'hair' on each cell (figs. 7, 8, 9, 11). The hair-cells
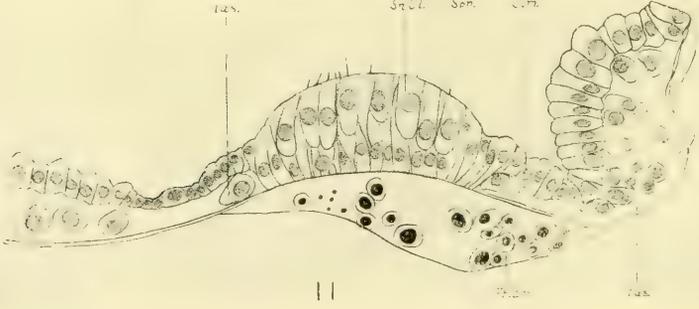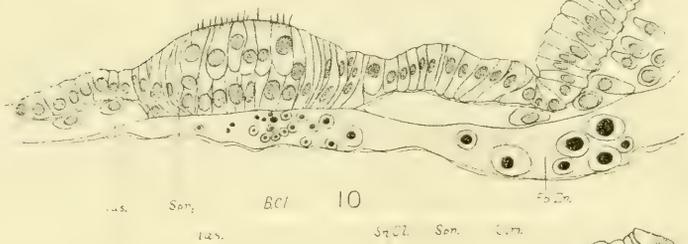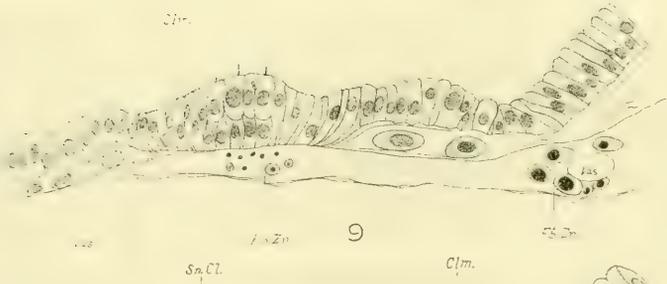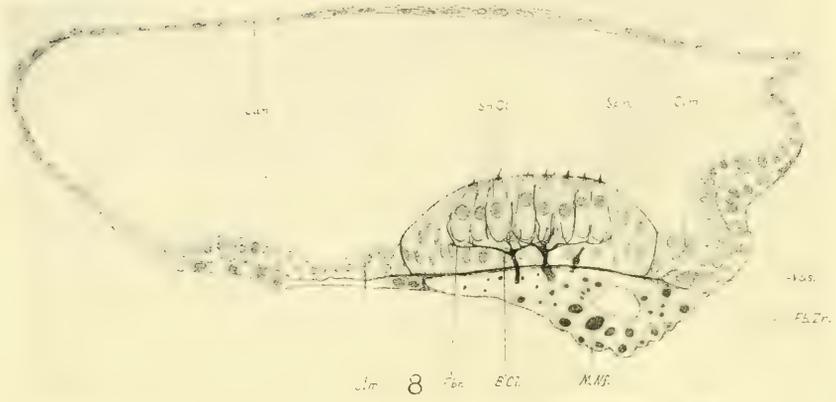
are not the same size throughout the length of the sensory column, but are considerably smaller in the posterior region (figs. 9, 11). They extend through about two-thirds the depth of the sensory column, and their distal ends abut against a limiting membrane which presents a great deal of variation in thickness in different preparations.

Basal cells. The boundaries of the basal cells are usually difficult to trace, except their proximal margins, which rest on a well developed basilar membrane. The cells usually appear somewhat triangular in shape and they extend distally between the proximal ends of the hair-cells. Their nuclei are round, centrally located, and frequently appear almost as large as the nuclei of the hair-cells (figs. 9, 11).

The spindle-shaped cells. These cells occur between and around the hair-cells (figs. 8, 9 to 12). They are very slender and greatly elongated, extending from the basilar membrane to the distal limiting membrane of the sensory column. The nuclei are small, much elongated, and are located near the proximal ends of the cells except in cells which lie immediatly between two adjacent clusters of hair-cells; in these the nuclei are near the distal ends of the cells (fig. 5).

Columnar cells. The columnar cells are the most numerous of all the cells in the sensory column. They extend from the basilar membrane to the distal limiting membrane and vary in length with the variation in thickness of the sensory column (figs. 9, 10). On each side of the sensory column proper the columnar epithelium becomes thinner and merges gradually into the thin, inner epithelial layer of the sensory canal (figs. 7, 8).

Fig. 8 Transverse section of lateral sensory canal, showing the histological structure of the sensory column, peripheral nerve terminations, and the longitudinal fiber zone (of nerve distribution). The figure was drawn with the aid of a camera lucida from a pyridine silver preparation counterstained with Delafield's haematoxylin. Mustelus canis garter. × 370.

Figs. 9, 10, 11 Transverse sections of the lateral sensory column of a small adult Mustelus canis, showing that, posteriorly, the sensory epithelium is considerably less extensive than anteriorly. Figure 11 was taken anterior to the first dorsal fin; figure 10, between the first and second dorsal fins; and figure 9, posterior to the second dorsal fin. × 370.

The nuclei of the columnar cells are small, round or elongated, and are located at various levels in the cells.

Cross sections show that for a short distance on each side of the sensory ridge the columnar epithelium is considerably modified and thickened where it overlies rather large longitudinal blood spaces (fig. 8). In many preparations some of the cells in this region present full, rounded outlines, while others appear greatly shrunken and vacuolated. The difference in appearance of these cells under like treatment and their proximity to an abundant blood supply suggests that they may have a secretory function.

The basilar membrane appears as a structureless sheet from 0.5 to 1 micron in thickness. It separates the sensory epithelium from the longitudinal fiber zone, the blood capillaries and other neighboring tissues.

c. Peripheral terminations of the nervus lateralis. Demonstration of the precise nature of the peripheral terminations of the lateral nerve has been one of the most difficult tasks in connection with the study of the sense organs of the lateral canal system. The earlier investigations on nerve terminations took place before the introduction of silver impregnation, of methylen blue, and of other modern methods. Retzius '92 and von Lenhossék '92–'93 made numerous impregnations by Golgi methods but they were unable to demonstrate the terminal fibers of the lateral nerve. They expressed the belief, however, that the nerve fibers ended, not in the hair cells, as Leydig believed, but in free fibrillations in the sensory epithelium.

Later investigators have held, largely, to the ideas expressed by Retzius and von Lenhossék. Johnston '06 says that the lateral nerve ends in complex fibrillations between the hair-cells of the sense-organs. His statement, however, is based on a brief unillustrated paper on the lateral sense organs of Ameiurus by F. S. Bunker '97. Heilig '12 worked on the peripheral terminations of the lateral nerve in a species of perch, but his histological illustrations are not completely satisfactory. His figures show the nerve fibers approaching the bases of the hair-cells, but their relation to the cells is not shown.

As far as I am aware no observations have been published on the peripheral terminations of the lateral nerve in selachians and, as indicated above, the work on other forms has not been entirely satisfactory. The following account is based on the study of a great number of preparations made from successfully impregnated sensory epithelium from the lateral canal of Mustelus canis. In order to disclose all possible relations, sections were cut transversely, obliquely, and longitudinally, to the long axis of the canal, and a few were cut in a plane at a right angle to the long axis of the hair-cells.

It has already been stated that each ramulus of the lateral nerve approaches the sensory canal obliquely (figs. 4, 13) and that the sensory column is developed on the medial superior wall of the canal. What is spoken of as the base of the sensory column is thus directed towards the superior boundary wall of the sensory canal (fig. 6).

Each ramulus of the N. lateralis is composed of eighteen to thirty or even more nerve fibers, varying in diameter from one to six micra (figs. 14, 15). The majority of the fibers are medullated and they are bound together by a comparatively thick connective tissue sheath. Upon reaching the base of the sensory column the outer sheath of the ramulus merges into the dermal tissue surrounding the sensory canal and the nerve fibers then pass in both directions, i.e., caudad and cephalad, along the sensory column outside the basilar membrane (fig. 5). The nerve fibers from adjacent ramuli overlap for a short distance on the base of the sensory column. A continuous and well-marked fiber zone is thus formed which extends throughout the length of the sensory column. Naturally this fiber zone is largest where the ramuli first reach the canal, and smallest approximately midway between two adjacent ramuli, where fibers from each territory overlap for a short space (figs. 5, 12). In no case has a complete break in the continuity of the fiber zone been observed. This condition is similar to an old sketch of Hexanchus griseus published in 1859 in Leydig's Lehrbuch der Histologie.
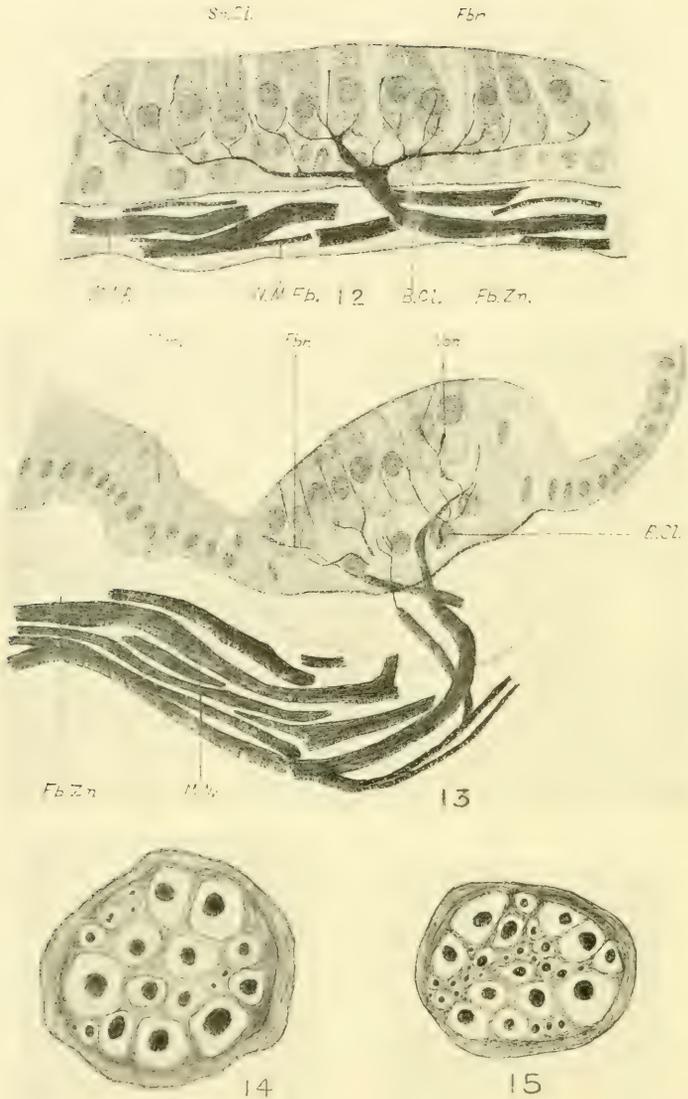
Fig. 12   Longitudinal section through one group of 'hair-cells' showing the extensive branching of fibrillae from one nerve fiber. Mustelus canis adult. (Pyridine silver and Delafield's haem.) $\times$ 565.

Fig. 13   Oblique section of the lateral sensory column, showing the lateral ramulus and several varicosities on the terminal fibrillae.   Mustelus canis adult. (Silver-pyridine tech.). $\times$ 555.

Figs. 14, 15   Transverse sections of lateral ramuli, showing variation in the number of fibers as well as in the size of the individual fibers.   Mustelus canis adult.   Figure 15 represents a silver-pyridine preparation; figure 16, a Delafield haematoxylin preparation. $\times$ 555.

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The usual manner of distribution of the nerve fibers to the groups of hair-cells is shown in figure 5. The fibers run along the basement membrane until they reach points beneath the particular groups of hair-cells which they innervate. Here they usually make a sharp bend and, losing their medullary sheaths, they penetrate the basement membrane (fig. 13), but in several cases they are seen to divide into two or more primary divisions while still outside of the sensory epithelium (figs. 17, 20, 21). The nerve fibers may divide at a variety of levels between the longitudinal fiber zone and the bases of the hair-cells. As a rule the finer fibers pass deeper into the sensory epithelium before dividing than the larger ones, but fibers of intermediate sizes are seen dividing at various levels between the basement membrane and the hair-cells. The primary branches of a single nerve fiber separate rather widely, and as seen in longitudinal section (fig. 12) they may cover approximately the base of one group of hair-cells. These branches divide repeatedly, often into extremely fine fibrillae which pass freely between the adjacent walls of the hair-cells to a distance ranging from about one-fourth to three-fourths or more of the length of the hair-cells (figs. 13, 16, 20). In a great number of cases the fibrillae are seen to terminate in little rounded enlargements or end-knobs, and varicosities of similar appearance occur at various levels on many of the terminal branches (figs. 13, 19). The largest are often seen at points of primary division and in some cases resemble the terminal enlargements of the ampullae of the ear described by Mullenix (1910) as 'calyses', though they do not bear the same relationship to the hair-cells as Mullenix has suggested they do in the auditory epithelium of Fundulus.

No cup-like expansions or close anastomosing networks around the bases of the hair-cells have been observed. The appearance of such conditions in the auditory epithelium, as reported by some investigators, seems likely to have been due to overstaining or over-impregnation, with resulting irregular deposits of silver between the finely dividing fibrillae.

The general similarity between the canal organs and those of the internal ear makes the nature of the terminations of the

eighth nerve of interest in this connection. A. D. Morrill, in a very exact piece of work, found conditions in the auditory epithelium of Mustelus which correspond very closely to the conditions here described for the sensory epithelium of the lateral canal, and from the morphological evidence now at hand it seems fairly safe to conclude that the peripheral terminations of the lateral and acoustic nerves are essentially alike, and that these terminations are not in the nature of 'cups,' 'calyses,' or
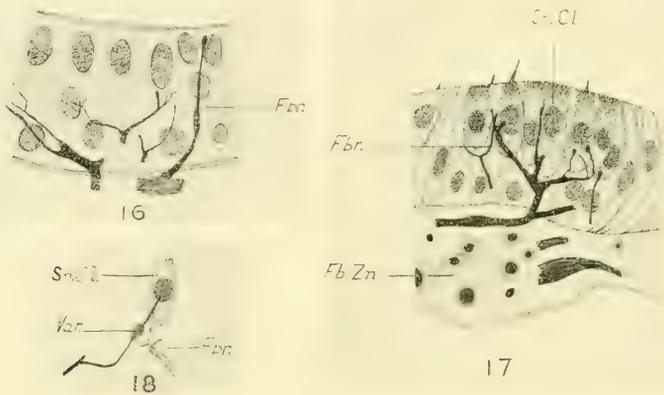
Fig. 16   Longitudinal section of the lateral sensory column showing free nerve terminations.   Mustelus adult.   Silver-pyridine tech.   × 555.

Fig. 17   Longitudinal section of sensory epithelium showing fiber zone, free terminations embracing bases of 'hair-cells,' and varicosities on fibrillae.   Mustelus adult.   Silver-pyridine tech.   × 555.

Fig. 18   'Hair-cell' and fibrillae bearing an unusually large varicosity.   Mustelus adult.   Silver-pyridine tech.   × 555.

complex anastomosing networks, but that the nerves terminate in numerous delicate fibrillae which surround the bases of the hair-cells to a greater or less degree, and penetrate freely and severally between the walls of the hair-cells.

3. Development of the sensory canals of Mustelus

A complete series of Mustelus embryos could not be procured and accordingly more detail of development will be brought out in the following division of this paper dealing with Squalus
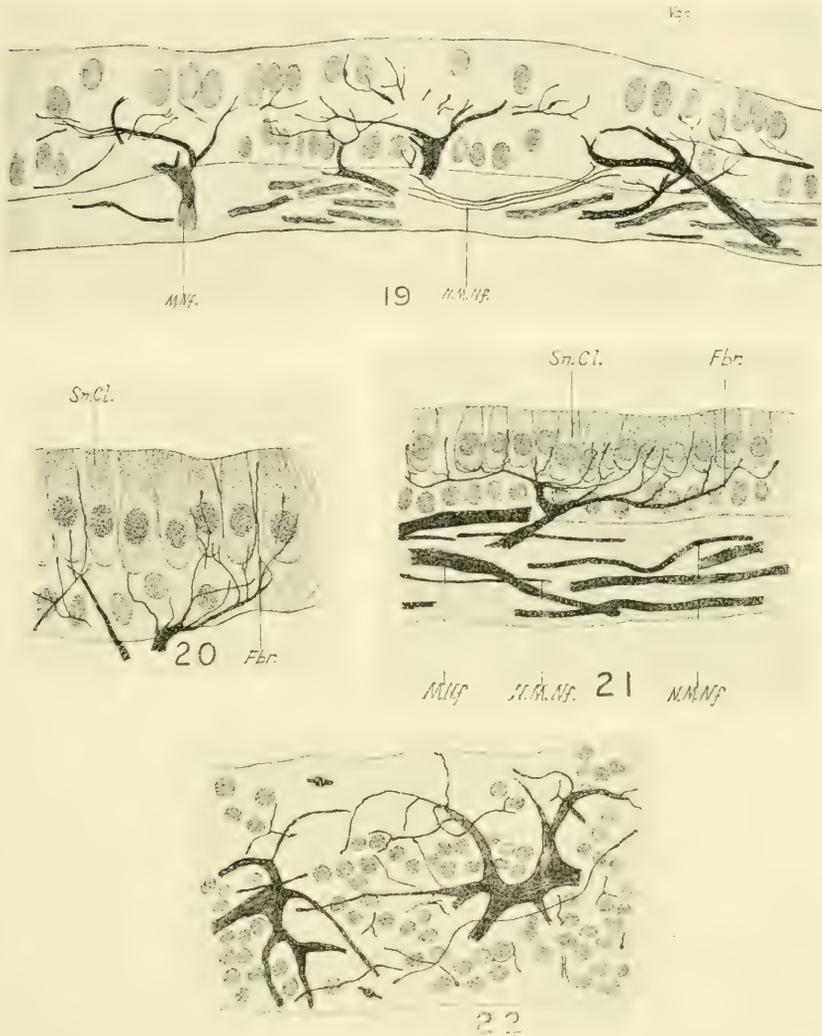
Fig. 19   Longitudinal section of the sensory column and zone of nerve distribution.   Mustelus adult.   Silver pyridine tech.   × 444.

Fig. 20   Longitudinal section of the sensory column showing very long and delicate fibrillations.   Mustelus adult.   Silver-pyridine tech.   × 592.

Fig. 21   Longitudinal section of lateral sensory column showing wide distribution of fibrillae.   Non-medullated fibers are to be seen in the fiber zone.   Mustelus adult.   Silver-pyridine tech.   × 440.

Fig. 22   Section of lateral sensory epithelium cut at right angles to long axis of the secondary sensory cells.   Cell boundaries could not be seen.   Mustelus adult.   Silver-pyridine tech.   × 440.

acanthias. The earliest stages of Mustelus available and in usable histological condition were 40 mm. in length. In these the head canals and their nerves are already formed. The lateral canal reaches to within 3 or 4 mm. of the caudal extremity and for a few millimeters in this region it has not yet closed, but is still in the form of an open groove.

The closure of the canal. There are no important differences in the method of canal formation in Mustelus and Squalus. It is essentially a process of the formation of a tube from an invaginated groove, though this fact is considerably obscured by irregularities due to unequal growth. As the lateral surface thickening grows posteriorly a groove appears on its surface and extends from the anterior end, where it first appears, posteriorly, lagging considerably behind the growing end of the thickening. As growth continues the groove becomes deeper and longer. At the same time the sensory epithelium is differentiating into a thickened ridge. Over this ridge the lips of the groove approach each other in short sections. Between the sections openings remain which become the canal tubules of the adult. The lips opposite each other fuse first at their central points so that the openings between the points of fusion appear, in the early stages of fusion, as longitudinal slits. In a specimen 55 mm. long the canal is closed to within a few millimeters of the caudal extremity. The surface openings near the end of the closed part of the canal, however, are still in the form of elongated slits. Passing forward the slits become gradually shorter until, below the second dorsal fin, they are minute, circular openings, which is their normal condition in the adult (fig. 23).

For a considerable length of time after the lateral canal is formed its lumen remains very small, in some cases appearing to be almost obliterated. Careful examination, however, always reveals its presence, although the inner walls of the canal may be nearly in contact with each other (figs. 24, 25). In these early stages the lumen of the canal must have been overlooked by Balfour as he held the opinion that it was formed by a lumen appearing in a solid cord of cells.
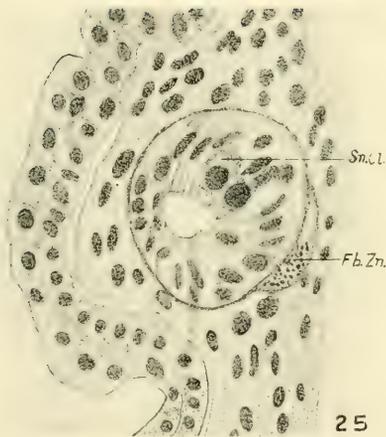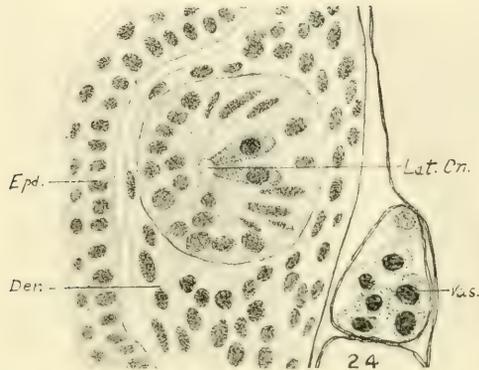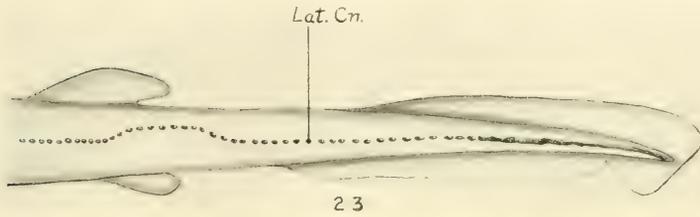
Fig. 23   Caudal end of a 55 mm. Mustelus embryo.   Note that the pores of the tubules become larger and somewhat elongated posteriorly to the point where the canal merges into an open groove. $\times$ 4½.

Fig. 24   Transverse section, lateral canal of a 65 mm. embryo, posterior to first dorsal fin.   Note the small lumen.   Mustelus $\times$ 592.

Fig. 25   From the same specimen as figure 24, but farther cephalad, where the lumen of the canal is much larger.

In specimens 65 to 70 mm. long the entire sensory canal system is completely mapped out. The canals are all closed and have reached their ultimate distribution, though in subsequent stages they become somewhat greater in diameter and, on the head, sink deeper in the integument, or even to a depth beneath the integument.

The differentiation of the sensory epithelium could not be studied in Mustelus owing to the lack of sufficiently early stages.

B. SQUALUS ACANTHIAS

1. Development of the sensory canal system

a. Early stages of development—9 to 36 mm. In tracing the development of the canal system of Squalus it will be convenient to consider the matter under two heads: (a) early stages of development, from the first definite appearance of sensory cords on the surface to the time the lateral cords have grown back to the tail, and (b) later stages of development, involving involution of the sensory cords, the closure of the canals and the differentiation of the sensory epithelium. For observations of the earlier stages I had embryos between 9 and 36 mm. body length and for the later stages those from 40 to 72 mm. The descriptions are confined chiefly to the development of the lateral cord and canal, since their growth is typical of the corresponding structures of the head, simpler for examination and not complicated by cephalic modifications.

Material was not available for independent study of the first phases of development of the canal system, but sketches of Squalus embryos from 3.25 to 8 mm. length made under the direction of Professor Locy by Mr. Otto Swezey seem to indicate that the epithelium of the sensory cords has a common origin with the epithelium of the ear. In tracing the primordia of the lateral line organs two things are to be kept in mind— the epithelium out of which the sensory cords are developed and the primordia of the ganglia. One comes from the surface ectoderm and the other from the neural crest. While the ganglia arise independently, the sketches of Mr. Swezey appear

to favor the conclusion that the thickened patches of the sensory cords were originally a part of the auditory thickening and such was the interpretation of Mr. Swezey.

It must be said, however, that my observations on the relation of the primordia to the auditory thickening are not of a definite character and, as a result, they do not throw light on the question of the independence of origin of the primordia of the lateral line system and of the auditory epithelium (Landacre '10, Reed '16).

My own observations of developmental stages began with embryos 9 mm. in length. In this stage definite rudiments of the lateral sensory cords had appeared as well as of the principal canals of the head. In a 36 mm. embryo the lateral sensory cord of the trunk has reached the tail region and the distribution of the sensory cords of the head, although not in their final arrangement, are so clearly outlined that the different lines can be easily defined.

Nine millimeter stage. The 9 mm. embryo, the head of which is shown in surface view in figure 26, has about sixty somites. The cranial ganglia from the fifth backwards are well developed and can be seen in surface view as elevations of more or less prominence. The ninth nerve, which may be adopted as a landmark, can be identified by its ganglion just behind the ear capsule and a long process extending from the ganglion into the first branchial arch.

Immediately posterior to the ninth are the lateralis and vagus roots, the latter connecting with the walls of the medulla in a broad sheet. The distal margin of this sheet exhibits, at this stage, three ganglionic enlargements that lie above the second, third and fourth branchial arches and have a placode connection with the thickened ectoderm.

A dissection of this region shows that the ganglion of the lateralis nerve is independent of the vagus. It is attached anterior to the vagus roots (figs. 27, 30) and crosses obliquely the expanded sheet of the vagus rootlets (fig. 30).

A cross section (fig. 28) taken through the plane of the line A-B of figure 27 shows the structure of the lateralis ganglion
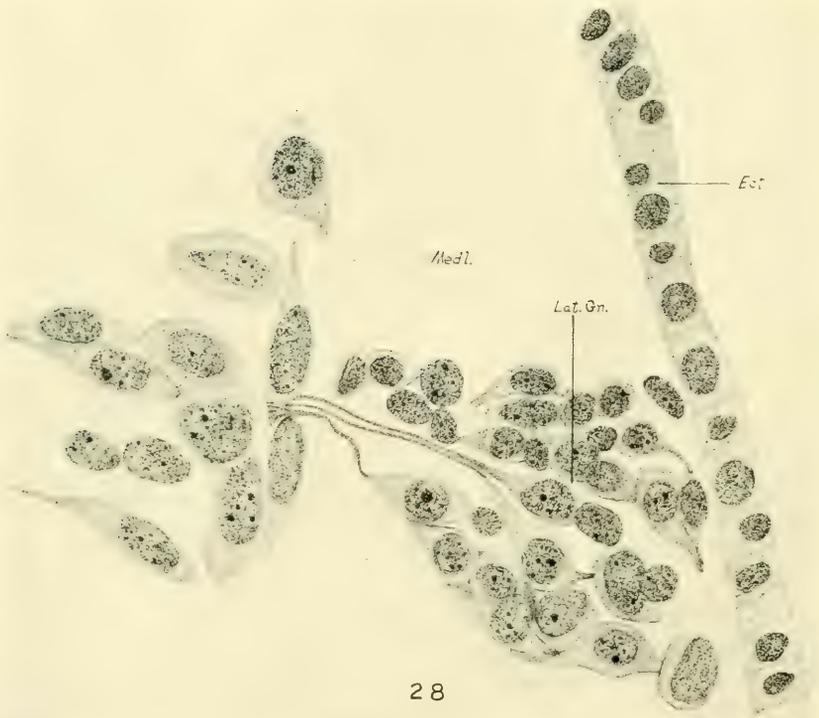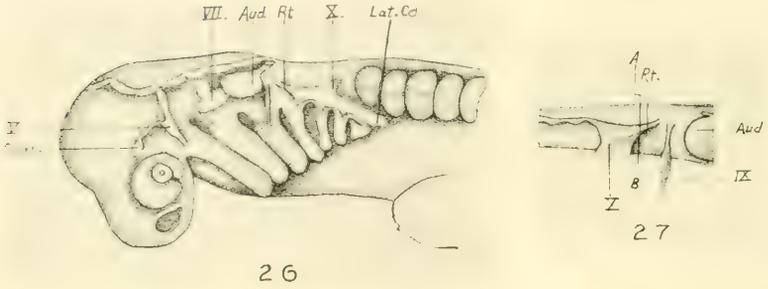
Fig. 26 Squalus acanthias. Surface view of a 9 mm. embryo. × 13. From a sketch by Otto Swezey.

Fig. 27 Lateral dissection of a 9 mm. specimen showing the roots of the ninth, the lateralis, and the vagus nerves. × 20.

Fig. 28 Transverse section through the region indicated by the line *A-B* in figure 27. Squalus. × 976.

and its connection with the brain wall. At this stage the ganglion cells are of the bipolar type and the section shows three central processes penetrating the wall of the medulla. The peripheral processes run obliquely backwards toward the cells of the ectodermal anlage of the lateral sensory cord.

Eleven millimeter stage. This stage (fig. 29) shows well marked advances in the development of cranial structures. Confining attention to the vagus and lateralis region, surface observations show the widely expanded sheet of vagus rootlets merging on the ventral margin into three well marked vagus ganglia. The lateralis ganglion is connected with the brain wall in front of this widely expanded sheet and the ganglion itself passes obliquely backwards over the sheet of rootlets and above the vagus ganglia. This is shown better in the dissection of this region of a slightly older embryo (fig. 30). The independence of the lateralis ganglion is clearly shown in this sketch and sections confirm this independence, although its ganglion cells and those of the vagus are closely associated in some of the sections. Distally the fibers arising from the ganglion cells of the lateralis pass out among the growing cells of the ectodermal cord. The terminals of the peripheral fibers end freely among the cells and have not as yet established dendritic relations with cells of the ectodermal cord. The condition is shown in a little later stage of development in figures 35 and 36.

In the 11 mm. stage the ectoderm at the growing end of the lateral sensory anlage is crowded with dividing cells which lie next to the surface (fig. 31) and the cells of this thickening are enlarged or elongated.

Thirteen millimeter stage. The sketch of this stage (fig. 32) is copied from a drawing by Mr. Otto Swezey. Four gill-slits have broken through and fringes of external gills are beginning to appear. Owing to thickening of the adjacent tissues, the cranial ganglia and their roots are not so clearly discernible from the exterior.

Two small thickened cords of ectoderm fork in front of the facial ganglion. One is directed above and the other below the
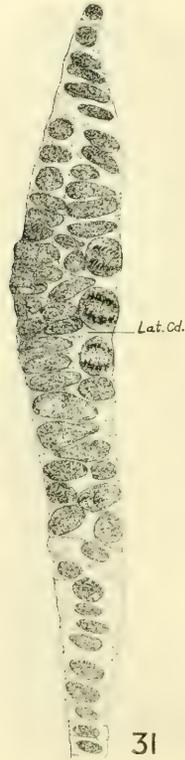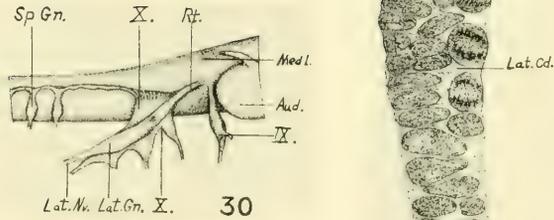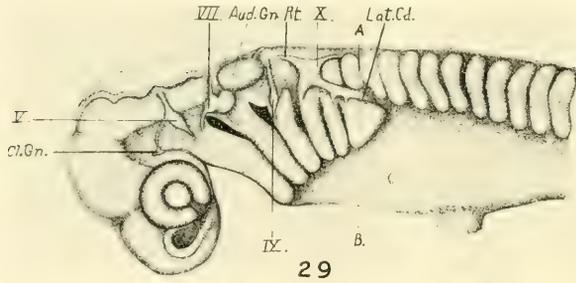
Fig. 29   Surface view of an 11 mm. embryo showing the various structures concerned in the formation of the sensory canal system.   Squalus.   From a sketch by Otto Swezey.   × 16.

Fig. 30   Lateral dissection of the medulla spinalis showing early separation of lateralis root and ganglia from the vagus complex.   Squalus, 11 mm.   × 20.

Fig. 31   Transverse section of the thickened ectodermal cord at position indicated by line *A-B* in figure 29.   × 466.

eye. These cords represent the rudiments of the supraorbital and the infraorbital sensory canals. Nerve fibers from two ganglionic enlargements of the seventh are distributed among the cells of the inner surface of the growing cords. The superior bundle of fibers is the rudiment of the ramus ophthalmicus superficialis VII and the inferior bundle the rudiment of the ramus buccalis VII. The cord of the lateral canal is obviously longer than in the earlier stages.

Fifteen millimeter stage. Surface observations of this embryo (fig. 33) show considerable growth of the sensory cords. The supraorbital and the infraorbital are longer and better defined. The nerve bundle of the infraorbital cord has begun at its proximal end to sink beneath the inner surface of the cord.

The lateral cord has increased in length and also in thickness. A new feature has made its appearance at the distal end of the cord in the form of an ectodermal fold (fig. 33, *Ep. Po.*) which at first is a shallow pocket, and in later stages becomes a tunnel. The condition produced is as if the distal end of the cord in growing backwards had pushed under the common ectoderm lying in its path, causing the latter to bulge into a crescent-shaped fold. A longitudinal section of this fold is shown in figure 36.

A dissection of the region back of the ganglion of the ninth nerve is shown in figure 34. The lateralis ganglion and its roots are now easily distinguishable from the ganglia and roots of the vagus. The vagus roots enter the brain wall in a continuous broad sheet and the inferior margin is separated into four distinct ganglia. The lateralis ganglion crosses the vagus-complex running obliquely above the four ganglionic enlargements. It is greatly elongated, its distal end reaching posterior to the fourth vagus ganglion.

Longitudinal sections of this stage show the nature of the growth of the lateralis nerve and the relation of its fibers to the cells of the sensory cord. Figure 35 represents part of a section taken lengthwise through the lateralis ganglion. The ganglion (*Lat. Gn.*) is composed chiefly of bipolar cells the central fibers of which pass into the brain (fig. 28). The peripheral
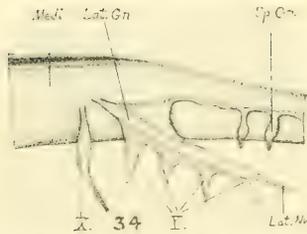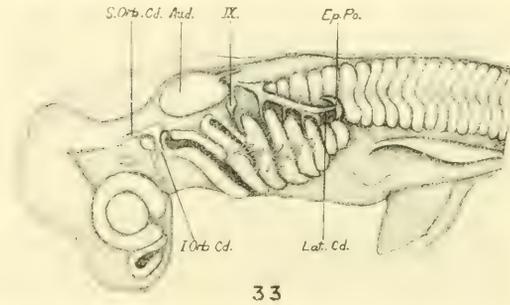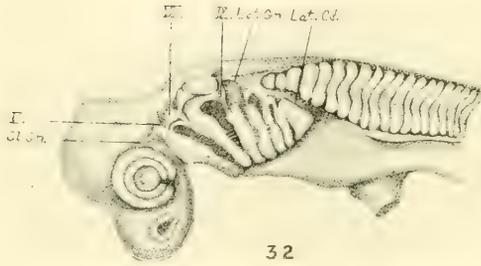
Fig. 32　Surface view of a 13 mm. embryo.　Squalus acanthias.　From a sketch by Otto Swezey.　× 10.

Fig. 33　Surface view of a 15 mm. specimen.　Note the epidermal pocket at the growing end of the lateral cord.　Squalus acanthias.　From a sketch by Otto Swezey.　× 10.

Fig. 34　Lateral dissection of the medulla of a 15 mm. embryo of Squalus showing roots and ganglia of the lateralis and vagus nerves.　× 20.

Fig. 35　Horizontal section through the posterior part of lateralis ganglion, showing fibers from the ganglion cells extending into the thickened ectoderm of the lateral cord.　Squalus, 15 mm. specimen.　× 492.

Fig. 36　Horizontal section, from the same specimen as figure 35, but taken at the growing end of the lateral sensory cord.　Shows the epidermal fold over the greatly enlarged growing end of the sensory cord; nerve fibers extend among the cells of the latter.　Squalus, 15 mm.　× 492.
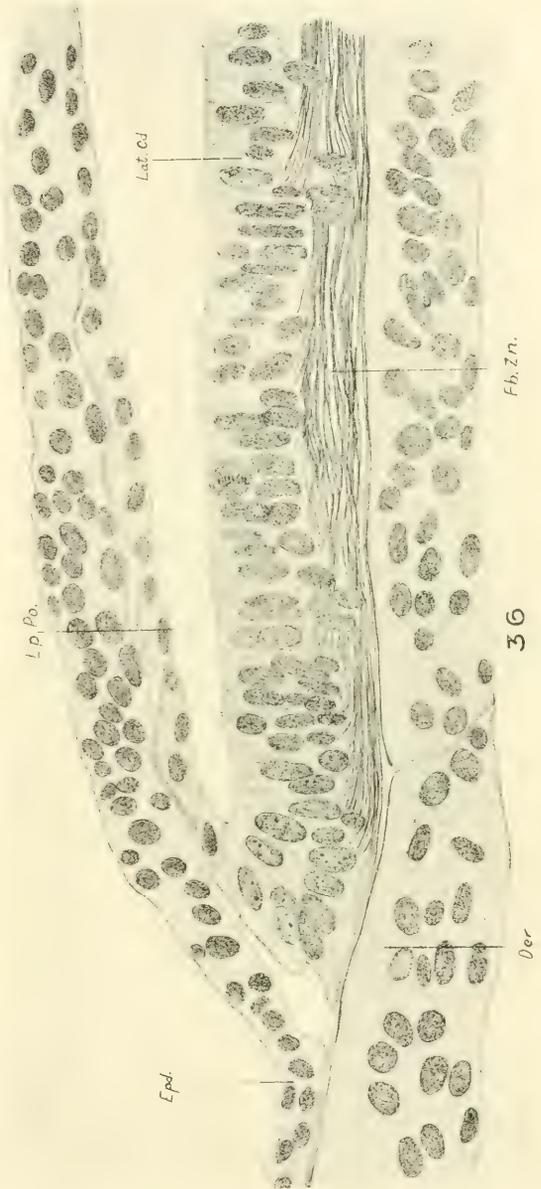
fibers of the ganglion cells pass directly to the inner surface of the ectodermal thickening, where they ramify amongst the cells which are already becoming differentiated to form the sensory column (figs. 35, 36). This primitive lateral nerve lies immediately subjacent to the cells of the sensory epithelium. The grouping of the fibers into ramuli is a secondary process of which there is as yet no indication.

Figure 36 is a longitudinal section of the growing end of the lateral sensory cord, showing the ectodermal pocket, the sensory column and the terminal fibers of the lateral nerve. The nature of the ectodermal pocket is well shown—it is a fold of the outer layer of the skin curving over the thickened growing end of the sensory cord. It takes no part in the formation of the canal which subsequently arises by a process of involution of the sensory epithelium and the closure of the groove thus formed. The overlying epidermal fold, although it becomes much elongated, breaks down and disappears before the true canal is formed.

The epithelium of the growing end is undergoing differentiation. Some of the cells are assuming a columnar or a spindle form, while others appear to be assuming the features of hair cells. Abundant mitotic figures indicate rapid growth of this part of the cord. The continuity of the sensory column is unbroken, there being as yet no differentiation of the cells into the little groups or clusters that a little later are characteristic of the sensory column.

Seventeen millimeter stage. This embryo is slightly in advance of the last stage.

On the head the supraorbital thickening has grown forward to a point above the middle of the eye. Its companion cord, the infraorbital, is about the same length and is directed forwards close to the inferior margin of the eye. Immediately subjacent to the thickened cords of ectoderm, and in close association with the cells of the latter, are large bundles of nerve fibers which arise from the ganglia of the seventh nerve and which accompany the growing cords of thickened ectoderm.

The lateral thickening has assumed more definite form and extends posteriorly to the anterior margin of the pectoral fin-

bud. It appears as a narrow, slightly elongated cord, and the epidermal infolding at its growing end has assumed the character of a little pouch with its crescent-shaped opening directed forwards. The shape of the fold is explained by the fact that the central part is carried backwards by the rapidly elongating thickened cord of ectoderm, while the upper and lower margins are retarded by the neighboring common ectoderm with which they are continuous.

The root of the lateralis ganglion has become more distinct and its superficial attachment to the brain wall is more widely separated from the vagus roots. From the posterior limit of the ganglion, which is sharply defined, a large bundle of nerve fibers passes to the inner surface of the lateral sensory cord.

Eighteen millimeter stage. In this embryo (fig. 37) all of the sensory cords have greatly elongated. The supraorbital reaches almost to the anterior margin of the eye; the infraorbital has attained an equal growth below the eye, and both cords appear as narrow ridges occupying perceptible grooves. For their greater length the nerves which supply these cords lie immediately subjacent; owing to the growth of the embryo, however, the proximal ends of the nerves now occupy a position slightly beneath the integument.

Ectodermal folds appear at the growing ends of the head cords, but their walls degenerate rapidly and for that reason they do not assume the character of an elongated tunnel such as develops over the lateral cord.

The lateral cord of this stage reaches as far caudad as the middle of the pectoral fin (fig. 37). It shows marked lateral convexity and lies in a slight groove. The epidermal tunnel is rather prominent. This primitive tunnel must not be confused with the lateral canal which arises later.

The growing end of the lateral cord is greatly enlarged with dividing cells and it is quite evident that the superficial ectodermal tunnel is formed by the ordinary ectoderm as it is pushed up by the growing sensory cord. The layer of ectoderm which was originally continuous with the lateral sensory canal anlage is carried along with the growing end, thus giving two layers to the outer wall of the ectodermal tunnel (figs. 38, 36).

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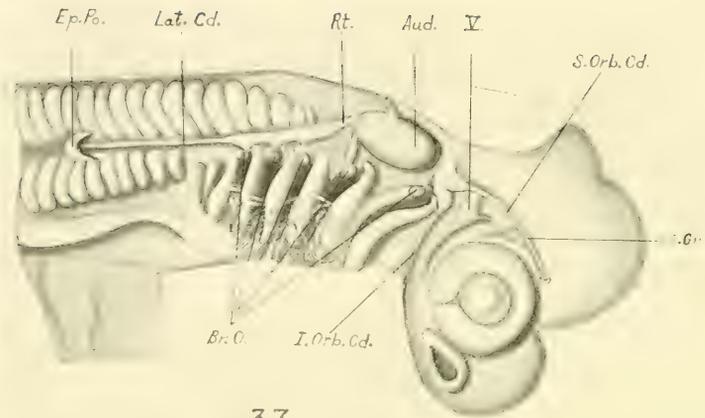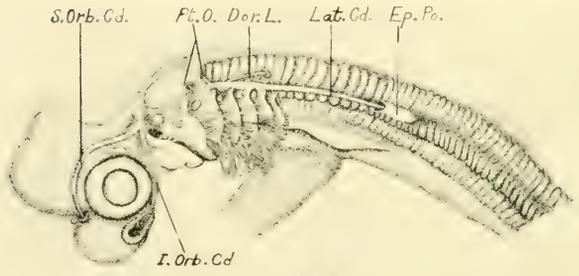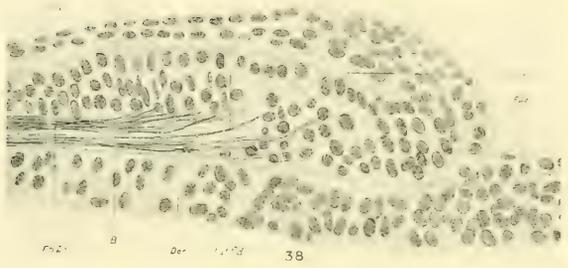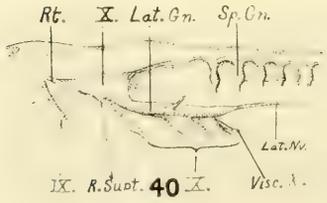
The nerve distribution to the sensory cord is continuous throughout its length. The cells of the cord have not taken on a definite arrangement, although the different types of cells characteristic of the adult appear to be differentiating.

Twenty millimeter stage. In this specimen (fig. 39) is first seen the rudiment of the dorsal series of pit organs. It is a narrow, slightly thickened cord of cells arising as an off-shoot from the suprabranchial ectoderm in a manner entirely comparable to the origin of the lateral and head cords. There is a little ectodermal pocket at its growing end and it carries nerve fibers from a branch of the lateralis ganglion (fig. 40).

The lateralis and vagus ganglia (fig. 40) are somewhat more advanced than in previous stages. The branch that innervates the series of pit organs mentioned above is shown in figure 40. It springs from about the middle of the lateralis ganglion and extends obliquely upwards and backwards.

As shown in sections the lateral nerve is still in close association with the inner surface of the sensory cord (fig. 41). Farther caudad the lateral nerve is separated into four bundles (fig. 42). Transverse sections (figs. 42, 43) of the sensory thickening show its relative size and degree of differentiation at different levels. A distinct enlargement is evident at the distal end where the thickening is composed of several layers of cells, many of which show the mitotic figure. Anterior to the epidermal tunnel (fig. 42) the cells of the sensory cord show considerable differentiation and longitudinal invagination of the sensory epithelium appears to have begun.

Fig. 37 Surface view of an 18 mm. embryo. The lateral, supraorbital, and infraorbital sensory cords have greatly extended in length. The branchial sense organs of Beard are also quite prominent. From a sketch by Otto Swezey. × 12.

Fig. 38 Horizontal section of the growing end of the lateral sensory cord. Squalus, 18 mm. See figure 43.

Fig. 39 Surface view of a 20 mm. embryo. In this specimen the dorsal cord is well defined and the rudiments of two supratemporal pit organs (Pt. O.) have appeared. Squalus. From a sketch by Otto Swezey. × 6.

Fig. 40 Lateral dissection of the medulla of a stage similar to that shown in figure 39. The rami which supply the supratemporal commissure and the dorsal line of pit organs appear as small offshoots from the lateralis ganglion. × 13.
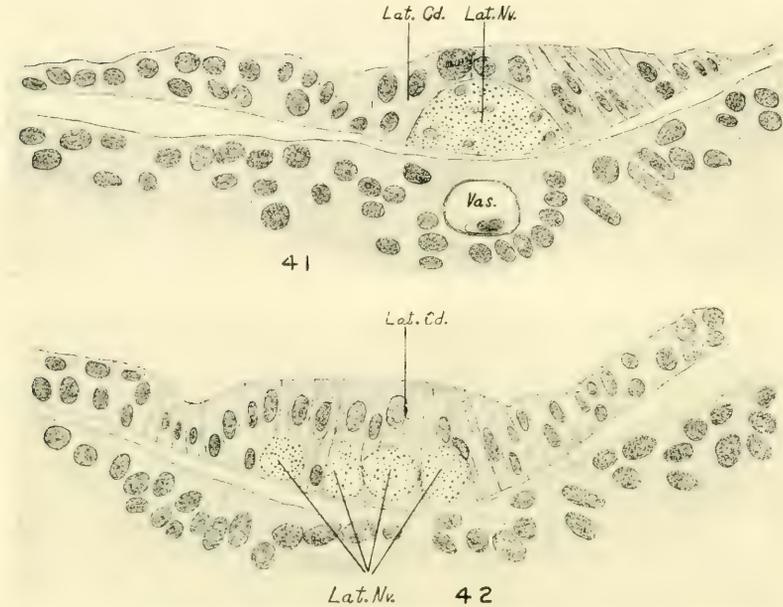
Fig. 41   Transverse section of the lateral sensory cord, of a 19 mm. specimen, near its anterior end.   The large lateral nerve has caused a slight bulging of the overlying ectoderm.   Squalus.   × 592.

Fig. 42   Transverse section of the sensory cord of the same specimen as shown in figure 41, further caudad.   The fibers of the lateral nerve have separated into four bundles.   × 592.

Fig. 43   Transverse section of lateral sensory cord at point indicated by the line A-B in figure 38.   Squalus, 20 mm. specimen.   × 300.

Fig. 44   Surface view, Squalus 21 mm.   From a sketch by Otto Swezey. × 8.

Fig. 45   Dorso-lateral dissection of a similar stage.   × 12.

Twenty-one millimeter stage.   An embryo 21 mm. long is represented in figure 44.   In it the structures described in previous stages are a little further advanced.   Figure 45 shows a partial lateral dissection of an embryo of the same age.   The ganglia of the seventh are exposed together with the nerves that pass from them to the supra- and infraorbital lines.   The independence of the very elongated lateralis ganglion is clearly exhibited.   In this figure should be especially noted the nerve trunk for the dorsal series of pit organs.

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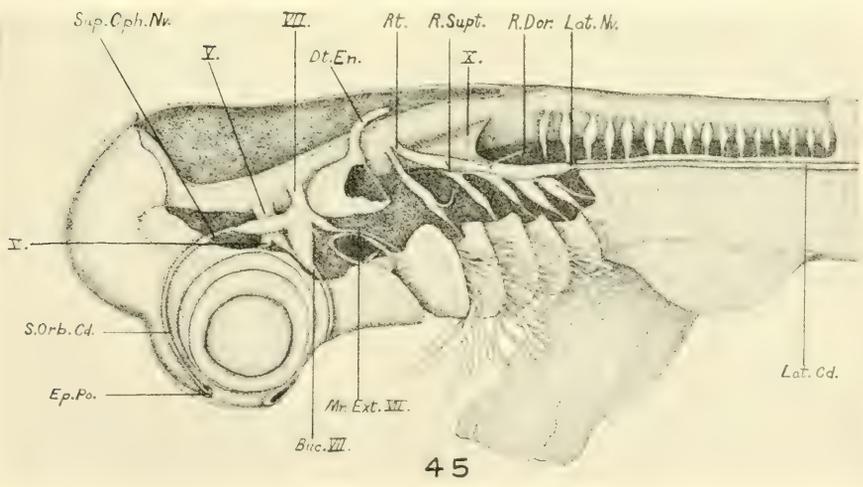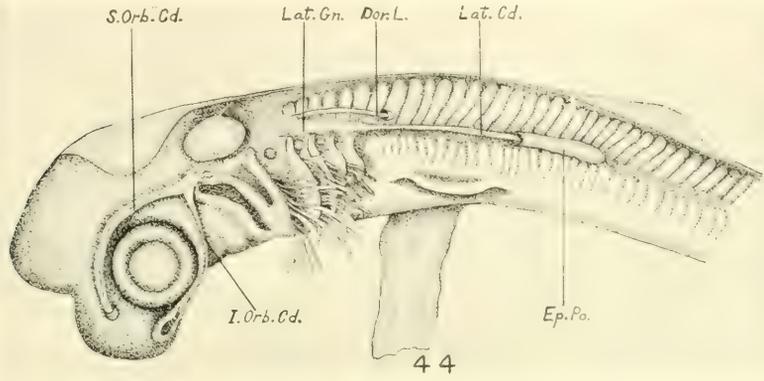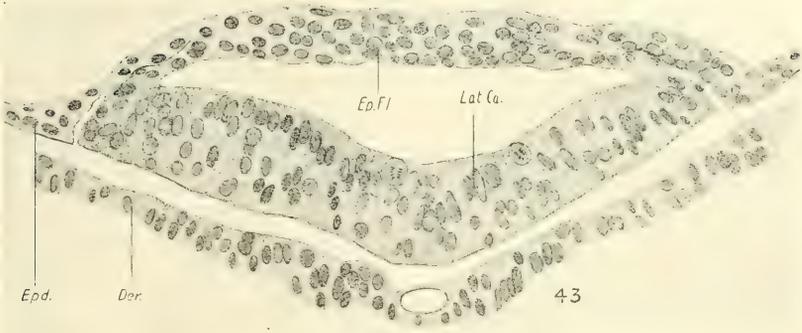
In horizontal section the growing end of the lateral cord of this stage appears practically the same as that shown in figure 36.

Figure 46 is a cross section in front of the ectodermal pocket near the pectoral fin. The nerve cord is well defined in this situation. It lies close against the sensory cord and produces a slight bulge on the surface.

Twenty-two and one-half millimeter stage. In this stage (fig. 47) it is important to note two little off-shoots of the thickened ectoderm which pass dorsalwards posterior to the ductus endolymphaticus. They take their origin from the suprabranchial ectoderm and ectodermal folds are pushed up at their distal ends. These small cords are substantially similar to the other sensory thickenings. Like the dorsal cord, they undergo subsequent partial degeneration or, at least, great modification. In the adult each of the two small cords is represented by single isolated pit organ, while the dorsal cord is represented by a series of separated pit or surface organs.

The dorsal cord is still short, although it has thickened considerably and has a prominent fold of ectoderm at its distal end.

The supraorbital and infraorbital cords have not only become greatly extended, but they have also begun to invaginate or sink beneath the surface preparatory to the formation of closed canals.

The lateral cord extends as far caudally as the posterior border of the first dorsal fin. The ectodermal tunnel forms a long tube over its growing end, although its anterior lip has also receded to a considerable extent.

Fig. 46   Transverse section of lateral sensory cord at level of pectoral fin. Note the slight bulging caused by the relatively large lateral nerve. Squalus, 21 mm. $\times$ 592.

Fig. 47   Surface view of a 22 mm. embryo. Squalus. From a sketch by Otto Swezey. $\times$ 625.

Fig. 48   Dorso-lateral dissection of a $22\frac{1}{2}$ mm. specimen showing the nerves concerned in the innervation of the various sensory cords. Squalus. $\times$ 13.

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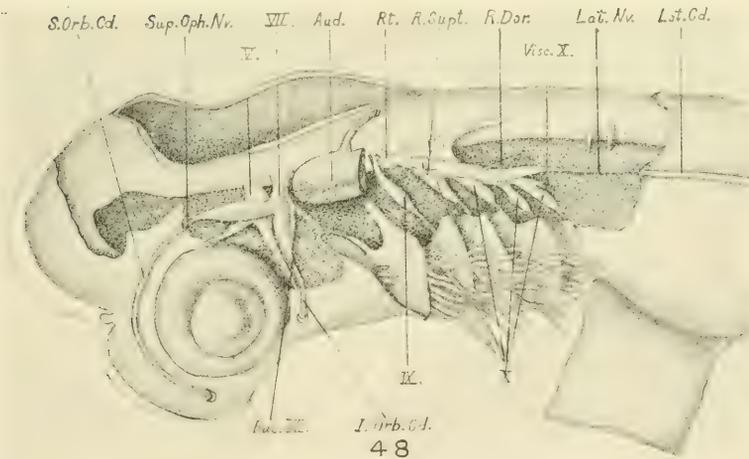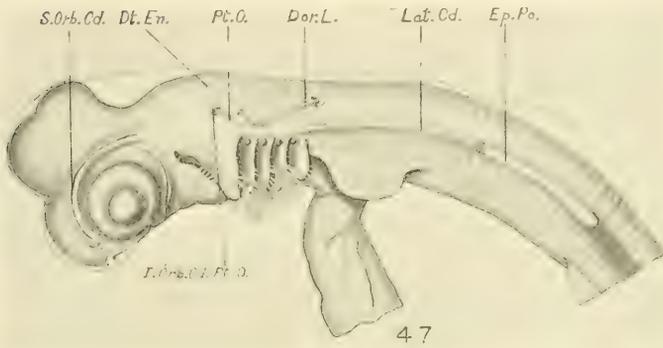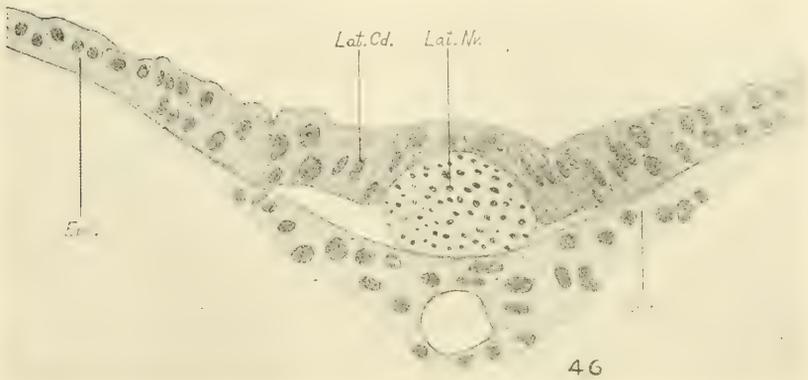
Figure 48 represents a lateral dissection of a 22.5 mm. embryo showing the ganglia and the nerves associated with the sensory cords. Note again in this stage the branch to the dorsal pit organs and the one to pit organs of the supratemporal region. Anteriorly the lateral nerve has now come to lie slightly below the integument, but a little farther back it is still in close contact with the ectoderm (fig. 49).

Twenty-five millimeter stage. In this embryo (fig. 50) marked growth has occurred in the supraorbital cord, its inferior limb now bending backward in front of the eye. The infraorbital is considerably extended also, and passes to the ventral side of the head. The infraorbital and supraorbital both present slight grooves, and at their proximal ends the lips of the grooves have begun to approach each other in short sections or segments. The spaces between the sections with approximated lips become smaller in advanced stages and remain as the pores of the tubules in the adult.

The dorsal cord has pushed back a little farther and has a long epidermal tunnel at its growing end.

The lateral cord now extends to the posterior border of the anal fin and the ectodermal tunnel extends forward to the first dorsal fin. It is to be kept in mind that the ectodermal tunnel is a temporary structure and is not to be confused with the canal which is a later formation.

A perceptible groove has appeared on the surface of the cord anterior to the opening of the ectodermal tunnel and the mouth of the tunnel has receded to the first dorsal fin.

A lateral dissection of a specimen of the same length is shown in figure 51. In front are shown the ganglia from which issue the nerves that supply the supraorbital and the infraorbital lines. Posteriorly is exposed the long lateralis nerve. Two branches are given off from the body of the ganglion. The anterior one goes to the supratemporal commissure and the posterior to the dorsal series of pit organs.

Twenty-seven millimeter stage. Surface views of this embryo are represented by figures 52, 53. The anterior canals have extended to the ventral surface of the head. The supraorbital
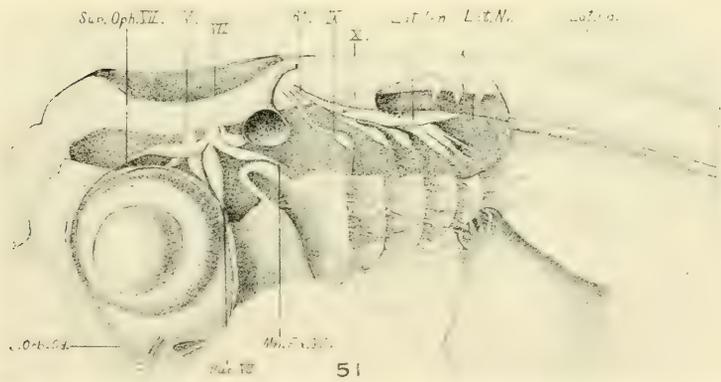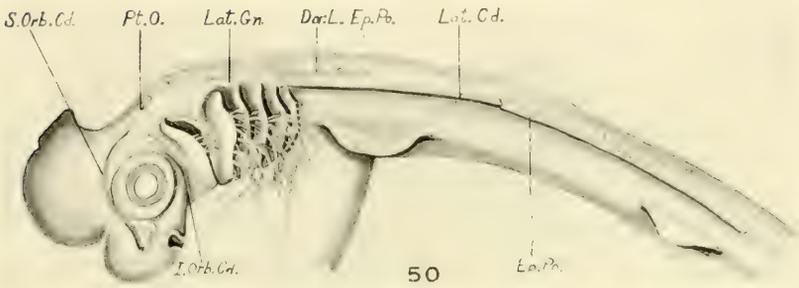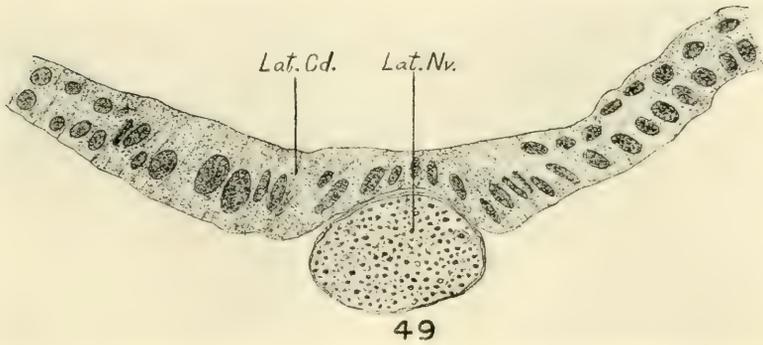
Fig. 49   Transverse section of lateral sensory cord and lateral nerve pectoral region.   Squalus 22 mm.   × 592.

Fig. 50   Surface view of a 25 mm. Squalus embryo.   From a sketch by Otto Swezey.   × 62.

Fig. 51   Dorso-lateral dissection of a similar stage.   × 13.

has lengthened and passes backwards between the eye and the nostril, while the infraorbitals draw close to each other in front of the buccal orifice and then bend forward a short distance between the nostrils (fig. 53). The grooves of the head thickenings are now quite deep and at their proximal ends the lips of the canals have begun to fuse or coalesce.

As seen on the ventral surface the hyomandibular sensory cord bifurcates at the angle of the mouth (fig. 53), one arm passing forward and the other backward.

The anlage of the supratemporal commissure has made its appearance as a short ectodermal cord passing dorsalwards immediately posterior to the opening of the ductus endolymphaticus (fig. 52). Two little rounded thickenings in front of the ductus endolymphaticus represent the remains of the two short thickenings shown in figure 47, and mentioned in the description of the 22 mm. embryo.

The lateral cord has extended posteriorly as far as the second dorsal fin, and the dorsal cord almost as far as the first dorsal. A new cord has arisen between the anterior end of the lateral cord and the point of origin of the supraorbital and infraorbital canals, with which it unites some time later.

A dissection of the lateralis ganglion and the adjacent territory is shown in figure 54. Arising from the body of the ganglion are the branches that supply respectively the supratemporal commissure and the dorsal series of pit organs. The lateralis nerve shows an interesting condition. It is divided into two strands. From the upper strand are given off ramuli of nerves that go to the accessory sense organs and from the lower strand similar ramuli whose fibers supply the canal sense organs. Dissections of other specimens make it clear that this division into two strands is not always present. It was observed in another specimen 29 mm. long, but in most of the specimens dissected these strands were combined into a single nerve.

A transverse section of the cord near the anterior end of the epidermal tunnel (fig. 55) shows that the fibers of the lateralis nerve have become gathered into a definite trunk which still lies immediately under the sensory cord. The sensory cord
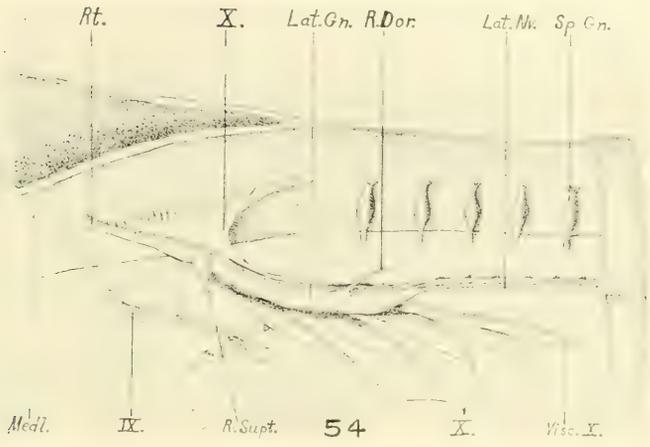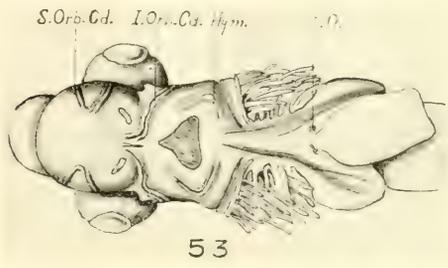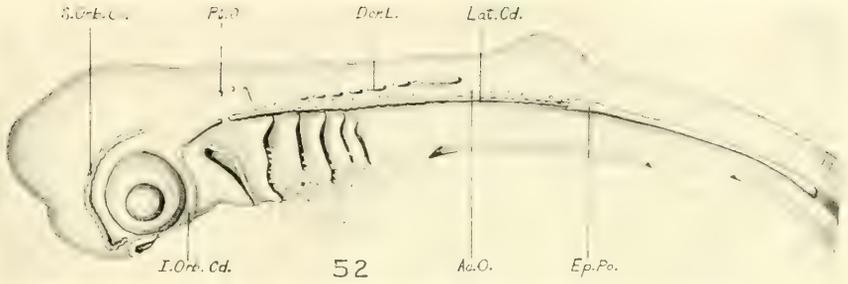
Fig. 52 Surface view of a 27 mm. embryo. Note that the dorsal cord has broken into short segments and that the accessory organs are now fairly prominent. Squalus. From a sketch by Otto Swezey.

Fig. 53 Ventral view of the same specimen showing distribution of the sensory cords on the under surface of the head. Note the rudiments of two short lines of pit organs (*Pt.O.*) just anterior to the yolk stalk. Squalus. From a sketch by Otto Swezey.

Fig. 54 Dorso-lateral dissection of the medulla of a stage similar to the one shown in figure 53. Note that the lateral nerve is separated into two main divisions. The upper division supplies the accessory organs. × 22.

has undergone considerable cellular differentiation so that the main features of the histology of the sense organs are discernible. There are columnar cells, basal cells and sensory cells. The outer wall of the ectodermal tunnel is very thin and shows evidence of degeneration.

Above the lateral sense organ proper is seen in this section an accessory organ which appears to have budded off from the lateral sensory cord and is still within the ectodermal fold (fig. 55). A section anterior to the ectodermal tunnel (fig. 56) exhibits the accessory and the canal organs more widely separated and the lateral nerve considerably removed from the inner surface of the ectoderm.

Accessory sense organs of this kind exist as a longitudinal series running parallel to the lateral sense organs. They are shown in figure 52 just dorsal to the sensory cord. In this specimen they are exposed from the first branchial arch to the dorsal fin and then disappear under the ectodermal fold.

One of the two small sensory cords lying near the yolk-stalk (fig. 53, also fig. 3) is shown in longitudinal section in figure 57. The general resemblance of this section to one of the growing ends of the lateral cord is striking. It has an ectodermal fold and the sensory cord is well provided with nerve fibers. These thickenings are similar to the other sensory cords except that they are more transitory and I was not able to trace their origin from the thickened suprabranchial ectoderm. Of these cords only a few isolated pit or surface organs remain in the adult.

Twenty-nine millimeter stage. In this embryo (fig. 58) the surface changes are not great and consist in the progressive

Fig. 55 Transverse section of the lateral sensory cord posterior to the first dorsal fin. Note that a lateral line organ and an accessory organ are both covered by the superficial epidermal fold. Squalus, 27 mm. × 492.

Fig. 56 Transverse section of the lateral sensory cord farther cephalad (anterior to the epidermal tunnel) than shown in figure 55. Here the lateral nerve lies at a deeper level, and the accessory organ is farther removed from the lateral line organ. Squalus, 27 mm. × 370.

Fig. 57 Longitudinal vertical section of one of the pit organ rudiments indicated near the yolk stalk in figure 53 (Pt.O.). Squalus, 27 mm. × 492.

Fig. 58 Surface view of a 28 mm. embryo. Squalus. × 3.

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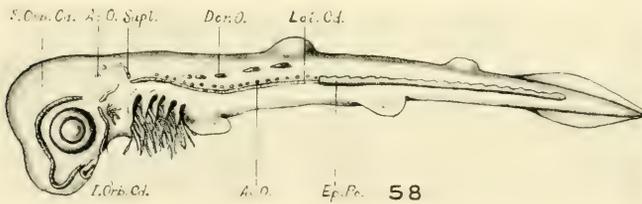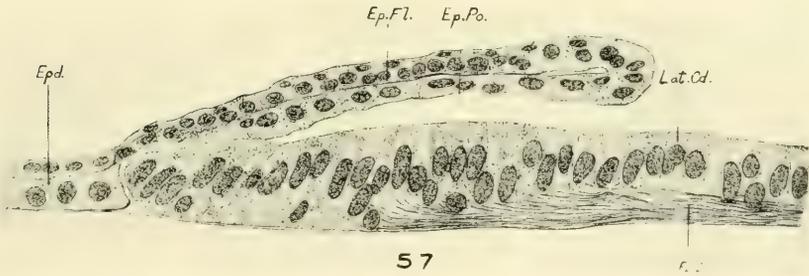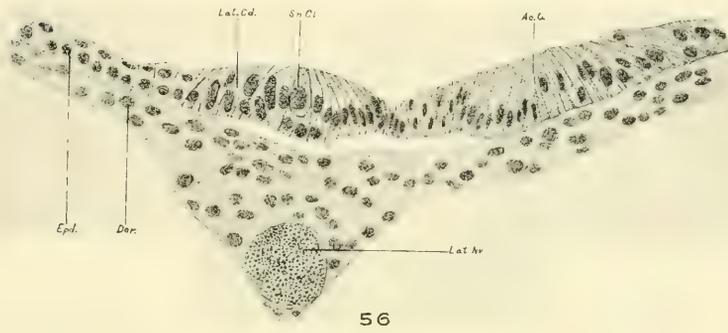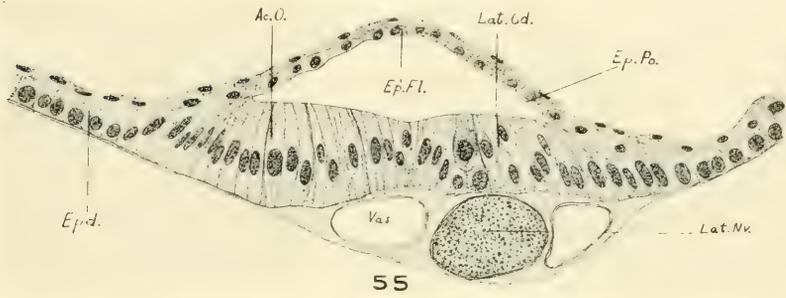
growth of all parts of the lateral line system. The head canals are closed at their proximal ends, and a short cord, sometimes designated the temporal cord, lacks only a little of uniting the lateral and the infraorbital canals.

In the trunk region we can readily distinguish (as also in earlier stages) a dorsal line, an accessory line and a lateral sensory cord.

The growing end of the dorsal cord, extending to the first dorsal fin is still prominent.

The growing end of the dorsal cord is still prominent, although intermediate parts of it have disappeared, leaving a series of separated sensory thickenings which are still enclosed in little epidermal pockets. This is shown in section in figure 59.

That part of the lateral sensory cord in front of the epidermal tunnel now appears as a continuous chain of minute 'swellings.' These swellings are the little clusters into which the hair-cells have grouped themselves and which are characteristic of the adult sensory epithelium. A longitudinal section of this part of the cord (fig. 60) shows basal cells, columnar cells and sensory cells. As shown in cross section (fig. 62) the sensory column is sunken below the surface but is not as yet enclosed within a canal.

Immediately above the lateral cord is a series of separated 'swellings,' just a little larger than the cell clusters of the lateral cord, and less numerous. These have been termed accessory lateral line organs. Their appearance in longitudinal section is represented in figure 61. The origin of these organs has not been traced from their earliest appearance. There appears to be little question, however, but that they arise from the superior margin of the thickening which gives rise to the organs of the lateral canal. Evidence in favor of this view is seen in figure 63, where the lateral and accessory organs are in close association, both lying within the epidermal tunnel (also fig. 55). As the epidermal fold recedes, the organs become uncovered and may be readily seen in surface studies under low magnification.

Thirty-six millimeter stage. In this embryo the course of the canals is completely mapped out with the exception of the su-
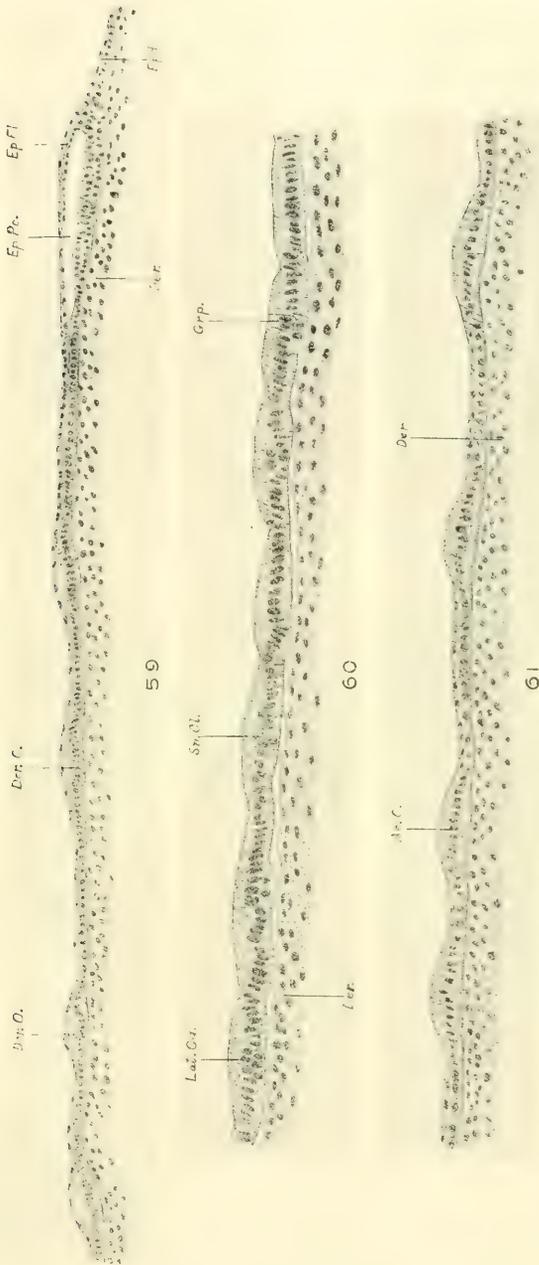
Fig. 59 Horizontal section of the dorsal sensory cord. Parts of this cord appear to have undergone degeneration. while the remaining portions become the pit organs of the adult. Squalus, 29 mm. × 114.

Fig. 60 Horizontal section, at the same level of the lateral sensory cord. The 'hair-cells' show definite arrangement into small groups. × 196.

Fig. 61 Horizontal section of the line of accessory organs taken at the same level as figures 59 and 60. × 196.

pratemporal commissure, the two arms of which have not yet grown together, and the infraorbitals, which have not yet reached their anterior limit (figs. 64, 65).

The lateral cord has united with the supraorbital and infraorbital canals, anteriorly, and at this stage, which precedes the formation of the lateral canal, it passes to the tip of the caudal fin. The sensory cord presents the appearance of a minute chain of beads lying at the bottom of a shallow furrow. The differentiation of the sense organs is more advanced in the anterior part of the sensory cord than in the posterior part.

The sensory canal system is now practically mapped out and, although great changes occur before it reaches the adult condition, these changes are of a secondary nature, such as changes in relations and closure of the canals. If the system were arrested in its development at this stage, or a little earlier, it would be comparable in a rough way to the lateral sense organs found in certain amphibia, although in the latter the sensory epithelium is much less abundant.

b. Later stages of development—40 to 72 mm. In the 36 mm. stage it was seen that the distribution of the sensory cords is practically completed, and the nerve connections apparently all established. Cellular differentiation of the sensory cords is also well advanced. Some changes in relations occur and surface tubules become elongated as growth proceeds but the important change which follows is the invagination of the sensory thickenings of the trunk and the gradual enclosure of the main lines of sense organs within epithelial canals. These changes are here described as they appear in embryos of 40, 45, 52, 67, and 72 mm. in length.

Forty millimeter stage. The head canals which were well on their way towards involution in the 36 mm. stage are now almost completely closed. The pores of the tubules are much smaller than they were and as a result the canals are less conspicuous in surface views.

The groove of the lateral line is closed for a short distance in its anterior region and is represented by a furrow for a consider-
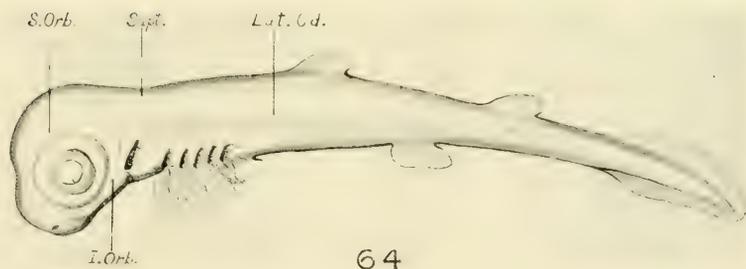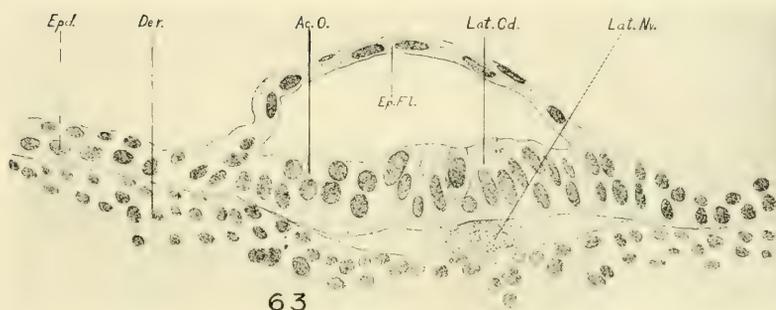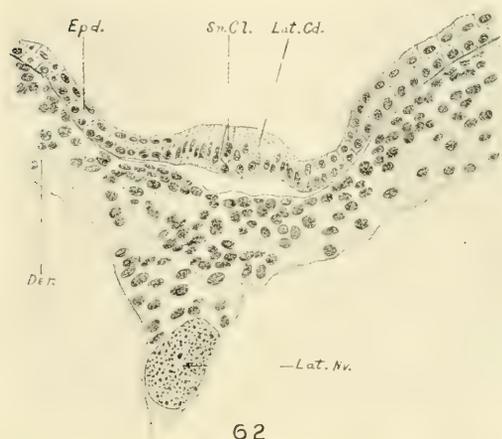
Fig. 62   Transverse section of the sensory cord at the level of the pectoral fin.   Squalus, 28 mm.   × 247.

Fig. 63   Transverse section, the same specimen, posterior to the second dorsal fin.   × 492.

Fig. 64   Surface view of a 36 mm. specimen.   Squalus.   × 24.

able distance posteriorly. The 40 mm. specimen was too badly shrunken to afford material for histological study.

Forty-five millimeter stage. In a 45 mm. specimen the trunk canal was closed for a short distance posterior to the pectoral fin. Figure 66 shows a transverse section of the canal anterior to the pectoral fin. Here the canal has just closed and has not yet separated from the epidermis. Figure 67 shows a transverse section a little posterior to the pectoral fin, where the canal lacks only a little of being closed. Considerably farther back the canal is represented by a furrow (fig. 68) which becomes shallower posteriorly. The formation of the canal resembles in its general features the closure of the neural groove to form the neural tube.

Fifty-two millimeter stage. In this specimen the canals of the head are all closed, although the surface openings of the infraorbital are still rather large and somewhat elongated.

The trunk canal as shown in figure 69 is closed nearly to the second dorsal fin. Figure 70 is a cross section of the canal a little farther forward where it has separated from the epidermis. Here the lumen is very small and the condition might easily be mistaken for a solid cord of cells. Posterior to the closed portion it is represented by an open furrow.

The separated organs of the accessory and dorsal series have reached their permanent number (dorsal series, 11 or 12; accessory, about 41) and the former have begun to sink slightly below the surface of the ectoderm. These structures, however, do not become enclosed within canals.

Sixty-seven millimeter stage. In this specimen (figs. 71, 72) the canal is tubular for a somewhat greater distance posteriorly. In the region of the first dorsal fin the lumen of the canal has become enlarged and its epithelial walls are relatively thin-

Fig. 65 Ventral view of the same specimen as represented by figure 64, showing distribution of sensory canals on the inferior surface of the head.

Figs. 66, 67, 68 Transverse sections of the lateral canal and cord at different levels of the body. Figure 66, anterior to the pectoral fin; figure 67, posterior to pectoral fin; and 68, posterior to second dorsal fin. All three figures from a 45 mm. specimen. Squalus. × 492.

Epa.

65

66

Lat.Cd.

Epa.

Den.

67

Lat.Cd.

Lat.Cd.

Den.

Epa.

68

61
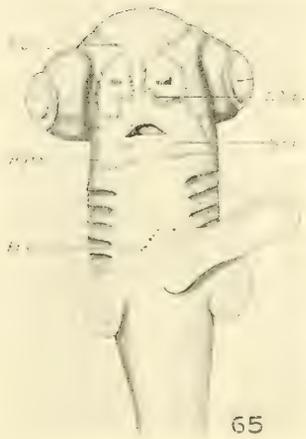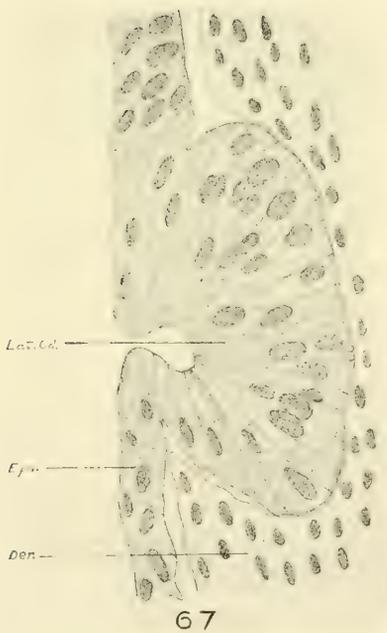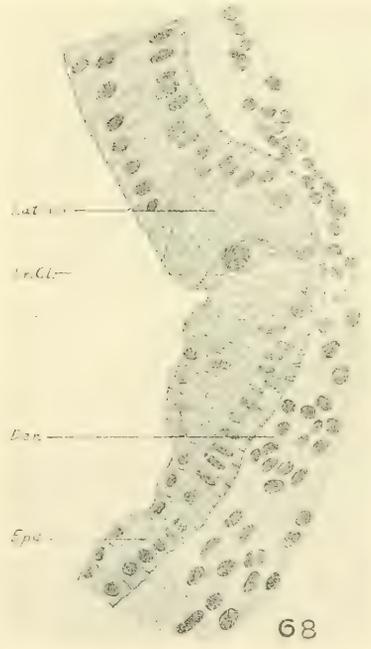
ner (fig. 73). Figures 74 and 75 show transverse sections of
the canal somewhat farther back. In figure 74 the trunk canal
is almost closed and in figure 75 it is still a wide open groove.
Figure 75 shows also an accessory organ which is now consider-
ably removed from the canal and is covered by a superficial
layer of ectoderm.

Seventy-two millimeter stage. In this embryo (fig. 76) the
canals appeared to be completely closed and could be traced
only by the openings of the tubules. Anterior to the first dorsal
fin the lateral canal lies considerably beneath the epidermis,
its lumen is greatly enlarged, and its epithelial walls relatively
thinner than in previous stages (fig. 77). Posteriorly the canal
has not yet separated from the epidermis (fig. 78), the lumen is
smaller, and here, as in other sections (e.g., fig. 79) it is seen that
the columnar layer of the canal wall is continuous with the
columnar layer of the epidermis.

In surface view the various lines of true canal organs are rep-
resented by continuous lines, the pores of the tubules being too
small for representation on the scale of the drawing. The ac-
cessory line organs, those of the dorsal line series and those in
front of the supratemporal commissure, are represented some-
what larger than their ratio to the outline of the embryo.

The 72 mm. specimen was the most advanced of the embryos
at my disposal and therefore it completes my study of the
method of formation of the sensory canals. The developmental
processes are complete in all essential features, however, as may
be seen by a comparison with the conditions found in the adult.

2. Structure of the sensory canals of the adult

Distribution. Particular attention has not been given in my
observations to the distribution of the various sensory canals in
Squalus, although on rather superficial examination enough
differences have been noted to enable one to distinguish the
two genera by this character alone (Garman '88). In Squalus
the lateral canal lacks the short upward bend at the level of the
second dorsal fin which is characteristic in Mustelus. The
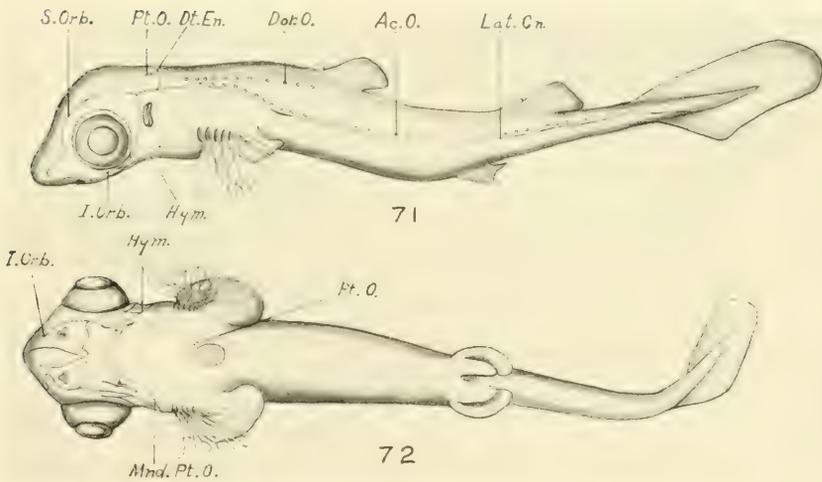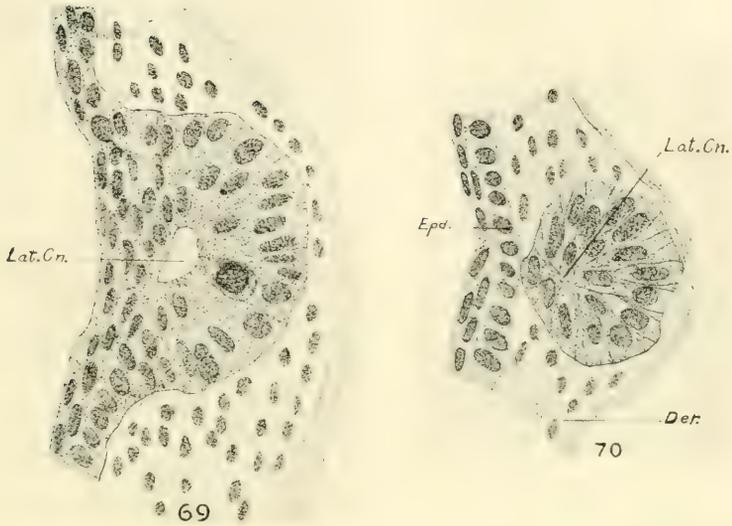
Figs. 69, 70 Transverse sections of the lateral canal at different levels show-
ing a great difference in the size of the lumen. Squalus, 52 mm. × 560.

Figs. 71, 72 Lateral and ventral views of a 67 mm. specimen. Squalus.
× 1½.

Figs. 73, 74, 75 Transverse sections at different levels, showing the lateral canal closed, almost closed, and wide open. An accessory organ is also shown in figure 75. Squalus, 67 mm. Figure 73, × 360; figures 74 and 75, × 296.

infraorbital canals do not appear to anastomose with the supraorbitals as they do in Mustelus, and there are other differences in the supraorbitals and hyomandibulars which I have not examined with sufficient care to describe in detail.
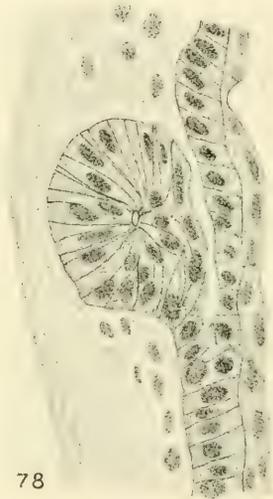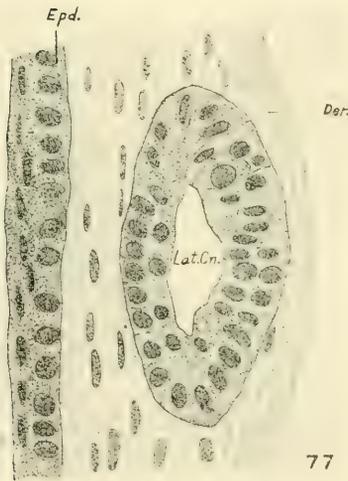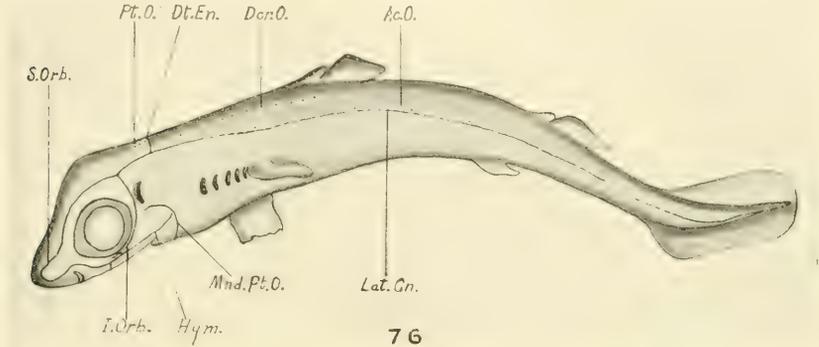
Fig. 76   Surface view of 72 mm. embryo.   Squalus. $\times 1\frac{3}{5}$.

Fig. 77   Transverse section of lateral canal, anterior region of body.   Squalus, 72 mm. $\times$ 360.

Fig. 78   A section similar to figure 77, but farther caudad.   Note the small lumen, and also that the canal wall is still attached to the ectoderm.   Squalus. $\times$ 360.

Structure. Differences of considerable extent are found in the form of the canals and in the histological structure of the sensory epithelium.

The lateral canals lie in the deeper layers of the dermis and their tubules pass ventralwards for a considerable distance (0.1 to 0.5 mm. or more, depending upon their position) before opening to the surface (fig. 80). The walls of the tubules consist of an outer columnar and an inner flattened layer of cells. These layers are continuous centrally with similar layers of the canal walls and externally with the columnar and stratified layers of the epidermis.

In transverse section the sensory canals are usually round or nearly so (fig. 82), and a small bundle of nerve fibers runs along the base of the sensory column (fig. 81). The sensory column lies in the dorso-medial wall of the canal (figs. 79, 80).

The structure of the sensory column is indicated in figures 81, 82, 83. The hair-cells are arranged in small groups (about 3 to 5) around which the supporting elements tend to group themselves in small concentric clusters. Usually only one hair-cell can be seen in transverse sections. The cells are relatively large and each appears to bear a single hair-like process (figs. 82, 83). The supporting elements consist of columnar cells, basal cells, and spindle-shaped cells. These are similar to the corresponding elements found in the sensory column of Mustelus.

Efforts to secure silver impregnation of the peripheral terminations of the lateral nerve of Squalus acanthias were not satisfactory. Fresh Squalus material has not been available and the fixation of the preserved specimens was not suitable for silver impregnation methods. By other methods (iron haematoxylin and Weigert-Pal), however, the lateral nerve has been traced from its central ganglion to the basilar membrane of the sensory column and it appears safe to assume that the terminations are essentially similar to those of the lateral nerve in Mustelus.

The question of the mode of growth of the lateral nerve should be considered. Regarding the growth of the lateral nerve,
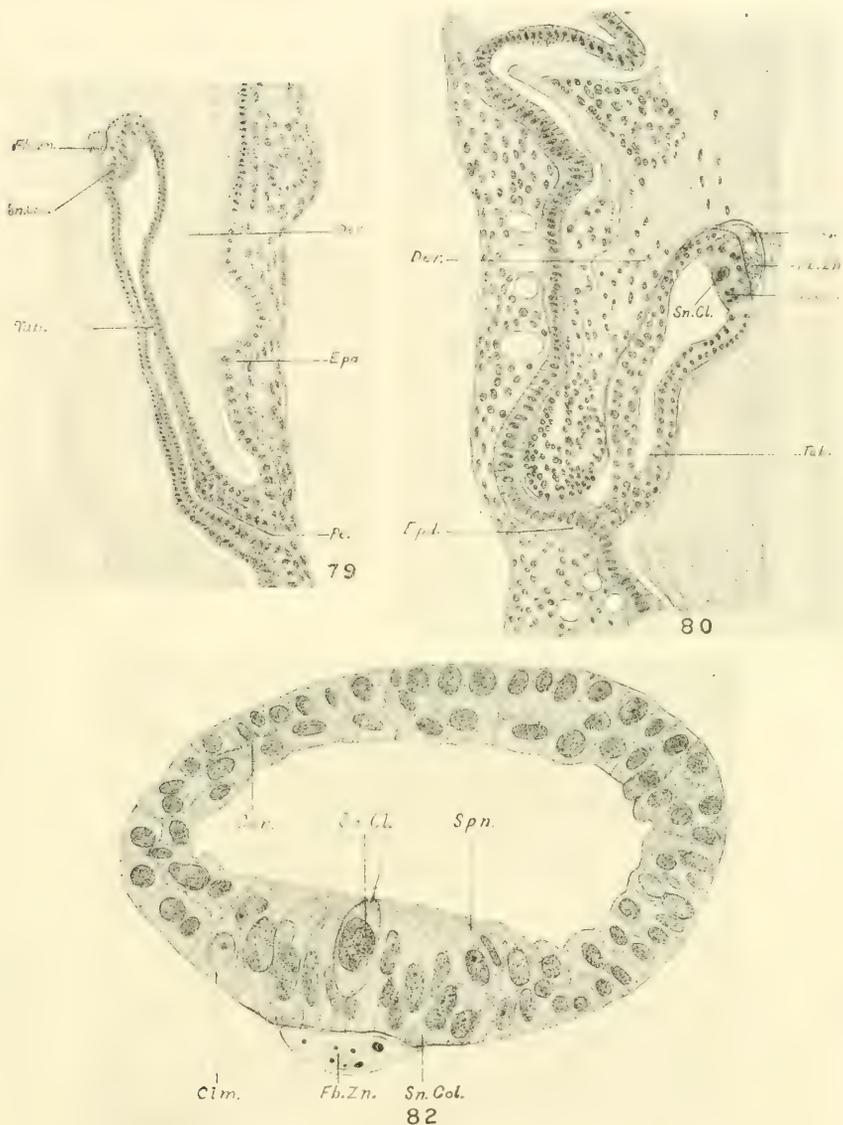
Fig. 79 Transverse section of the lateral canal of a Squalus acanthias (garter stage) showing relations of fiber zone, sensory column, surface tubule, dermis and epidermis. × 120.

Fig. 80 Transverse section of the lateral canal just anterior to the caudal fin. Squalus pup. × 136.

Fig. 82 Transverse section of the lateral canal of a Squalus pup. × 592.
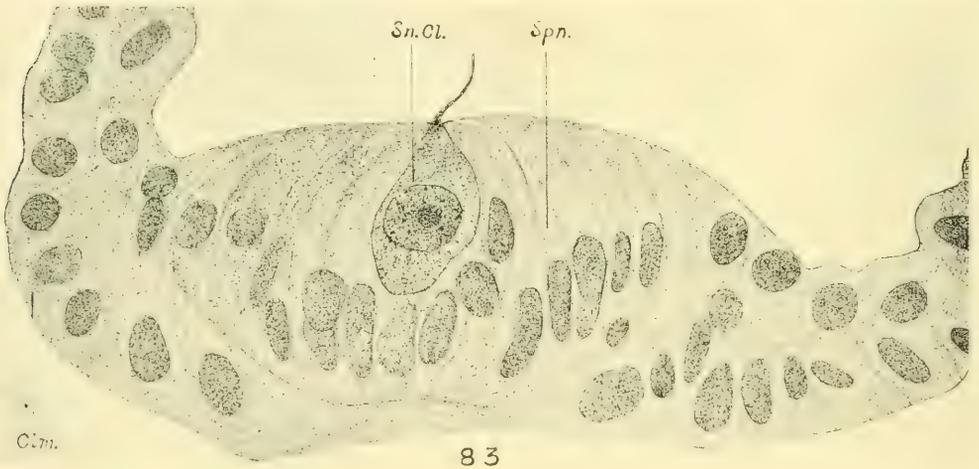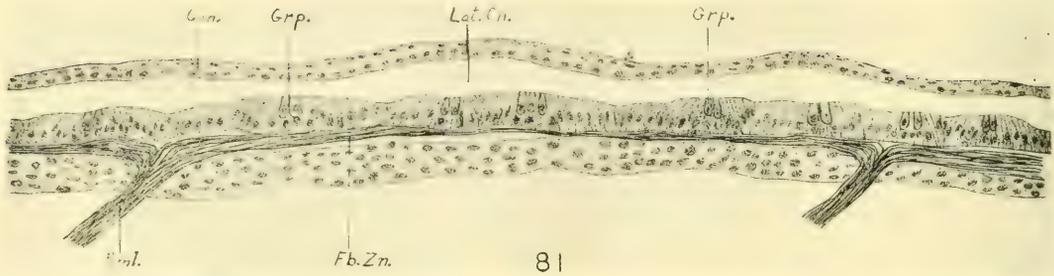
Fig. 81 Longitudinal section of the lateral canal of a Squalus pup, showing two lateral ramuli, fiber zone, and sensory epithelium.   × 136.

Fig. 83 Transverse section of lateral sensory column of the same specimen (fig. 82).   × 1160.

observations on both Mustelus and Squalus show that the nerve fibers grow from the ganglion cells.   There is no evidence of addition to the nerve from cells of the integument.   The latter are developed into secondary sense cells (Harrison '03).

V. SUMMARY, CONCLUSIONS AND COMPARISON OF THE FORMS STUDIED

The sensory canal system embraces two sets of organs designated canal organs and surface or pit organs respectively.   As the terms imply, the former are enclosed within epithelial canals and the latter lie at the surface of the skin or in shallow pits.

The principal canals are the supraorbital, the infraorbital, and the lateral. Two shorter ones are the mandibular and hyomandibular canals. The lateral canals are connected by a short canal, the supratemporal commissure. Only slight differences exist in the distribution of the canals in Squalus and Mustelus.

Four lines of pit-organs have been found in Mustelus and five in Squalus. The dorsal series is the most extensive, in Mustelus, extending from the supratemporal region to the caudal extremity of the body. In Squalus this series extends only to the region of the first dorsal fin. In both Squalus and Mustelus two small pit organs are located immediately in front of the pores of the endolymphatic ducts. Their method of development gives evidence of their relationship to the canal system. Two short lines of organs have been found just in front of the scar left by the yolk-stalk in Mustelus pups and in Squalus embryos up to 72 mm. They have not been found in the adult of either species. Another series of organs common to both forms extends from the lower margin of the spiracle ventralwards around the angle of the mouth and almost to the midventral line. The fifth series of sense organs included in this group is the 'accessory line.' These organs are not strictly homologous to the other organs of the group as they do not have an independent development, but appear to arise from the superior margin of the lateral sensory cord. Accessory organs have not been found in Mustelus but it is my opinion that their presence could be demonstrated, especially in early embryos.

In structure the sensory canals are epithelial tubes, one wall (usually the superior) of which is specialized to form the sensory epithelium. The canals lie superficially within the dermis or somewhat deeper (on the head) and numerous minute tubules lead from the canals to the surface, passing in a ventro-lateral direction, thus permitting considerable elongation of the tubules, especially in the anterior region of the body. In adult Squalus and Mustelus the tubules of the lateral canal outnumber the vertebrae in the ratio of approximately four to three.

In both Squalus and Mustelus the sensory epithelium constitutes a continuous column of cells along the superior or supero-medial wall of the sensory canals. In the sensory ridge, however, the hair-cells (sense end-organs) are arranged in little groups or clusters which are separated and surrounded by the supporting cells.

In Squalus the sensory epithelium is less extensive than it is in Mustelus. There are approximately three to five hair-cells in each group and about eight groups between adjacent tubules and ramuli of the lateral nerve. In Mustelus there are several times as many hair-cells in each group and approximately twice as many groups.

In Squalus the sensory canals are somewhat smaller in diameter, they are more nearly round in transverse section, and the sensory epithelium does not occupy as wide a part of the canal wall as it does in Mustelus. The peculiar cells found at each side of the sensory ridge in Mustelus have not been observed in Squalus, nor do the blood capillaries occupy as prominent a position in the latter.

The nerve supply of the sensory canals is practically the same in the two forms considered. The innervation of the lateral canal, which has received more careful attention than that of the other canals, is by a large cranial nerve, the N. lateralis. This is unique among the cranial nerves, extending from the medulla to the caudal extremity of the body.

The cells of origin of the lateral nerve are located in a greatly elongated central ganglion which is partly enclosed in a common sheath with the vagus roots and ganglia, but is, however, independent. From this ganglion the nerve passes posteriorly between the muscles of the lateral body-wall at a considerable depth from the surface. At intervals corresponding to the surface tubules bundles of fibers (ramuli) pass to the base of the sensory column and then branch forward and backward, forming a continuous longitudinal fiber zone from which distribution to the sensory epithelium takes place.

In Mustelus it has been shown that the peripheral terminations of the lateral nerve are in the nature of free ramifications

between the hair-cells. Owing to the lack of fresh material the peripheral terminations of the lateral nerve in Squalus have not been demonstrated, although the nerve fibers have been readily followed as far as the basilar membrane of the sensory column, and are similar in arrangement to those of Mustelus.

The sensory canals begin their development as linear extensions from certain thickened patches of ectoderm. Two of these thickenings, the suprabranchial and the preauditory are well defined. The points of origin of the mandibular surface organs, and the surface organs which appear in front of the yolk-stalk are not so evident.

By linear extensions the suprabranchial thickening gives rise to the lateral sensory cord, the dorsal series at pit organs, the supratemporal commissure, and two isolated pit organs in front of the pores of the endolymphatic ducts.

Similarly the preauditory thickening gives rise to the supraorbital, infraorbital and hyomandibular sensory cords.

As the sensory cords elongate in their respective directions the ectoderm which lies in their path is pushed up to form little pockets or even long tunnels over the growing ends of the cords. These tunnels are not the canals.

Early in their development cellular differentiation begins in the sensory cords and by the time the thickenings have practically reached their full linear extension they begin to sink into a groove at their proximal ends. As the infolding continues the sensory cords are gradually enclosed within epithelial canals which, however, retain communication with the exterior by means of numerous delicate tubules.

By the time the suprabranchial thickening has become fairly prominent and considerably before the lateral sensory cord has definitely appeared, the suprabranchial ectoderm is separated from the wall of the medulla by a mass of cells, the neuroblasts of which give rise to the lateralis and vagus ganglia. No evidence has been seen to indicate that any of the cells in the ganglionic mass are derived from the external ectoderm. This point, however, has not been the subject of special examination. While the external ectoderm is still in contact with the ganglionic

mass fibers grow out from the cells of the latter and become associated with the overlying ectodermal cells. Very early in development a longitudinal mass of the ganglionic cells becomes partly separated from the rest. This is the embryonic lateralis ganglion. Its central fibers form a definite root which enters the medulla anterior to, and slightly above, the roots of the remainder of the ganglionic (vagus) mass. Peripheral fibers of the lateralis ganglion have already become associated with the ectodermal cells which proliferate and extend backward as the lateral sensory cord. The fibers which accompany the sensory cord constitute the lateralis nerve. In early embryos the nerve lies immediately subjacent to the sensory cord, but as growth proceeds it recedes from the surface and comes to occupy a position at a considerable depth medial to the ectoderm. As the nerve becomes farther removed from the inner surface of the ectoderm the fibers, which have become associated with the sensory cord, do not pass to the latter in a continuous sheet, but arrange themselves into small bundles, the lateral ramuli.

Mode of formation of the canals. By the time the nerve connections are established as outlined above the canals are closed and the sensory canal system has practically reached the adult condition.

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THE DEVELOPMENT OF THE PARAPHYSIS AND PINEAL REGION IN MAMMALIA

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THIRTY-FIVE FIGURES

CONTENTS

INTRODUCTION

The object of this paper is to describe the appearance of the primary arches in the roof of the forebrain which have not as yet been reported in mammalia and also the presence of a mammalian paraphysis, as there has been much doubt as to its existence in any members of this class of vertebrates. The other features of this region, the velum, choroid plexus, epiphysis and commissures will only be discussed in a general way, espe-

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cially in the older stages, as a considerable amount of research has already been devoted to their study and description.

Part I. In Part I the development of the pineal region in sheep embryos will be considered, as these embryos in practically every case studied from 21 mm. up to 48 mm. possess a distinct paraphysis and differ in this respect from all other mammalian embryos that were available for study in the Harvard Collection.

Part II. In Part II the development of certain special features only of the pineal region in human embryos will be discussed. These are the formation of the primary arches, which can be readily demonstrated in early human embryos; the formation of the paraphysis, which can be seen in a few specimens only as a very inconstant and a relatively rudimentary structure as compared with that of vertebrates below the mammalia; finally attention will be called particularly to a peculiar set of tubular outgrowths springing from the velar end of the postvelar arch and overhanging the paraphysal arch and the paraphysis when present. These will be referred to as the postvelar tubules or diverticuli.

Part III. Part III will contain a brief review of the development of the forebrain roof in embryos of the opossum, rat, rabbit, cow, pig, deer, cat and dog. Wax models on a scale of 40 diameters have been made to demonstrate the whole of the forebrain in sheep embryos from 9 mm. up to 48 mm. and additional models on a scale of 100 diameters to show the details of the paraphysis only in embryos of 28 mm. and 48 mm. Two drawings on the same scale are added to point out the histological structure of the organ in those two specimens. To illustrate the development in human embryos models were reconstructed of the whole forebrain at a magnification of 40 diameters of embryos of 15 mm., 16 mm., and 23 mm. Other models have also been reconstructed on a scale of 80 diameters of the paraphysal arch, velum and oral or velar end of the postvelar arch only and are intended to demonstrate details of the paraphysis and of the velar end of the diencephalic roof. The figures of the wax models are in all cases reduced to one-half the linear dimensions of the wax models themselves.

PART I. SHEEP EMBRYOS

1. *The primary arches and the subdivisions of the forebrain*

The primary arches were first described by Minot (16) in Acanthias and have since been demonstrated in practically all vertebrates below Mammalia. In a previous article, Warren (24), the primary arches and the three main subdivisions or segments of the forebrain in vertebrates were considered and the previous work on these subjects was reviewed at length. The primary subdivisions of the forebrain consist of a telencephalic segment whose roof is formed by the paraphysal arch. This is separated by the velum from the first diencephalic segment (Kupffer's parencephalon) with the postvelar arch and later the epiphysal arch in its roof. This in turn is followed by the second diencephalic segment bounded above by the pars intercalaris (Kupffer's synencephalon) which contains a portion of the posterior commissure and is limited caudally by the groove and ridge between the fore- and midbrain. These subdivisions were demonstrated in Reptilia and also in the pig and sheep, Warren (24), figures 28 to 37. For further details on this subject and on the question of neuromeres readers are referred to the review and discussion of the previous investigations on the subdivisions of the forebrain in the above article.

Figure 1 is from a model of a sheep embryo of 9.9 mm. sagittal series which shows the subdivisions of the forebrain as outlined in the previous paragraph into the telencephalic segment anterior to the velum, *V*, with the paraphysal arch, *P.A.*, forming its roof, the first diencephalic segment the roof of which is made up of the postvelar arch only, *P.V.A.* (the epiphysal arch is not as yet formed) and separated by an internal ridge from the second diencephalic segment. The roof of the latter, the pars intercalaris, *P.I.*, is practically filled by the posterior commissure, *P.C.* The midbrain is composed of two segments separated from the forebrain and from each other by distinct internal ridges. Compare this model with those for Reptilia, Warren (24), figures 3, 4, 17. All of the primary arches and the velum are shown in figure 2 taken from an embryo of 14 mm.

sagittal series and they correspond to the conditions already described in other vertebrates, Minot (16), Terry (22), Neumayer (17), Warren (24). The extension of the posterior commissure into the forebrain is very striking and it is evident that this belongs partly to the forebrain and partly to the midbrain. The primary arches, including the pars intercalaris, and the three primary subdivisions of the forebrain can therefore be said to be definitely established in sheep embryos.

2. Paraphysal arch and paraphysis

The paraphysal arch is clearly seen in the last two figures and passes over into a relatively low velum. The earliest signs of a paraphysis could be made out in an embryo of 21 mm. (fig. 3). It is a tiny conical structure, P., springing from the telencephalic roof plate immediately in front of the velum. Figure 4, an embryo of 26 mm., shows a good sized paraphysis, P., with a relatively wide opening lying between the foramina of Monro and the anlagen of the lateral telencephalic plexuses and inclined backwards towards the velum.

The next stage appears in figure 5 and is from an embryo of 29 mm. In the last stages the wall of the paraphysis consisted of cells similar to those in the roof plate of the forebrain. Here the wall of the organ has become much thicker, the distal end more solid and the cavity much reduced. The outline is quite irregular and this feature is shown clearly in figure 6, which represents a model of the paraphysis of the same embryo at 100 diameters   The view in the picture is from the front and outside of the brain looking in between the walls of the hemispheres in the direction of the guide line from the letter P in figure 5. The base of the paraphysis where it is attached to the telencephalic roof plate, is noticeably wide and thick and is prolonged into a narrow tapering extremity, which contains a partial cavity and is apparently the main portion of the original outgrowth.   On each side are two lateral prolongations from the base showing a striking uniformity which are practically solid.   Directly behind the paraphysis in the mid-line

the postvelar arch inclines forward and upward above the organ. A swelling on its right extends into the cavity of the diencephalon. This represents the right half of the diencephalic choroid plexus, *D.C.P.* The velum, *V*, is indicated by a slight fold which is much better marked towards the mid-line and is not shown well in this view. This stage was selected on account of the peculiar appearance of the paraphysis, which was the most irregular specimen seen in any of the sheep embryos, though there were several other cases, notably in embryos of 21 mm. frontal series, H. E. C. no. 1686; 25 mm. frontal series, H. E. C. no. 1690; and 40 mm. H. E. C. no. 1691, where the structure was almost as irregular but varied in size. Its markedly solid formation differentiates it sharply from the tubular and glandular types so characteristic of Reptilia and Amphibia. In the following two embryos no paraphysis could be found, no. 1111, 24.4 mm. transverse series, no. 1240, 26.6 mm. transverse series, so that the paraphysis is not absolutely constant in the sheep embryos. Figure 7 gives the details of a section along the line *A-B* (fig. 6), taken just above the opening into the brain. The thickness of the main part of the organ is well shown and the cavity has gradually faded out into two narrow prolongations, the most peripheral being its main continuation. One of the solid projections on the right is cut separately and both of these on the left. The general detail of structure can be seen in the drawing and compared with that of the brain wall and the double commencement of the diencephalic plexus also can be made out, *D.C.P.*

In an embryo of 34 mm., H. E. C., no. 1692, there is only a rudimentary paraphysis not as large as that shown in figure 4, while in an embryo of 40 mm., H. E. C. no. 1691, the organ is relatively small as compared with that shown in figure 5 and has a central projection with two smaller ones in front and behind which are nearly solid and contain only a slight trace of a cavity. The last three figures are taken from the oldest specimen in the collection, an embryo of 48.4 mm., H. E. C. no. 1696. In figure 8, representing a model of the forebrain, there is a picture of the paraphysis in median section, and in figure 9 a view of the

paraphysis is given practically similar to that seen in figure 6.
One is looking from the front and a bit to the right of the mid-
line at the outer surface of the lamina terminalis, *L.T.*, and of the
paraphysis, *P.* Behind the paraphysis the diencephalic choroid
plexus, *D.C.P.*, bulges backward into the diencephalon. The
space between the paraphysis and the wall of the plexus is of
course filled with vessels and connective tissue which could not
be modeled. The base of the paraphysis is nearly as broad as
that shown in figure 6 and is likewise prolonged into a narrow
tube which is hollow up to the tip, but no lateral projections
are seen. A distinct fold, *V*, marks the position of the velum.
Figure 10 is a section through the paraphysis and the dien-
cephalic plexus at the line *A-B* (fig. 9). The section is through
the broadest part of the organ, the wall of which is thick and
well defined and stands out in contrast to the cells covering the
folds of the plexus. The cells in the paraphysis form a double
row while those covering the folds of the plexus are for the most
part arranged in a single row. This specimen is hollow and dif-
fers in this respect from the more solid types seen in the em-
bryos of 29 mm. and 40 mm. and a good view of the character
of the tufts of the diencephalic plexus is given in the picture.
The paraphysis is thus shown to be practically a constant struc-
ture in embryos of 21 mm. to 48.4 mm. and differs markedly
from the elongated tubular organ of Reptilia and the compli-
cated glandular structures of Amphibia, its chief characteristics
being its short, broad and irregular form and its solid character.

3. Velum transversum

The velum appears in some of the earliest embryos and can
always be clearly followed up to the oldest stages studied for
this article. At first it forms a comparatively slight fold in
the roof of the forebrain which, with the elongation of the post-
velar arch, becomes more accentuated especially towards the
median line, but as the choroid plexuses develop it tends to be
more obscured. The observations made here agree with those
previously made in other Mammalia, Johnston (12), and with
Neumayer's pictures of early sheep embryos (17). See also
Ziehen (25).

4. Postvelar arch

At first the postvelar arch forms a short curve in the roof of the first diencephalic segment and later with the appearance of the epiphysal arch becomes more elongated. The diencephalic choroid plexus begins to form at the velar end of the arch and appears at first in embryos of about 25 to 26 mm. (fig. 4, *D.C.P.*). The plexus develops at first on either side of the median line forming two marked tufts of tissue bulging into the diencephalon and beginning just behind the diencephalic leaf of the velum. After a short distance the separate tufts become more or less condensed into a single median tuft which invaginates the diencephalic roof plate in the mid-line (*D.C.P.*, figs. 6 and 7). In an embryo of 32 mm. the plexus has involved a large part of the postvelar arch and in the oldest specimens has extended dorsally nearly to the epiphysis. The size and character of this formation are well shown in figure 10. The lateral choroid plexus is first seen in embryos of about 20 mm. arising on either side of the telencephalic roof plate and of the opening of the paraphysis. Bailey in two excellent papers, the one on the "Morphogenesis of the Choroid Plexuses," the other on the "Morphology of the Roof Plate of the Forebrain and Lateral Choroid Plexuses in the Human Embryo" has considered so carefully the origin of the plexuses, especially that of the lateral plexus, that it is unnecessary to add anything further here.

5. The epiphysis

The epiphysal arch appears first in an embryo of about 14 mm. (fig. 2) and is relatively short but well marked. It continues in approximately this condition up to about 24 or 26 mm., when its walls thicken and it becomes more elevated above the surface of the brain. It now may truly be called the epiphysis and both commissures are placed close against its anterior and posterior walls (fig. 4). In figure 5 it is more clearly defined and begins to incline slightly backward a position which is more strikingly shown in the oldest embryos (fig. 8). In the specimens studied there was no sign of the differentiation of a pineal

eye and an epiphysis, a condition which seems to be peculiar to Reptilia, and the structure is relatively much suppressed as compared with its development in birds, Reptilia and Amphibia. As it is intended to indicate only the early appearance and topographical position of the epiphysis in this paper, no attempt will be made to consider its histological structure. For histological details see Jordan (13) on the epiphysis of the sheep and Jordan (14) on the structure of the same organ in the opposum. For a review of the appearance of the epiphysis in other vertebrates see Warren (24).

6. The commissures

The posterior commissure appears relatively early in sheep embryos and at first begins partly in the forebrain, in the hinder end of the pars intercalaris. In this respect it resembles the early form of the posterior commissure in Reptilia but is even more precocious in its development. It apparently must be regarded as belonging partly to the forebrain and is not wholly confined to the midbrain as is usually stated. It is often difficult in the younger stages to determine accurately its limits, which tend to gradually fade out into the outer layer of the brain wall, but in all the younger stages it had approximately the extent as shown in figures 1 and 2. In embryos of about 20 mm. the commissure has completely filled up the pars intercalaris and its cephalic end lies directly against the dorsal wall of the epiphysis. This same condition is also found in young human embryos of from 10 to 20 mm. The extent of the pars intercalaris in the roof of the early mammalian brain, where it really should be considered as one of the primary arches or segments in the roof of the brain, is one of the most striking features in these embryos. It is constant in all vertebrates and always precedes the superior commissure except in ammocoetes.

The superior commissure can first be observed in sheep embryos of 24 to 26 mm. and lies directly against the anterior wall of the epiphysis. It occurs in practically all vertebrates including man and its development has been thoroughly described by Minot (16), Neumeyer (17), Cameron (3), Osborn (18), and Dexter (4).

No trace of the velar commissure described in Reptilia by ·Elliot Smith and others occurs in the Mammalia studied for this paper.

7. Conclusions

1. The primary arches consist of the paraphysal arch, the postvelar arch, the epiphysal arch and the pars intercalaris (synencephalic arch) and together with the velum are formed in the roof of the forebrain of early sheep embryos.

2. The paraphysis can be followed in practically all sheep embryos from 20 mm. up to 48 mm. It is characterized by its short, broad and irregular outline and its solid structure, the cavity being in most cases reduced to a minimum.

3. The diencephalic choroid plexus and lateral telencephalic plexuses are well marked and develop essentially as described in other vertebrates. There is no trace of the median telencephalic plexus so noticeable in Amphibia.

4. The epiphysis forms a short hollow stalk with thick walls and inclined slightly backward over the posterior commissure.

5. The superior and posterior commissures are formed as in other vertebrates. The posterior commissure is characterized by its precocious development and by the extent that it invades the pars intercalaris of the forebrain in early embryos.

PART II. HUMAN EMBRYOS

1. Primary arches in the roof of the forebrain

These structures have not as yet been described in human embryos and their present demonstration fulfils the prophecy of the late Professor Minot made in 1901 that they would eventually be found in all vertebrates, Minot (16). In an embryo of 10 mm. (fig. 11), the primary arches in the roof of the forebrain are as clearly marked as in any of the lower vertebrates. The postvelar arch is relatively short and thick, while the epiphysal arch is, on the other hand, rather longer than in lower forms. The pars intercalaris is quite extensive and contains in its posterior end a portion of the posterior commissure as was the case in the corresponding stage in sheep embryos. In

an embryo of 15 mm. (fig. 12), a slight hint of a paraphysis appears in the paraphysal arch. The postvelar arch has become greatly elongated and thinner, while the epiphysal arch has become much condensed with thicker walls. There is a long pars intercalaris and the posterior commissure has continued its encroachment therein. In figure 13, a picture of the median section of the forebrain in an embryo of 23 mm., the arches have passed out of their primitive condition and have assumed their more advanced characteristics. The long extent of the postvelar arch is noticeable and the peculiar modification of its velar end has commenced. The velum is small but distinct and a small paraphysal outgrowth is seen in the much reduced paraphysal arch. The epiphysis has been definitely formed and the posterior commissure has developed forward through the whole extent of the pars intercalaris up to the dorsal wall of the epiphysis. No sign of a superior commissure could be seen in the embryo, though Bailey (2) shows it in an embryo of 19 mm. The pars intercalaris has now become much reduced in length due to the increased development of the midbrain and corresponds in this way to a similar process in lower vertebrates.

2. Paraphysal arch and paraphysis

Very few observations had been made on the pineal region in human embryos until the appearance of Bailey's (2) paper in 1916. Selenka (19) gave the first description of the paraphysis in opossum embryos, but showed no figures of the structure. D'Erchia (5) has described the paraphysis as a simple fold in an embryo of about 30 mm., and Francotte (8) shows a picture of a section through a tubular paraphysis in a three weeks embryo. In a previous article, Warren (24) figure 39, there appeared to be a distinct paraphysal outgrowth in a section of an embryo of 28.8 mm., H. E. C. no. 1598, but as will appear later this observation was incorrect. Bailey (2) described the roof of the forebrain in three embryos of 19 mm., 28 mm., and 32 mm. respectively. His observations were based especially on the development of the choroid plexuses. As regards a

paraphysis, he states that no glandular structure was present
in his specimens but that the paraphysal arch was in all cases
well defined. The velum was also clearly traceable in his em-
bryos and my own observations as to the velum and the para-
physal arch coincide with his results. I have been able, how-
ever, to demonstrate the presence of a paraphysis. It exists
in a rudimentary form as compared with that of lower verte-
brates and is very inconstant. Out of the embryos in the Har-
vard collection used in the preparation of this paper I have been
able to find it in only eight cases. These specimens were em-
bryos in good preservation and any that were at all damaged in
the region of the forebrain were excluded, though two or three
of these showed signs of a possible paraphysal outgrowth. It
is owing to the relatively large number of human embryos in
the Harvard Collection that the writer was fortunate enough to
find the few specimens containing a paraphysis, which will here
be described.

In an embryo of 15 mm. (fig. 12), a slight median elevation is
seen in the paraphysal arch in the true morphological position
of a paraphysis. It is composed of a solid clump of cells with
no cavity with the exception of a very tiny dimple on the under
side of the arch just beneath it A similar structure could be
found in other embryos of about the same size, H. E. C. no. 2044,
16 mm. and H. E. C. no. 1707, 16.4 mm. From the above
stages up to embryos of 23 mm. no signs of a paraphysis could
be found with the exception of three doubtful cases in embryos
of 19 to 20 mm. which were, however, excluded as the roof of
the forebrain was somewhat damaged. In an embryo of 23
mm. (fig. 13), however, a tiny paraphysis with a slight cavity
is clearly seen. This model was made from a sagittal series and
the paraphysis only extended through four sections and was at
first overlooked. Three other embryos of approximately this
same size showed no signs of any paraphysal outgrowth and
had a simple paraphysal arch as shown by Bailey (2), figure 18.

Figures 14, 15 and 16 are taken from two models of the para-
physal and velar region of the roof of the forebrain of an embryo
of 25 mm. This embryo was in an excellent state of preserva-

tion and has the best specimen of a paraphysis of any in the
Collection.

Figure 14 shows a median section of this part of the roof of
the forebrain. The velum, V, forms a deep fold and immediately
in front of it can be seen a well marked paraphysis, P, with a
wide opening and a large cavity. On the opposite side of the
velum the diencephalic roof bulges forward and overhangs the
paraphysis. In figure 15 there is a view of the external surface
of the roof of the forebrain in this same region seen from in
front and a little to the right as in figures 6 and 9. The para-
physis, P, overlies the telencephalic roof plate. Immediately
behind the velum, V, arise several outgrowths from the dien-
cephalon, the so-called postvelar tubules to be considered later.
It is easy to see that in a series of sections at right angles to the
telencephalic roof plate these prolongations might be mistaken
for a paraphysis if that organ was actually absent, and it is not
easy in such a series to determine at once on what side of the
velum these outgrowths really belong until an actual model has
been made.

Figure 16 represents the same model seen from the interior of
the brain to show the relative positions of the opening of the
paraphysis and the openings of the postvelar tubules with ref-
erence to the velum and the telencephalic and diencephalic
roof plates. The extent of the remainder of the diencephalic
roof and the position of the epiphysis and commissures are well
shown in Bailey's models of 28 and 32 mm. embryos ((2) figs.
20 and 22). The next stage in which a paraphysis could be
shown was an embryo of 36 mm., again a specimen in excellent
condition. Between this specimen and the previous one the
collection contained several embryos of 28.8, 29, 30 and 31 mm.
In an embryo of 31 mm., sagittal series, there was a very slight
elevation in the paraphysal arch close to the velum which might
be regarded as a mere rudiment of a paraphysis (fig. 17). In
the others there was no sign of a paraphysis. The telencephalic
roof plate formed a well defined paraphysal arch similar to the
condition shown by Bailey (2), figures 20 and 22. For misin-
terpretation of the paraphysis and velum see Warren (24),

figure 29, where the velum is incorrectly placed and what is labelled paraphysis should really be a postvelar outgrowth from the diencephalon.

Figure 18 is from a model of a median section of the forebrain of the 36 mm. embryo similar to figure 14. The velum corresponds to that in the 25 mm. embryo and in front of it a relatively small paraphysis, *P*, projects forward from the telencephalic roof plate. It is connected to the telencephalon by a solid stalk with a slight depression indicating the original opening and a small cavity persists in the distal end only. The paraphysis and telencephalic roof plate are completely buried under the mass of tubules which spring from the postvelar arch and extend forward. In three embryos of 37, 40, and 42 mm. respectively, all in good preservation, there was no sign of any sort of a paraphysis. An embryo of 44.3 mm., the oldest embryo in the collection, is represented in figure 21, which gives an external view of the roof of the telencephalon and diencephalon similar to figures 15 and 19. Here a tiny paraphysis, *P*, can be seen in the midline of the telencephalic roof buried under the mass of diencephalic tubules. It extended through a very few sections, only one of which had a cavity, and is attached by a thin solid stalk to the brain. The paraphysis in this specimen is about to degenerate and entirely disappear.

Of all the embryos studied, excluding doubtful cases and slightly damaged specimens, a more or less rudimentary paraphysis could be found in only eight instances. It therefore may be said to exist in human embryos as a very inconstant and variable structure.

3. Postvelar arch and postvelar tubules or diverticuli

The earliest sign of this peculiar modification of the velar end of the postvelar arch appears in embryos of from 19 to 23 mm. In figure 13, an embryo of 23 mm., it forms a simple transverse fold somewhat tent-shaped in the mid-line. This was earlier mistaken by the author for a paraphysis, as the real paraphysis was here so small that it was at first overlooked.

In three or four other embryos of approximately this same size
a similar formation could be seen which in some cases was carried
still further forward into a finger-like projection in the mid-line,
which gave in the section the impression of a small rounded tube
whose walls were rather thicker than the diencephalic roof.
The embryo of 25 mm., a median section of whose brain is seen
in figure 14, shows this projection more distinctly. Figure 15
gives an external view of the same specimen, where the details
of the tubules can be clearly seen. There are two main pouch-
like projections on either side of the mid-line, which show a
tendency to give off smaller tubules and to cover up the para-
physis. They are limited below by the fold in the brain wall
formed by the velum and have quite definite limits above, the
extreme lower end of the postvelar arch being alone concerned
in this formation. Their relations to the brain cavity are best
seen in figure 16, which gives a view from the interior of the
brain. In the lower part of the figure is the lamina terminalis,
L.T., which passes over into the forebrain roof, *T.R.P.* The
opening of the paraphysis, *P*, lies just cephalad to the velum,
V, and there can be no doubt of its telencephalic origin. On
the diencephalic side of the velum appear the two large pouches
in the depths of which are the openings of the smaller tubules.
This outgrowth as a whole begins at the diencephalic lip of the
velum, involves practically the whole width of the postvelar
arch at this point and ends fairly definitely above. Beyond this
point the diencephalic roof arches upwards with a perfectly
smooth and gently curved surface. This structure is clearly
of diencephalic origin and should never be mistaken for the
paraphysis.

Embryos of 28.8, 29, 30, and 31 mm. were next examined and
in all of them some similar formation was to be seen, which
varied from a relatively simple fold like that in figure 13 to a
more complicated replica of what has just been described. The
embryo of 31 mm., H. E. C. no. 1706, was selected in order to
show the separation of some of the tubules from the brain and
their formation into blind cysts or vesicles. Figure 17 shows a
model of this embryo seen from the front, as is the case with

figures 15, 18 and 21. The paraphysal arch or telencephalic roof plate is here rather sharply elevated and then bends abruptly downward into the lamina terminalis, *L.T.*, between the median walls of the hemispheres. In the depths of the model a very rudimentary paraphysis, *P*, can just be seen. The diencephalic roof is prolonged forward into a large projection a little to the left of the mid-line, below which several smaller tubules can be seen lying nearly side by side on either side of the mid-line. Tubule number 1 represents an absolutely blind vesicle, which is fused to a hollow stalk, number 2, that communicates by means of a narrow opening with the brain cavity. Number 3 also opens by a wide opening into the lower part of the large projection. Number 4 is a closed sac and is fused to the wall of number 5. It is almost exactly similar to number 1, while number 5, like number 2, communicates with the brain by a narrow opening. Histologically the walls of vesicles 1 and 4 are thinner than those of the others and show signs of degeneration. It is evident that they represent the distal ends of the original tubular outgrowths which are about to become detached entirely from the brain and probably will eventually disappear.

Figure 18 is from an embryo of 36 mm. which was in an excellent state of preservation and, together with the 25 mm. embryo shown in figures 14, 15 and 16, represent two of the best specimens in the Harvard Collection. It shows a median section of this part of the brain. The velum forms here a well developed fold and beginning immediately above it on the diencephalic side a large tubule partly subdivided is thrust forward, which extends over nearly the whole of the telencephalic roof plate. Above this tubule is seen the section of a larger diverticulum. In figure 19 an external view of these tubules is seen similar to that in figure 15. Two large diverticuli are thrown out above from either side of the mid-line enclosing a smaller one between them, a section through which appeared in the previous figure. Below appears a number of smaller and more irregular tubules lying on the telencephalic roof and completely burying the paraphysis. At least two of these have become blind vesicles, though still fused to the wall of a

neighboring tubule. Their walls are thinner than those of the others and are evidently about to separate off and probably degenerate. Figure 20 gives a view of the same model seen from the inside of the brain. The velum, V, forms a well marked ridge, in front of which a slight depression in the telencephalic roof plate marks the former opening of the paraphysis. On the diencephalic side of the velum the roof plate shows two large irregular openings separated by an irregular ridge in the mid-line, the one on the right being much larger than the one on the left. In the depths of these larger diverticuli are seen the openings of the smaller tubules, the majority of which communicate with the right diverticulum. It can clearly be seen that all of these represent outgrowths or evaginations from the roof plate and differ in this respect from the usual invaginations or ingrowths of the brain wall found in plexus formations. Above the larger prolongations the diencephalic roof plate sweeps gradually upward with only a slight median depression. In the depths of these tubules were two or three closed vesicles either entirely cut off from the brain wall or still partly fused and showing the same signs of degeneration as was seen in the 31 mm. embryo (fig. 17). An embryo of 37 mm., H. E. C. no. 820, has a much simpler arrangement in this region. The evaginations from the diencephalic wall formed three fairly large pouches, one in the mid-line with two smaller ones on either side, and there was no sign of any smaller tubules or detached vesicles, while in an embryo of 40 mm., H. E. C. no. 1917, there is a simple medial pouch pushed forward over the telencephalic roof plate with a very few tiny outgrowths from it. An embryo of 42 mm., H. E. C. no. 838, showed practically the same conditions as in the 36 mm. embryo (fig. 19), and in the oldest embryo in the collection which is shown in figure 21. There was in this embryo, however, some little shrinkage in the walls of the postvelar tubules, which must have caused some slight distortion, but the general plan however is essentially similar to that shown in figure 19. There is one especially prominent tubule appearing below and in the mid-line. The other specimens almost always had some similarly placed tubule which at first

caused confusion in studying sections as it was so similar to
sections through a long tubular paraphysis. However it, to-
gether with all the others, opens into the larger outgrowths from
the brain wall on the diencephalic side of the velum and is of
diencephalic and not of telencephalic origin. The view from
the inside of the brain is essentially similar to that shown in
figure 20 and immediately above these diverticuli the roof plate
runs smoothly up and back. Real plexus infoldings in the roof
plate do not appear until a point is reached roughly at least half
way to the supra-pineal recess.

In all the specimens this tubular formation begins just at the
diencephalic lip of the velum and involves in all cases relatively
about the same extent of the velar end of the diencephalic roof
with quite definite limits caudally. When one takes into con-
sideration the distance from the velum to the superior com-
missure the formation as a whole is quite compact and definitely
limited.

The question at once arises as to the character of this forma-
tion and its homologies in lower forms. It seems first of all to
be in the nature of a transitory affair as shown by the tendency
to the formation of detached and degenerating tubules and
vesicles. The only specimen of an older stage available for
study is shown in figure 22. This is a model of the forebrain of
a human embryo of 80 mm. at a magnification of 20 diameters,
for which I am indebted to the kindness of Prof. G. L. Streeter
who loaned me this specimen about four years ago when the
model was made. The low magnification is unsuited to bring
out details but gives a general topographical view of the roof of
the forebrain. The velum, *V*, is very thick and dense and the
paraphysal arch almost entirely suppressed. On the dienceph-
alic side of the velum the diencephalic roof plate is prolonged
forward in the form of a wide pouch overhanging the roof of
the telencephalon to a marked extent. Irregularities are seen
in the lateral wall, which are apparently choroidal in character
or may be due to shrinkage, though the brain roof as a whole
was in good condition. Streeter in his account in the Keibel-
Mall Embryology states that the oral end of the roof plate of

the diencephalon forms a large choroidal pouch overhanging
the telencephalon and suggests that this is the homolog of the
paraphysis of other vertebrates. In figure 22 a picture corre-
sponding to this description appears, but as the pouch is on the
diencephalic side of the velum it cannot be compared with the
true paraphysis which always is of telencephalic origin. His
(11), figure 56, shows a frontal section through that part of the
diencephalon prolonged forward over the velum, in which a few
choroidal folds appear, but no trace of any tubules or diverticuli
and the picture corresponds closely with that seen in the sections
of the embryo modeled in figure 22.

As regards the appearance of this formation in lower verte-
brates, there is but little evidence on which to base conclusions.
I have examined carefully the Harvard Collection of models
of the forebrain of Necturus, Lacerta (agilis and muralis),
and Chrysemys marginata. In all of these specimens the di-
encephalic roof plate is invaginated by the plexus formation in
the roof of the forebrain. Here one finds solid projections into
the brain cavity instead of hollow tubules or diverticuli growing
out of that cavity. This is especially the case in Amphibia,
where practically all of the postvelar arch is absorbed into a
huge mass of plexus, which extends back to the hindbrain. In
Chrysemys there is a slight projection of the diencephalic roof
plate forward on either side of the paraphysis, but this seems to
be due rather to the impression made by the latter on the dien-
cephalic roof and practically the whole roof plate is invaded
by masses of plexus. In Lacerta the plexus formation affects
the roof of the diencephalon in the neighborhood of the epiphysis
only and the velar portion is absolutely smooth and flat. In birds
Dexter (4), figures 5 and 7, shows an oval or a triangular shaped
vesicle lying dorsal to the paraphysis and close against the wall
of the diencephalon. He finds this as an inconstant structure
in chick embryos from 60 mm. up to young birds after birth.
He mentions also the presence of detached vesicles referred to
by Dendy in Sphenodon and by other authors in some of the
Lacertilia. These latter, however, all seem to belong to the
epiphysal part of the forebrain roof. Whether the vesicle seen

in certain chick embryos could be homologized with any of the detached tubules or cysts shown in the human embryos will require further study and confirmation. As regards Mammalia, there are no signs of these postvelar tubules in any of the sheep embryos here described. Heuser (9) shows several excellent models of the ventricles of the pig from early stages up to 260 mm., but nothing comparable to what has been described in human embryos occurs there and no traces can be found either in rat or rabbit embryos.

In dog embryos of 14 mm. and 17 mm. a median pouch-like prolongation from the diencephalic roof is clearly seen, figures 31 and 32, which resembles the similar projection in the human embryo in figure 13. A somewhat similar arrangement appears in cat embryos of 10 to 12 mm. (fig. 33) and in older embryos there is a marked projection of the diencephalic roof forward over a greatly reduced velum and paraphysal arch. In the cat, however, folds of plexus invade the prolongation in a manner quite different from that seen in human embryos, and no tubular formation can be seen. These are apparently the only instances occurring in other forms which are in any way comparable to the condition just described in the human embryos. It might be asked justly whether this formation was not wholly pathological. It, however, occurs so constantly, although in a variable degree of complexity, that it would seem to be a normal phase in embryos between the ages considered here. It is also suggested that the possible persistence of some of these detached vesicles might account for some of the pathological conditions found in this general region. Conditions prevailing at the time when this paper was written prevented the author from following out the comparative morphology of these postvelar tubules as thoroughly as should have been done and more extensive study of comparative forms and of older human embryos will be needed to reach a satisfactory conclusion. The terms 'postvelar tubules' or 'diverticuli' are used here for lack of more suitable expressions and are rather unsatisfactory. It is hoped, however, that with further investigation a better description can be obtained.

4. Conclusions

1. The primary arches can be demonstrated in early human embryos from 10 to 15 mm. in length.

2. Of the embryos of 15 mm. and over examined in preparing this paper there were about thirty in which the brain was in suitable condition to warrant making observations and in addition to these a number of others were studied but excluded on account of injury or distortion of the forebrain. In the thirty specimens only eight showed any possible signs of a paraphysis and most of these were mostly rudimentary in character. By counting every possible case we get a result of 27 per cent. The fact remains, however, that the structure can be found in human embryos, though in a rudimentary and inconstant condition.

3. The so-called postvelar tubules or diverticuli can be clearly followed in every degree of complexity in embryos of 19 mm. up to 44 mm. and appear in every specimen studied in those stages. They begin at the diencephalic lip of the velum, have definite limits and involve a relatively short extent of the oral end of the diencephalic roof plate. They always appear as outgrowths from the brain roof and are to be distinguished from ingrowths due to plexus formation.

PART III. MAMMALIAN EMBRYOS IN GENERAL

As was mentioned in the introduction, it is intended to review generally the main features of the development of the pineal region in the other mammalian embryos which could be examined in the Harvard Collection. Of these only a few specimens of opossum, cow, deer, and dog embryos were available and the statements on them must necessarily be incomplete.

1. Marsupials

Opossum. In an opossum embryo of 11 mm. (fig. 23) the primary arches are all recognizable. The paraphysal arch is short and is succeeded by a very low velum. The postvelar

arch is long and flat, the epiphysal arch just developed and the pars·intercalaris of relatively large size with the posterior commissure partly in it and partly overlapping into the midbrain. The next stage to be described is an embryo of 26 mm. shown in figure 24. The paraphysal arch, *P.A.*, is reduced to a deep narrow fold which passes over into the velum, *V*, which has been wholly involved in the plexus formation. The roof plate above the velum swells forward over the telencephalon but this seems to be chiefly choroidal in character and is similar to what is seen in rat embryos (figs. 25 and 26) and especially in cat embryos (figs. 34 and 35). As is the case in the cat, the plexus has involved all of the diencephalon up to the deep suprapineal recess. A peculiar feature of this stage is the absence of an epiphysis, only the low arch just behind the pineal recess representing it. This however develops later. See Jordan (13) who gives a good account of the histology of the organ. The commissure shown here is the posterior commissure which fills up all of the pars intercalaris.

This was the oldest stage in the Collection with the exception of a set of sections through the head of an opossum after birth which were in too poor condition to be of any value. The original description of the paraphysis was given by Selenka in the opossum, but he contented himself with making the statement that it existed and gave no pictures of it as far as I can discover. It is hoped that some investigations will be published on older embryos and on the adult opossum in order to confirm the presence of the structure in this species.

2. Rodents

A. Rat. The Harvard Collection contains an excellent series of these embryos up to 25 mm. in which the earlier development of the pineal region can be advantageously studied. Figure 25 shows the primary arches in an embryo of 9.6 mm. with the posterior commissure partly in the pars intercalaris of the forebrain, as is always the case in Mammalia. An embryo of 14.4 mm. is shown in figure 26. In the paraphysal arch there

is a fold which runs across the brain at this point, in the position of a paraphysis, but which cannot be regarded as the real structure. The velum is very small and the postvelar arch is now filled by the diencephalic plexus. The epiphysis is of large size and forms a long sack with rather thick walls and a wide opening into the brain. Just in front of its opening appears a small superior commissure and the posterior commissure fills all of the pars intercalaris and extends back into the midbrain. Figure 27 is a median section of an embryo of 25 mm. constructed from three different sections and is the largest one in the collection. Immediately in front of the small velum is seen the same transverse fold that appeared in the previous figure, which however is deeper and extends entirely across the brain cavity. The postvelar arch has become long and more dome-shaped and contains masses of plexus. Above the superior commissure is a very deep suprapineal recess folded backward against the anterior wall of the epiphysis. Both commissures are well developed but the posterior seems to be much crowded together by the encroachment of the midbrain which has shortened up the pars intercalaris. The epiphysis is of unusual size and has developed backward over the pars intercalaris and the midbrain. Its cavity is at first wide but narrows towards the distal end, which however is thick and expanded laterally. There is apparently no real paraphysis in rat embryos up to 25 mm., its place being occupied by a simple fold running across the full width of the brain cavity in the paraphysal arch. The size and shape of the epiphysis are also very striking.

B. Rabbit. Early rabbit embryos give a picture of the primary arches essentially the same as that shown above for rat embryos. Figure 28 is a transverse section of an embryo of 6 mm. showing the primary subdivisions of the forebrain into a telencephalic segment, *T*, and two diencephalic segments, *I.D.*, and *IID.*, as described in the sheep and pig embryos earlier in this paper and in a previous article (Warren (24), figs. 34 to 37).

An embryo of 14.5 mm. (fig. 29) has a much reduced paraphysal arch. The velum is rounded and as yet the postvelar arch is without any plexus formation. The epiphysis is much

enlarged, contains a well marked cavity, with its tip directed forward and the whole structure is of somewhat irregular outline. The posterior commissure has completely filled the pars intercalaris and has developed backward into the midbrain.

The oldest stage at my disposal was an embryo of 22 days about 36 mm. in length, which was not very well cut or preserved, but an embryo of 19 days, 30 mm. (fig. 30) shows essentially the same conditions. This was from a special series not catalogued in the collection. In the median plane the velum forms a distinct angle with a low fold or groove in the paraphysal arch which passes immediately over into the lamina terminalis. On either side of the median line the velum is much obscured by the plexus which has become much increased, leaving clear however a small suprapineal recess. The superior commissure is of good size, while the posterior commissure and the pars intercalaris seem somewhat reduced in length by the pressure from the midbrain behind. The epiphysis consists of a long tubular body, which ends in an enlarged tip and the central cavity in the body is prolonged into numerous smaller tubules in the extremity of the organ. The whole resembles somewhat the epiphysis of birds. The striking feature in rat and rabbit embryos is the extreme development of the epiphysis which differentiates them from the other mammalian specimens in the Harvard Collection, where the organ remains in a more rudimentary condition.

3. Ungulates

No additional description is needed here to any extent, as the development in the sheep has been already discussed. As regards pig embryos Heuser's (9) work on the shape of the ventricals covers the main points in the development of this part of the brain. See also Johnston (12) on the morphology of the forebrain in vertebrates for additional details, especially the velum. The formation of the primary arches and the three main subdivisions of the forebrain correspond to the account already given for sheep embryos. Heuser found no sign of a paraphysis but there is a deep fold running the whole width of

the paraphysal arch, somewhat similar to the condition in rodents, which lies in the morphological position of the true paraphysis. Of two specimens of cow embryos one of 17 mm., sagittal series, H. E. C. no. 1126, showed beautifully all the primary subdivisions in the roof of the forebrain, and the older, a transverse series, was not reconstructed. The same is true of several early deer embryos which at 7 to 9 mm. showed the arches and subdivisions of the forebrain as previously described. See H. E. C. no. 1514, 9.8 mm. sagittal series and H. E. C. no. 1516, 7.3 mm. transverse series. The oldest deer embryo of 18.6 mm., H. E. C. no. 1230, had a very rudimentary epiphysis, velum and paraphysal arch and no sign of any paraphysis.

4. Carnivora

A. Dog. Of the three sets of sagittal sections in the collection those of 14 mm. and 17 mm. are here shown, as the youngest, 12.5 mm., was somewhat damaged in the paraphysal region. Figure 31 is a median section of the forebrain of the 14 mm. embryo and shows the primary arches, although the postvelar arch is already somewhat invaded by the plexus, which was not the case in the 12 mm. embryo. The velum forms a well developed fold with a low paraphysal arch in front. On the diencephalic side of the velum the velar end of the roof plate bulges somewhat forward over the velum. It should be noted that the posterior commissure has filled nearly the whole of the pars intercalaris and extends practically up to the low epiphysal arch. In figure 32, embryo of 17 mm., there is no sign of a paraphysis, the velum forms a sharp fold and the diencephalic roof protrudes above it in a pouch-like projection very similar to what is seen in a median section of some of the human embryos. This pouch makes a fold extending across the whole width of the roof. Owing to lack of material it was impossible to follow the development further, but it can be stated that the primary arches are present and that there is a hint of the diencephalic outgrowth described in human embryos.

B. Cat. The primary arches in cat embryos are quite similar to the pictures already shown here in other forms and there is

no need of adding a special picture. The same is true of the three main subdivisions of the brain as seen in transverse sections (fig. 28). In figure 33, a cat embryo of 10.7 mm., there is a very low paraphysal arch and only a slight fold for the velum, which is less well marked than in earlier stages. Above it appears a wide projection in the diencephalic roof somewhat similar to that seen in figure 32. The rest of the brain roof shows merely the other arches. In an embryo of 24 mm. (fig. 34) the paraphysal arch is reduced to a mere slit, the velum is also very insignificant and immediately above it there is a large forward projection of the diencephalon followed by a mass of plexus which involves the whole of the roof. This projection has the same relative position as the one shown in human embryos but seems to become more involved in the plexus formation and to lack the tubular outgrowths which are so characteristic of the former. A small epiphysis which can be seen in embryos of 15 mm. is shown here inclined backward between the superior and posterior commissure. The largest embryo cut sagitally is shown in figure 35. It is an embryo of 39 mm. and is in the main an exaggerated picture of that seen in figure 34. The greatly reduced size of the paraphysal arch and velum is to be noted and also the excessive plexus development which involves the whole of the diencephalic roof up to the suprapineal recess. The pars intercalaris occupies a very prominent space in the roof of the diencephalon in this stage and in that shown in figure 34 and is not as much suppressed by the pressure from the midbrain roof as is usually the case. No paraphysis could be found in any of the cat embryos in the collection. Owing to the small size of the paraphysal arch and of the velum and to the extreme development of the diencephalic plexus, it is often difficult to fix accurately the true position of the velum and misinterpretations are easily made. The great temptation is to place the velum rather further back along the brain roof and describe the fold or folds which then seem to belong to the telencephalon as the paraphysis. The author feels convinced, however, that the velum in the above figures is properly placed, thereby confirming all the above folds to the diencephalon and

having only a rudiment of a paraphysal arch in the roof of the telencephalon, with absolutely no sign of a paraphysis, which must be morphologically of telencephalic origin. The important features in the development of the pineal region in these embryos are the almost total suppression of the paraphysal arch, the much reduced velum and the luxuriant development of the plexus. An excellent account of the development of the forebrain in the cat is given by Tilney (23) and figures of embryos up to 70 mm. are there shown.

CONCLUSIONS

1. The primary arches in the roof of the telencephalon and diencephalon are all seen in early stages of mammalian embryos, and are all present at the same time. The marked extent of the synencephalic arch or pars intercalaris and its early appearance is a very striking feature in Mammalia.

2. The three main subdivisions of the forebrain, the telencephalic and the two diencephalic segments or subdivisions, can be followed in all the Mammalia referred to here.

3. The paraphysis is found definitely developed in sheep embryos only. In all other Mammalia except man it is represented merely by the paraphysal arch either with or without a transverse fold which passes across the whole width of the telencephalic roof. In human embryos it can be found in occasional cases as a rudimentary and very inconstant structure.

4. In all the earlier stages of mammalian embryos the velum is fairly well developed. In later stages it becomes reduced, especially towards the mid-line, to a mere angle and is more or less absorbed in the folds of the diencephalic plexus.

5. The postvelar arch is always well developed. Later the diencephalic roof plate becomes incorporated with the tufts of the diencephalic plexus which occupies the greater part of its extent. There is in most cases a well marked suprapineal recess extending upward and backward over the supracommissure.

6. The epiphysis of ungulates and carnivora even in the oldest embryos studied is very small possessing thick walls and a small lumen. It is almost buried between the two commissures and

covered anteriorly by the arched suprapineal recess. In rodents it undergoes a much more extensive development and resembles somewhat the large epiphysis of reptiles. In the cat and rabbit it is characterized by a long hollow stalk with fairly thick walls and an extended distal end, which shows distinct tubule formation and inclines backward over the posterior commissure.

7. The superior commissure is well developed and present in all forms studied. It is deeply placed between the epiphysis and the suprapineal recess. The posterior commissure makes a very early appearance, being present in almost all forms in the stages where the primary arches are present in the roof of the forebrain. Its first traces can be seen in both the forebrain and the mid brain and it soon occupies all of the pars intercalaris, extending close upto the dorsal wall of the epiphysis.

8. The diencephalic and lateral telencephalic plexuses are well developed in mammalian embryos but there is no trace of the median telencephalic plexus which is so distinctive in Amphibia.

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ABBREVIATIONS

D., diencephalon
I.D., first diencephalic segment
II.D., second diencephalic segment
D.C.P., diencephalic choroid plexus
D.R.P., diencephalic roof plate
E., epiphysis
E.A., epiphysal arch
F.B., forebrain
F.M., foramen of Munro
H., hypophysis
Hm., hemisphere
L.C.P., lateral choroid plexus (telencephalic)
L.T., lamina terminalis
L.V., lateral ventricle

M., mesencephalon
M.B., midbrain
O.C., optic commissure
P., paraphysis
P.I., pars intercalaris
P.V.A., postvelar arch
P.V.T., postvelar tubules or diverticuli
P.C., posterior commissure
S.C., superior commissure
T., telencephalon
T.R.P., telencephalic roof plate
Th., thalamus
V., velum transversum

PLATE 1

EXPLANATION OF FIGURES

1 Sheep, 9.9 mm. H. E. C., sagittal series, no. 1339. × 20.
2 Sheep, 14 mm. H. E. C., sagittal series, no. 1330. × 20.
3 Sheep, 21 mm. H. E. C., sagittal series, no. 1687. × 20.

8   Sheep, 48.4 mm. H. E. C., transverse series, no. 1696. $\times$ 20.
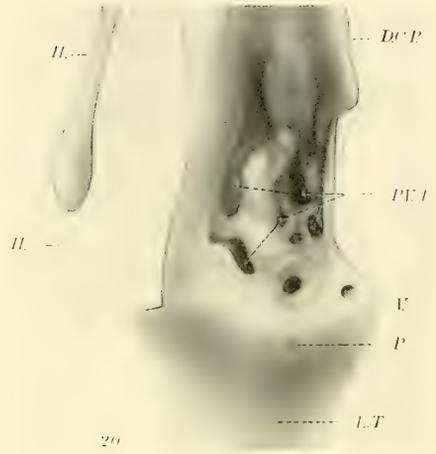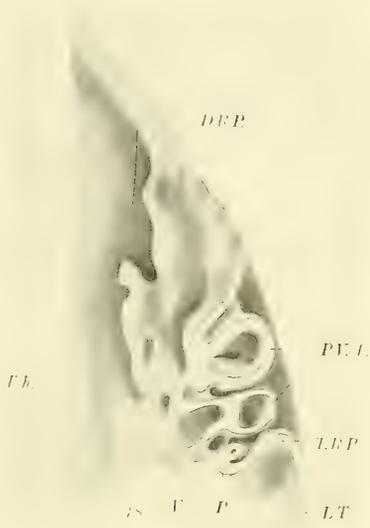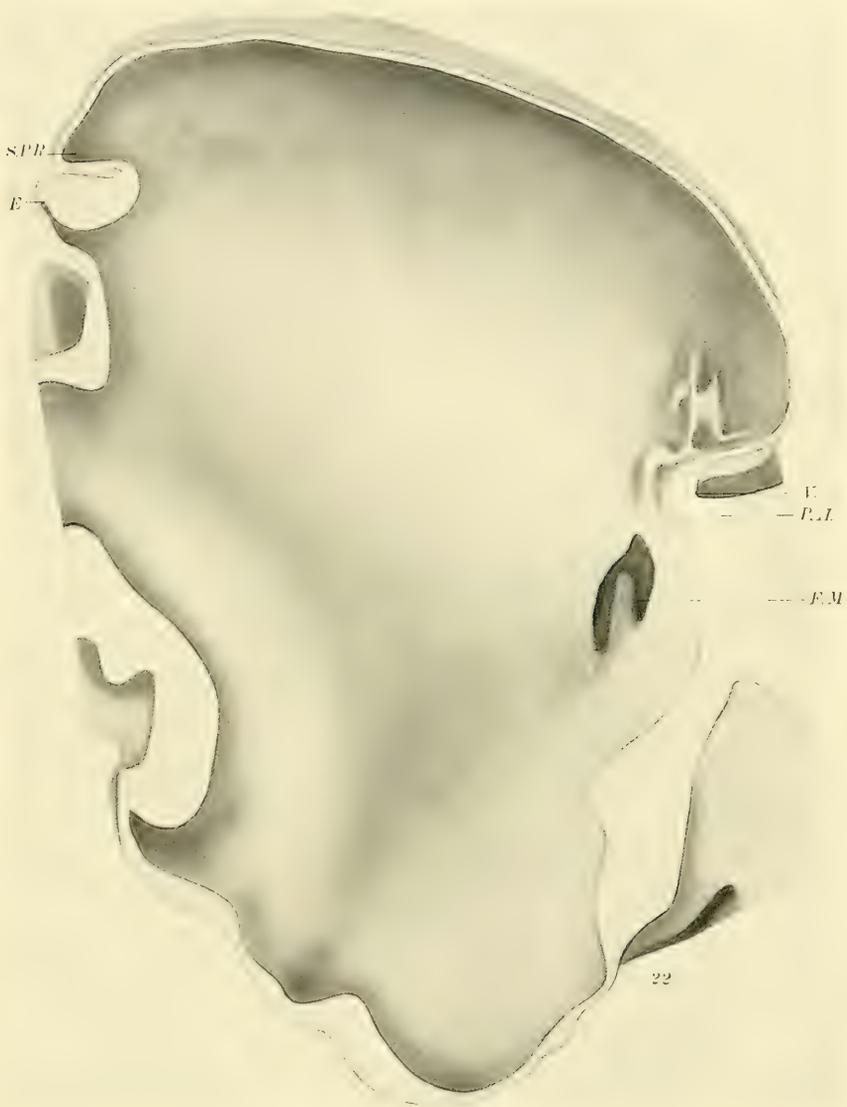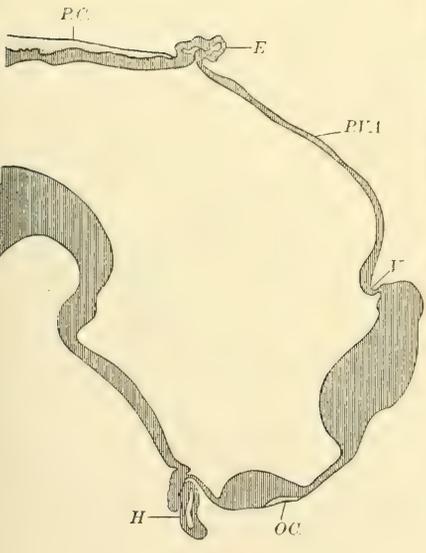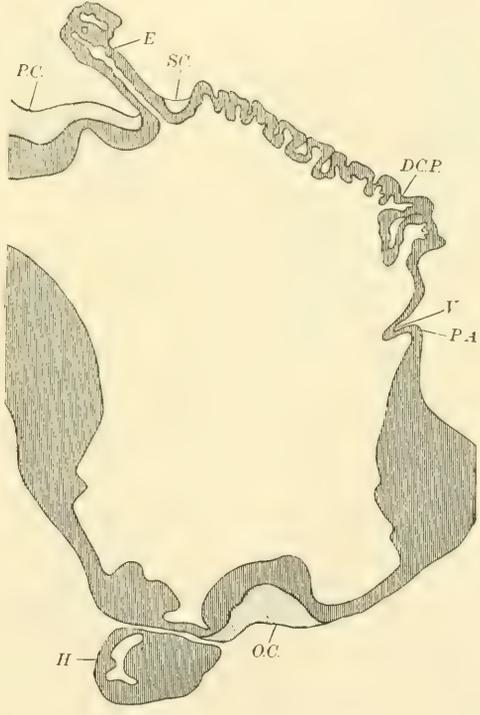
PLATE 11

23 Opossum, 11 mm. H. E. C., sagittal series, no. 925. × 20.
24 Opossum, 26 mm. H. E. C., sagittal series, no. 2077. × 20.
25 Rat, 9.6 mm. H. E. C., sagittal series, no. 1824. × 20.
26 Rat, 14.4 mm. H. E. C., sagittal series, no. 1925. × 20.
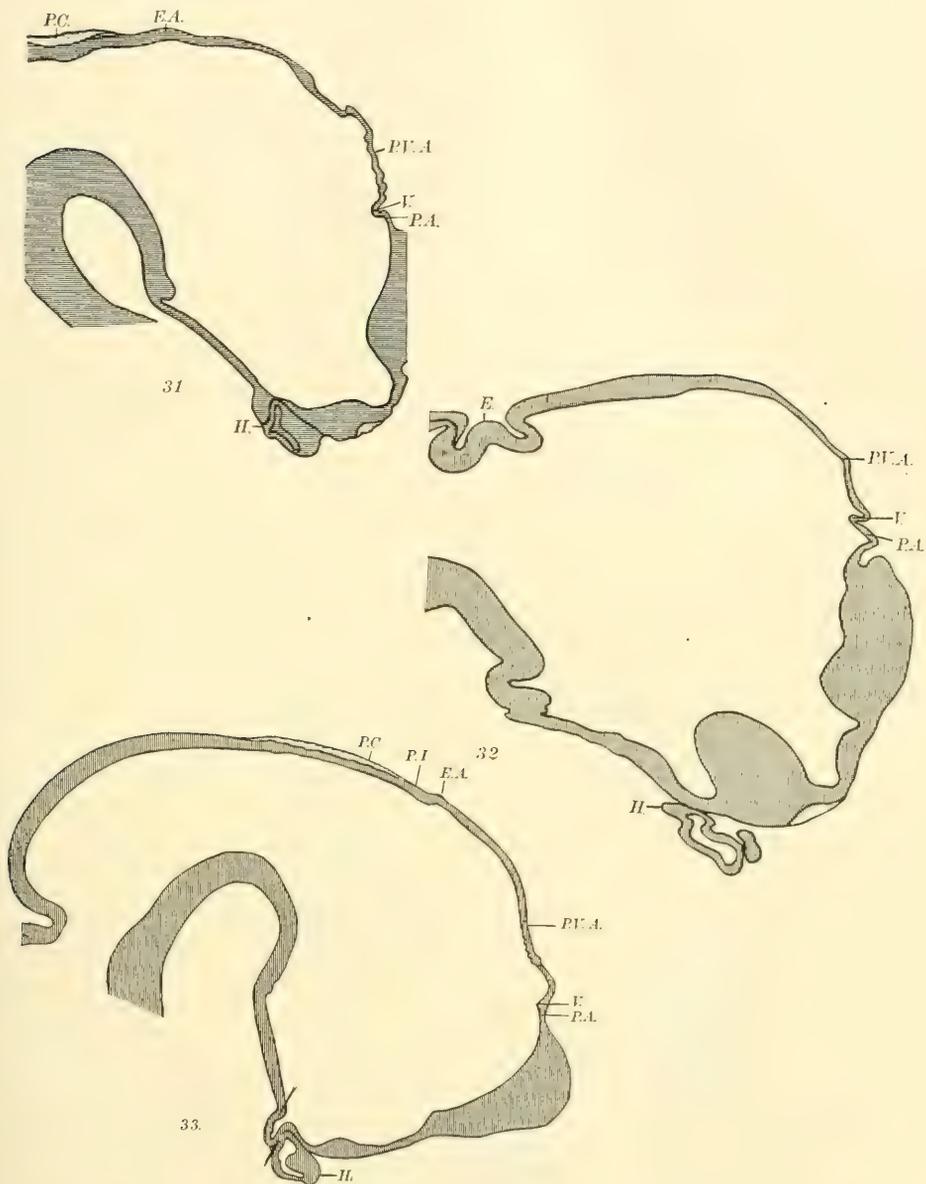
DISTRIBUTION OF THE SPINAL NERVES IN POLISTO-
TREMA AND SOME SPECIAL STUDIES ON THE
DEVELOPMENT OF SPINAL NERVES

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THIRTY-FIVE FIGURES

CONTENTS

INTRODUCTION

This paper is the result of a continuation of a study of some
problems suggested from the distribution and development of
the peripheral and central nervous system in Polistotrema
(Bdellostoma). A recent publication dealt with the origin of
the tela choroidea of the fourth ventricle and the causes con-

tributing to the formation of the ribbon-like spinal cord of Cyclostomes. In this paper the questions of chief interest are concerned with the manner and cause for the union of the motor and sensory components of the spinal nerves in vertebrates above Amphioxus and Petromyzon, the appearance of muscle sense endings in the specialized M. cordis caudalis outside the caudal heart, and the presence and possible significance of certain peripheral ganglion cells found along the course of the vagus, glossopharyngeal and some of the spinal nerves.

Material and methods

The material for this paper consisted of several sets of serial sections taken from various levels of adult Polistotrema (Bdellostoma) and Amphioxus; together with serial sections of a number of Polistotrema, Squalus acanthias, turtle, pigeon and pig embryos. I am indebted to Prof. R. E. Scammon for the use of his very complete serial collection of Squalus embryos. The results were obtained largely from a study of graphic reconstructions which were prepared after the usual method. Careful projection drawings were made of each section. These were later checked up for errors using a higher magnification. A common base or projection line was drawn horizontally across the ventral surface of the notochord in the first drawing from which all structures were measured off and plotted on millimeter paper. In order that some of the minor details of description might be eliminated from the text, very complete and detailed descriptions of the figures have been given at the end of this paper to which the reader's attention is directed.

MANNER OF DISTRIBUTION OF THE MOTOR AND SENSORY SPINAL NERVE FIBERS IN ADULTS AND EMBRYOS OF VARIOUS VERTEBRATES

1. Comparison of the two extremes, Amphioxus and Petromyzon, with mammals

An examination of figures 29 and 30 discloses the well-known differences between the spinal nerves of Amphioxus and a

mammal. It will be seen in Amphioxus that the motor and sensory elements are widely separated, a motor nerve appearing opposite each muscle plate, and a sensory nerve at the intermuscular septum. The corresponding nerve roots of the two sides do not enter and leave the spinal cord in the same plane. A section passing through a motor root on one side would in all probability pass through the sensory root on the opposite side. The arrangement shown in figure 30 for mammals will hold for all forms from Amphibia up and is too well-known to require more than the mere statement, that the dorsal (sensory) fibers which enter the spinal cord in the same plane as the corresponding ventral (motor) fibers leave, intermingle with the latter after passing through a spinal ganglion, and the mixed fibers form the three characteristic rami, dorsal, ventral, and communicans (fig. 30, *R.D.*, *R.V.*, and *R.C.*).

Upon closer examination of figure 29 it will be seen that the fibers of the ventral root, radix anterior (*V.R.*), after leaving the ventro-lateral surface of the spinal cord and penetrating the neural arch, spread out on the inner surface of the myotomes somewhat after the manner of a cone. In my haematoxylin sections the light marginal areas bounded by a dotted line in figure 29 have the appearance of connective tissue more than nerve fibers. A similar appearance may have led Balfour' (80) to affirm that the ventral roots, accurately described by Schneider in 1879, are mere processes of the muscle plate. This cone-like distribution of the motor fibers to the myotomes could be described also as forming a motor ramus dorsalis and a motor ramus ventralis (*R.D.M.* and *R.V.M.*).

As will be seen from figures 28 and 29 and from the descriptions of Schneider ('79), Hatschek ('92), Dogiel ('02), Johnston ('05), Miss Kutchin and others, the sensory fibers of Amphioxus are collected by dorsal and ventral rami. These trunks follow the outer surface of the intermuscular septum, and when a little above or below the level of the dorsal surface of the spinal cord they penetrate the connective tissue septum to unite on or near the inner surface of the septum, in forming a short dorsal root, radix posterior (*D.R.*), which pierces the neural arch to enter

the dorso-lateral surface of the spinal cord. The important peculiarities in the arrangement and course of the sensory fibers in Amphioxus are: (1) there is no centralization of ganglion cells in the dorsal root to form a spinal ganglion, although scattered ganglion cells are found (figs. 28 and 29, N.C.) in the dorsal region of the spinal cord, in the dorsal root, throughout the ventral ramus to the skin, and in the dorsal ramus. (2) The dorsal and ventral sensory rami do not follow a circuitous course along the inner surface of the intermuscular septa and around the myotomes to reach the skin, but penetrate immediately the intermuscular septa.

From the work of Ransom and Thompson, Schneider ('79), Julin ('86) and ('87), Johnston ('05), and others it is evident that the arrangement of the motor and sensory fibers of the spinal nerves of Petromyzon agrees with Amphioxus, except for the passage of the dorsal and ventral sensory rami around the intermuscular septa and myotomes to reach the skin and the concentration of ganglion cells in the dorsal root directly outside of the neural arch to form a spinal ganglion. Julin ('86) and ('87) found in a 15 to 18 mm. Ammocoetes that the ventral sensory rami from the region of the heart to the anus received visceral fibers from a ramus communicans, and that sympathetic cells were present near the aorta as superficial and profundus sympathetic ganglia.

2. Conditions in various regions of adult Polistotrema

J. Müller found that the spinal nerves of Myxine arose from two roots which united in crossing the notochord. He was unable to determine whether these roots were motor or sensory, nor was he certain of the presence of ganglia, but thought that they were present on the most cephalic nerves. Ransom and Thompson state that in certain regions of Bdellostoma there are two anterior (ventral) roots to one posterior (dorsal) root. The posterior root is said to possess a distinct spinal ganglion, which divides into a dorsal and a ventral ramus. Each of the two anterior roots was found to give off a dorsal branch, which

join to form the motor ramus for the back. Likewise the two anterior roots unite in a ventral motor ramus, which joins the ventral sensory ramus in forming a mixed ramus ventralis. The dorsal sensory rami remained entirely separated from the motor rami, and the condition in Myxine is said to be similar to that in Bdellostoma. Miss Worthington's description for the distribution of the first spinal nerve in Bdellostoma resembles in the main the description of Ransom and Thompson, but is more complete, and the nerve is figured in her reconstruction of the cranial nerves (fig. 14). She finds that the first spinal nerve in Bdellostoma has one sensory and two motor roots, and states that the motor roots emerge from a Y-shaped foramen as one trunk. The spinal ganglion is said to be elongate, having a dorsal and a lateral arm. The dorsal arm gives rise to the dorsal sensory ramus, and the ventral, to the ventral sensory ramus. The ventral root continues ventrad as the ventral motor trunk, and unites with the corresponding sensory trunk to form the ramus ventralis. Close to the origin of this trunk, a branch, said to represent Fürbringer's dorsal motor trunk, is described and figured as passing dorsad to penetrate the body muscles. From its source this dorsal branch might be expected to possess sensory as well as motor fibers.

Cole in his paper on the muscles of Myxine figures a typical abdominal transverse section, incorporating in it all of the nervous elements found in that segment. A dorsal (sensory) root is figured as passing through the neural arch and expanding on the outside in a ganglion, from which a dorsal and a ventral ramus are given off. The motor fibers are figured as issuing by four rootlets into two roots, which apparently penetrate the neural arch through separate foramina. Outside the neural arch one of the roots is represented as giving off a dorsal branch, which joins a corresponding sensory trunk in forming the ramus dorsalis. The two ventral roots unite opposite the notochord, producing a short motor trunk, which joins the ventral sensory ramus in the formation of the ramus ventralis. Both the ramus dorsalis and the ramus ventralis are shown in his figure as traversing the inner surface of the myotome, the former pierces the

upper portion of the muscle, and both send off numerous branches into the muscle. The dorsal ramus after leaving the myotomes is represented as dividing into a branch for the dorsal fin region and a branch designated as the ramus cutaneus superior, which passes laterad and then ventrad along the inner surface of the skin. At the ventral margin of the myotomes the ramus ventralis sends off a branch to the obliquus and rectus muscles, and a sensory bundle continues onward a short distance to separate into a dorsal branch designated as the ramus cutaneus medius and a ventral branch designated as the ramus cutaneus inferior. Both rami follow the inner surface of the skin, the former pursuing a general dorsal course and the latter after crossing the mucous sac ends in the anal fin region.

As a result of a study of the elements of a spinal nerve from a Myxine series, taken from the region of the anus, I have been able to confirm everything Cole figured in his transverse section (fig. 1), excepting the union between the dorsal motor and the dorsal sensory rami. In this region the motor ramus was the more cephalic, and it was clear from the sections that it terminated in the myotome before the sensory ramus reached the same level.

Realizing that some of the discrepancies in the descriptions of the distribution of the motor and sensory components of the spinal nerves might be due to a study of different regions and to different genera, I began a careful study of the spinal nerves at different levels of adult ,Polistotrema (Bdellostoma). A reconstruction was made of the first two spinal nerves appearing behind the auditory capsule. A second reconstruction was made of a spinal nerve in the region of the caudal end of the M. retractor mandibulae. Three reconstructions were made of a number of spinal nerves in the tail region, one of which was from a late embryo, and finally a dissection was made of several of the spinal nerves in front of the caudal heart. Since considerable variation is shown in the distribution of the motor and sensory components in these reconstructions, a complete description will be given of each, beginning with the most cephalic.

One of the first spinal nerves is shown from a graphic recon-
struction (fig. 6) to have one sensory and two motor roots.
The dorsal or sensory root fibers penetrate immediately the
membranous neural arch, the point of entrance being indicated
by dotted lines. Within the neural canal their course is at first
caudad for some little distance, then bending medially they
terminate in the latero-dorsal surface of the spinal cord, the
space from the arrow to the dotted line (D.R.) representing the
area of the cord where the fibers enter. The spinal ganglion
(Sp.G.), which is much smaller than the one behind, is decidedly
compressed, not more than two cells thick. From the outside
the ganglion is somewhat spindle-shaped the two poles being
dorsal and ventral in position, and the distance between them
is little more than the dorso-ventral diameter of the spinal cord.
From the poles peripheral fibers leave to form the dorsal and
ventral sensory rami (R.D.S. and R.V.S.). In this nerve the
ventral sensory ramus is but little larger than the dorsal. Each
follows the inner surface of the intermuscular septum of the
myotomes, which bends, both dorsally and ventrally, slightly
cephalad at first and then caudad. Throughout its course the
dorsal sensory ramus is entirely separated from the dorsal motor
ramus, but the ventral sensory ramus is joined by the correspond-
ing motor ramus opposite the notochord to form a mixed ramus
ventralis (R.V.). The extreme peripheral course of the dorsal
sensory ramus of the first spinal nerve is not shown in figure 6;
it is, however, very similar to the dorsal sensory ramus of the
succeeding nerve, which will be described later.

As previously stated, the first spinal nerve in figure 6 has two
motor roots. They are more difficult to isolate than the corre-
sponding roots of a more caudal region. The more cephalic
root (V.R. (1)) leaves the latero-ventral surface of the spinal
cord almost opposite its foramen in the neral arch, which will
be seen to be some little distance cephalad of the spinal ganglion
for this nerve. In contrast, the caudal root may be said to
take origin from four rootlets. The two last leave the spinal
cord almost directly below the entrance of the sensory root
fibers, which appear in figure 6 to be midway between the spinal

ganglion of this nerve and the following ganglion, and they are almost the distance of a segment behind the exit of the cephalic root fibers. The two caudal rootlets soon unite in a common stem, which continues cephalad in the neural canal about on a level with the ventral surface of the spinal cord, to the region of the spinal ganglion, where it receives a common stem formed from the union of the two cephalic rootlets. This common trunk is designated as the caudal root. It continues forward in the neural canal to the exit of the cephalic root, and then leaves the neural canal through a foramen immediately behind the foramen for the cephalic root. Outside the membranous neural arch the two roots unite in the formation of a trunk, which passes dorsad a short distance to separate into a dorsal and a ventral motor ramus. The dorsal motor ramus (*R.D.M.*) pursues a dorsal course along the inner surface of the myotomes, to which it sends numerous branches. At first its course is considerably cephalad of the corresponding sensory ramus, but when near the level of the roof of the neural arch it is approached by the dorsal sensory ramus, and at this point terminates in a cephalic and a caudal branch, which soon disappear in the muscle. In figure 6 the cephalic branch may have the appearance of joining the dorsal sensory ramus; this, however, is not the case, for the two are widely separated by connective tissue. The motor ramus is the more lateral and traverses the inner surface of the myotomes; while the dorsal sensory ramus follows closely the outer surface of the neural arch. Since the myotomes (*D.Myo.*) extend some distance above the neural arch, it might be expected that the dorsal motor ramus of the first spinal nerve would extend farther dorsad than is shown in figure 6. The course of the ventral motor ramus (*R.V.M.*) is in general lateral, caudal and ventral. After cutting through a median portion of the myotome it unites with fibers of the ventral sensory ramus, opposite the notochord and a little lateral of the combined glossopharyngeal-vagus trunk, in forming a mixed ramus ventralis (*R.V.*). From this point on the course of the ramus ventralis is along the inner margin of the intermuscular septum of the myotomes. Since the further

distribution of the ramus ventralis is identical to the ramus ventralis of the spinal nerve in reconstruction 5, this nerve was not reconstructed further than the level of the dorsal aorta in figure 6.

Several facts remain to be recorded in connection with the components of the second spinal nerve in figure 6. They are in the main either differences from the condition described for the first spinal nerve or additional points of detail. First, the second spinal ganglion is much larger than the first. It extends dorsally nearly to the apex of the neural arch and ventrally to the notochord. In this region the myotomes follow very closely the outer surface of the neural arch and the myotomes of each side are attached above the neural arch to a median dorsal connective tissue septum; so that there is a wall of muscle above the neural arch of considerable thickness. It is between this wall of muscle and the dorsal median septum that the second dorsal sensory ramus in figure 6 passes to reach the skin. Upon arriving at the dorsal surface of the myotomes it bends laterally finally to pass ventrad along the inner surface of the skin as the superior cutaneous nerve of Cole. No peripheral ganglion cells were found along the course of this nerve outside the spinal ganglion. The dorsal motor ramus of the second spinal nerve in figure 6 does not arise as a branch from the common trunk formed from the union of the cephalic and caudal roots, but rather branches off from the cephalic root outside the neural arch shortly before it joins the caudal root to form the ventral motor ramus. The dorsal motor ramus of the second nerve can be traced to a much higher level on the myotome, but crosses the sensory ramus much closer to the ganglion. Throughout, it is lateral to the sensory ramus and there is no exchange of fibers. The caudal motor root takes origin from more than four rootlets. Attention should be called to the number and the distance apart of the motor rootlets in this region. There may be from five to eight taking part in the formation of the two motor roots of a spinal nerve and their extent is nearly equal to the length of a segment. In fact, in figure 6 the distance between the last motor rootlet of the first spinal nerve and the

first motor rootlet of the second spinal nerve is but little greater than the distance between two rootlets of the first spinal nerve. In the caudal region the position and arrangement of these rootlets was shown in figure 1 of a previous paper.

A reconstruction of a spinal nerve taken from the region of the caudal end of the retractor mandibulae muscles (fig. 5) presents a number of differences from the spinal nerves of the more cephalic region described above.

It will be seen that the dorsal root (*D.R.*) does not at first pursue a caudal course within the neural arch, but immediately passes inward to enter the latero-dorsal surface of the spinal cord. The fibers for this root arise from the inner central surface of the spinal ganglion from a dorsal and a ventral bundle (see previous paper, fig. 71). These bundles soon unite, forming the dorsal root, which immediately enters its foramen in the neural arch. This foramen is situated about on a level with the central axis of the spinal cord. The dorsal sensory ramus (*R.D.S.*) does not follow the contour of the neural arch so closely as the more cephalic rami do in reconstruction 6. Instead, it cuts obliquely dorsad through that portion of the myotomes which is situated above the roof of the neural arch, and approximates the inner surface of the myotomes more closely than the median dorsal septum. Throughout its entire course the dorsal sensory ramus is well separated from the corresponding motor ramus or any of its branches. The point of union between the ventral motor and the ventral sensory ramus is considerably more distal in this nerve than is the case of the more cephalic nerves. As was noted for the more cephalic nerves, the ventral sensory ramus is situated behind the ventral motor ramus, and both appear to gradually approach each other, join, and form a mixed ramus ventralis. Near the ventral border of the myotome there is a conspicuous ganglion cell in the ramus ventralis. This cell, which is shown in section in figure 11, is seen to be no different in size or structure from any of the spinal ganglion cells.

Several variations in the arrangement of the motor components are to be recorded for this nerve. It is clear from figure 5 that all of the motor rootlets, some eight in number, lie cephalad

of the spinal ganglion of this nerve. The cephalic ventral root
($V.R$ (1)) takes origin from two rootlets, the first, which is not
figured, leaves the latero-ventral surface of the spinal cord a
little behind the level of the preceding spinal ganglion. The
course of the cephalic root is at first caudad within the neural
arch. Its exit through the membranous neural arch lies be-
tween the two dotted lines in figure 5. At least five rootlets
take part in the formation of the caudal ventral root ($V.R.$ (2)).
Two of the more cephalic rootlets pursue a caudo-lateral course
and unite in a common stem which passes through a foramen in
the neural arch, situated on a level with the dorsal surface of
the notochord and about equidistant between the foramen for
the cephalic ventral root and the spinal ganglion. Three of the
most caudal rootlets travel cephalad and laterad to join in a
common stem, which leaves the neural arch through a foramen
immediately behind the common stem formed from the union
of the two preceding rootlets. Directly outside their foramina
these two motor stems unite, forming a caudal ventral root,
which assumes a general ventral course to unite with the cephalic
ventral root a short distance cephalad of the lower corner of
the spinal ganglion. The short common trunk thus formed,
is the ventral motor ramus ($R.V.M.$). Its fibers soon inter-
mingle with those of the ventral sensory ramus, lateral and a
little below the level of the central axis of the notochord. From
the above description it will be seen that the motor components
of this nerve pierce the membranous neural arch through there
separate foramina. Through the first, the cephalic ventral
root fibers emerge, and through the second and third, motor
bundles leave to unite outside the neural arch in forming the
caudal ventral root. It is also clear that all of the motor com-
ponents are situated in front of the sensory. The mixed ramus
ventralis ($R.V.$) presents no special peculiarities except for the
presence of a distal ganglion cell noted in the previous para-
graph. In this nerve the dorsal motor ramus ($R.D.M.$) has a
double origin, differing in this respect from the more cephalic
nerves in figure 6. A bundle of fibers branches off from the
upper surface of the cephalic ventral root close to its exit from

the neural canal and a second dorsal branch arises in like manner from the more cephalic stem of the caudal ventral roo˙ to unite with the first dorsal branch opposite the spinal cord in forming the dorsal motor ramus. This ramus continues in a dorsal direction in front of the corresponding dorsal sensory ramus and entirely separate from it. In figure 5 a caudal branch will be seen passing median to the dorsal sensory ramus, and terminating in that part of the myotome which is bounded by the dorsal median septum, roof of the neural arch and dorsal sensory ramus.

Figures 1 to 4 show a very different arrangement for the sensory and motor components of the spinal nerves for the tail region. Most significant, is the fact, that the majority of the motor and sensory rami remain separate throughout, resembling Petromyzon in this respect. The above mentioned figures include graphic reconstructions of some of the caudal spinal nerves from a transverse series of a small adult Polistotrema, from an 85 mm. Polistotrema embryo that had attained nearly adult conditions, and from a full grown adult.

In figure 1, where eleven of the last spinal nerves were reconstructed, seven have separate motor and sensory rami. The ventral motor rami of the last three nerves assume a gradual caudal course in crossing the notochord so that they eventually become located closer to the succeeding ventral sensory rami than they do their own. The third from the last ventral motor ramus follows the inner surface of a myotome about equidistant between its sensory ramus and the following one. It accompanies a branch from its sensory ramus when on a level with the lower surface of the caudal artery. The three most cephalic of the last seven ventral motor rami follow at first the inner surface of the myotomes a short distance behind their respective sensory rami until considerably below the level of the caudal artery, then bend cephalad, cross the outer surface of their sensory rami without any exchange of fibers and terminate in the myotomes. A conspicuous cephalic branch (*R.M.C.C.*) is given off from the last of these motor rami to pass along the inner surface of the M. cordis caudalis for a distance of at least

four segments. Apparently in this specimen it was a very important effective or motor nerve for the M. cordis caudalis. The first four ventral motor rami in figure 1 cross their respective sensory rami as the three more caudal rami did, but immediately after crossing they bend ventrad and probably join the sensory rami or branch of the same. It is impossible to state whether the fibers mix or not, although there is nothing to prevent them from so doing. A little below the ventral border of the myotomes the motor components of these ventral rami form a sort of plexus from which branches are given off to the M. cordis caudalis (R.M.C.C.), to certain longitudinal muscle bundles (L.M.B.) and to the M. transversus caudalis.

Regarding the distribution of the dorsal motor rami (R.D.M.) of the tail region, at least one important difference from the cephalic region should be noted. It is that the motor rami do not follow the general course of their respective sensory rami so closely as they do their succeeding sensory rami, a characteristic which was also recorded for the last three ventral motor rami. As in the cephalic region there is considerable variation concerning the mode of origin of these nerves. They usually branch off from or with the ventral motor rami at the point of union of the cephalic and caudal motor roots or they may arise directly from the motor roots upon emerging from the neural arch. Near the point of origin a lateral branch of considerable size is given off from each of the dorsal motor rami to the myotomes. It is much larger than any of the other lateral branches of the nerve and might be designated as the motor ramus lateralis. No dorsal motor rami were found corresponding to the last three ventral motor rami, resembling in this respect the occipitospinal nerves. This may have been brought about by the scarcity of dorsal musculature in the tail region. As was noted in a previous paper, that portion of the spinal cord which is situated behind the exit and entrance of the last motor and sensory roots is non-nervous. It consists of supporting and undifferentiated cells. From sections, the spinal cord was found to end in a number of processes, which intermingle and become lost in a mass of connective tissue at the end of the membranous neural canal.

Except for the extreme caudal region, the distribution of the sensory rami in figure 1 is essentially the same as in a more cephalic region. The primitive condition of the last seven nerves, where the sensory and motor rami remain entirely separated, has been noted. A second possibly primitive condition remains to be recorded for the caudal spinal nerves, namely, a tendency, as in Amphioxus, for the spinal ganglion cells (*N.C.*) to migrate peripherally along the course of the nerves. They are found along the course of many of the dorsal and on some of the ventral rami, and are most numerous on the extreme caudal nerves. The dorsal and ventral sensory rami entering the last four spinal ganglia exhibit considerable variation as to number and arrangement. It frequently happens that more than one sensory ramus will enter the dorsal or ventral surface of one of these ganglia or two or more nerves may unite before a ganglion is reached. In a number of instances there is evidence of a partial fusion of two sensory rami, and several other irregularities are shown in figure 1 for the last dorsal sensory rami. At the base of the fin the sensory rami unite and receive a sort of plexus from the fin. The right side (fig. 1) has one less sensory nerve than the left side. Its ganglion receives a sensory ramus from the rear in addition to others from above and below. The last sensory nerve on the left side (fig. 2) is of a very different type than any of the others. It has no ventral ramus and its root fibers enter the spinal cord at least a segment behind the last sensory root on the opposite side. To trace peripherally from the spinal cord, this nerves passes laterally to the neural arch, where it has one large nerve cell (*N.C.*). Its course is then dorso-caudad for some distance within the membranous neural canal and the position of its foramen in the neural arch is indicated by two dotted lines. Outside the neural arch it has six nerve cells, which may represent its spinal ganglion. On the contrary, its extreme dorsal position, the scattered appearance of its few cells, together with the relatively larger number of fibers than cells, are striking differences from ordinary spinal ganglia. To compromise for the absence of a ventral ramus, the so-called spinal ganglion of this nerve receives three

branches from above and one from the rear, one of which joins
the peripheral plexus above. A conspicuous lateral branch
(fig. 1, *R.Cut.S.*) is received by each dorsal sensory ramus at the
dorsal border of the myotomes, coming from the inner surface
of the skin. This is doubtless the ramus cutaneus superior of
Cole's figure 1. In like manner a lateral-dorsal branch is given
off to the skin from each ventral sensory ramus, a little below
the ventral border of the myotomes. It is apparently Cole's
Ramus cutaneus medius (fig. 1, *R.Cut.M.*). In this particular
region, where the main sensory rami continue upward and down-
ward to supply the dorsal and anal fins and the above cutaneous
rami appear to be branches of the main trunks, a change
of nomenclature might prove advantageous. For the dorsal
branch I would suggest Ramus cutaneus lateralis superior and
for the ventral branch R. cutaneus lateralis inferior.

The long distance that the motor and sensory roots (fig. 1)
travel within the membranous neural canal certainly supports
the supposition that the skeletal axis continues to grow after
the neural axis has obtained its growth or to be more specific
grows more rapidly than the neural axis. It will be seen from
figure 1 that the motor or ventral roots extend for longer dis-
tances than the dorsal or sensory within the neural canal, which
in some instances is nearly a segment. As in the cephalic region
the sensory fibers enter the latero-dorsal surface of the spinal
cord as a single bundle or root, and the point of entrance is about
on a level with the posterior border of the previous spinal gan-
glion. Occasionally it may be slightly cephalad or caudad of
this point. Like the more cephalic nerves, the caudal nerves
usually have a cephalic and a caudal motor or ventral root
(*V.R.* (*1*) and *V.R.* (*2*)), which ordinarily take origin from sev-
eral rootlets, generally more for the cephalic root. The last
three motor rami show an irregularity in having but one motor
root each. Of these three roots the first one has two rootlets and
the last two, one rootlet each. Usually both motor roots of a nerve
have separate foramina, situated a little behind their respective
ganglia, about on a level with the upper surface of the noto-
chord. In one or two of the first nerves in figure 1 the caudal

root appears to furnish the majority of fibers for the dorsal motor ramus, and the cephalic root the bulk of fibers for the ventral motor ramus, suggesting, possibly, that the dorsal and ventral motor rami of more primitive vertebrates may have arisen separately from the spinal cord. There is, however, nothing in the development of the spinal nerves in Polistotrema or in the adult condition of Petromyzon or Amphioxus to support this supposition.

A comparison of the reconstruction of seven of the spinal nerves in the region of the caudal heart of an 85 mm. Polistotrema embryo (fig. 3) with figure 1 discloses very few differences. Those that occur are apparently embryonic. The fact that the motor and sensory roots run for relatively shorter distances in the membranous neural canal can probably be attributed to a more rapid growth of the skeletal axis and myotomes over the neural axis. Also it will be seen from figure 3 that fewer of the motor and sensory rami have joined in the region of the M. cordis caudalis. This union has taken place only in the first nerve in reconstruction 3, while in reconstruction 1 it occurred in the first three nerves that cross the muscle; the motor rami of the remaining nerves in both reconstructions cross the sensory rami without any exchange of fibers. As in reconstruction 1, ganglion cells are present along the course of some of the nerves. A cluster of cells is shown encircling four out of seven of the dorsal sensory rami at the base of the dorsal fin.

In the reconstruction of three spinal nerves (fig. 4) taken from the region of the caudal end of the M. cordis caudalis of an adult Polistotrema it will be seen that there is almost no difference in the distribution and arrangement of the sensory and motor fibers when compared with similar nerves taken from reconstructions 1 and 3. A branch (R.M.C.C.) from the next to the last motor ramus that crosses the M. cordis caudalis enters this muscle and apparently innervates it, but no branch was found going to the inner surface of the muscle as was described for reconstructions 1 and 3. Whether this branch was absent in this specimen or undifferentiated was not determined. Isolated cells and clusters of ganglion cell are visible along the

periphery of some of the sensory rami. A description and dis-
cussion of these cells and similar cells of the glossopharyngeal
and vagus nerves is reserved for a separate subhead.

It is clear from the previous description that there is a striking
difference in the mode of union of the ventral motor and sensory
rami of the cephalic region and those of the caudal region, where
such union takes place. In the cephalic nerves (figs. 5 and 6)
the motor root foramina and the ventral motor rami are all
cephalad of their corresponding sensory rami and ganglia, and
the ventral motor rami join their respective sensory rami from
in front, while in the caudal region (figs. 1 and 3) the foramina
and the ventral motor rami are caudad of their corresponding
sensory rami and ganglia, and the motor rami join the sensory
from the rear. The point of union is much further peripherad
in the caudal nerves. In figures 5 and 6 it is opposite the noto-
chord, while in the tail region (fig. 1) it took place considerably
below the notochord. It occurred to the writer that a dissec-
tion of the spinal nerves of an adult might explain how the
change from one type to the other happened; whether it was
gradual or abrupt, and if the latter, where it took place. Fig-
ure 13 is a drawing of a part of this dissection. It includes the
distribution of the sensory and motor components of six of the
spinal nerves situated immediately in front of the M. cordis
caudalis. It will be seen that the third ventral sensory ramus
divides at the lower surface of the notochord into two branches,
which supply sensory fibers for two rami ventrales. The cephalic
branch receives the ventral motor ramus from in front and the
caudal one, from behind. All of the ventral motor rami cephalad
of this branched sensory ramus join their corresponding sensory
rami from in front and all caudad, that unite with sensory rami,
join them from the rear. It is clear in these six spinal nerves
that the point of union between the ventral motor and the ventral
sensory rami is rapidly becoming more proximal in passing
cephalad. So that it is only in a few of the caudal nerves that
the union of the ventral sensory and motor rami occurs below
the level of the notochord.

The main points of the previous section may be summarized as follows: (a) For certain regions of the body in Polistotrema there is a certain specific distribution of the sensory and motor components in the spinal nerves. Those in the extreme caudal region have the most primitive arrangement of their fibers and closely resemble the condition found in Petromyzon. (b) The characteristics which are common to all of the spinal nerves are as follows. Each spinal nerve has one dorsal or sensory root and two ventral or motor roots (exceptions to be noted for the last three nerves). The sensory root fibers enter the latero-dorsal surface of the spinal cord as a single bundle and leave the inner surface of their ganglia to enter the neural arch on a level with the central axis of the spinal cord. The two motor roots take origin from a number of rootlets scattered along the latero-ventral surface of the spinal cord for a distance of nearly a segment They emerge from the membranous neural canal through separate foramina on a level with the dorsal surface of the noto-chord, and soon unite on the outer surface of the notochord, or below to form a ventral motor ramus. A dorsal motor ramus usually takes origin as a branch from the motor ramus ventralis close to its origin from the union of the motor roots or in a few instances it may arise from either or both of the motor roots outside the neural arch. It distributes itself to the inner surface of each myotome. The spinal ganglia are more or less spindle-shaped and somewhat flattened in a vertical plane. From their two poles dorsal and ventral sensory rami take origin. They follow at first the inner surface of the intermuscular septa of the myotomes. Occasional ganglion cells, isolated or in clusters, occur along the peripheral course of these fibers. (c) The following characteristics will hold for all of the true cephalic spinal nerves down to a region a little below the anus. All of the motor components are cephalad of their corresponding sensory components, and all of the ventral motor rami join their respective sensory rami from in front, opposite the notochord. Upon reaching the dorsal border of the myotomes the main portion of the dorsal sensory fibers bend laterally and ventrally to form the R. cutaneous superior, a few fibers, however, con-

tinue dorsally to supply the skin. Ventrally, a mixed ramus ventralis divides at the lower border of the myotome, sending the R. cutaneus medius laterad and dorsad along the inner surface of the skin and a mixed ramus, the R. cutaneus inferior of Cole, to the constrictor muscles of the mucous sac and to the ventral integument. (d) A few spinal nerves situated in the region of the cephalic end of the M. cordis caudalis present the following peculiarities. The long motor roots of each nerve leave the neural arch behind their corresponding ganglion. Each dorsal motor ramus follows the course of its succeeding sensory ramus rather than its own, and every ventral motor ramus joins its corresponding sensory ramus from the rear instead of in front. This union also occurs much further peripherally, taking place below the level of the caudal vein. (e) A dissection of the spinal nerves of one specimen (fig. 13) discloses that the change relating to the ventral motor rami joining the ventral sensory rami from behind, is brought about abruptly, through the third ventral sensory ramus in front of the M. cordis caudalis dividing on a level with the lower surface of the notochord into cephalic and caudal branches, which furnish sensory fibers for two rami ventrales. In the first the motor ramus joins from in front and in the second it joins from behind; thereby introducing a different arrangement for the following caudal nerves. (f) At least seven of the spinal nerves in the region of the M. cordis caudalis differ from the more cephalic nerves in that their sensory and motor rami remain separate throughout. The motor rami frequently cross the sensory rami, but always without exchange of fibers. 'In most of the spinal nerves of the tail region the dorsal and ventral sensory rami extend to the dorsal and anal fins, and when outside the limits of the myotomes they give off lateral cutaneous branches, designated as the R. cutaneus lateralis superior and the R. cutaneus lateralis inferior. (g) The nerve supply for the M. cordis caudalis, the pulsating muscle for the caudal heart, is from certain of the rami ventrales. (h) Several variations were found in the last three spinal nerves. These nerves were without dorsal motor rami and had but one motor root, while their spinal ganglia received more than one

dorsal or ventral ramus.   (i) The last nerve to enter or leave the
spinal cord is a sensory nerve on the left side (fig. 2).   It lacked
a ventral ramus and a typical spinal ganglion, but a few scat-
tered ganglion cells were found outside the neural arch and one
within it.   (j) As was noted in a previous paper, the extreme
caudal end of the spinal cord is non-nervous, consisting of sup-
porting cells and undeveloped embryonic cells.   It culminates
in several processes, which terminate in a mass of connective
tissue at the end of the membranous neural canal.

3. Embryonic arrangement in Polistotrema

Serial sections of 20, 25, 27, 57, 60, and 70 mm. embryos were
available for this study.   The spinal nerves of the 20 to 27 mm.
embryos were found to be in an embryonic state; while those of
57 to 70 mm. had reached practically adult conditions.   Un-
fortunately the interesting gap between these extremes could
not be filled.   Of the material representing a purely embryonic
arrangement of the spinal nerves, reconstructions were made of
the spinal nerves of the tail of the 20 mm. embryo and of two
nerves from segments a little behind the anus from both the 20
and the 27 mm. series, the latter representing a region in the
adult where the ventral motor and sensory rami always unite
in forming mixed rami ventrales.

An examination of the 20 mm. reconstruction (fig. 12), which
includes the distribution of the spinal nerves of the tail region
and two nerves from the neighborhood of the anus, disclosed an
arrangement of the motor and sensory components which is
directly comparable in many respects to the primitive disposi-
tion found in Petromyzon and Amphioxus.   It is clear from
this reconstruction, that for the two most cephalic spinal nerves
(shown here) to attain adult conditions, the ventral motor rami
($R.V.M.$), which traverse the inner surfaces of their respective
myotomes about equidistant between two sensory rami ($R.V.S.$),
must sometime in their future join their following sensory rami.
In like manner the first two or three ventral motor rami in the
caudal portion of the reconstruction will have to unite with their

more cephalic sensory rami, while some of the extreme caudal
ventral motor rami will ultimately cross over their more cephalic
sensory rami without exchanging any fibers.

The following additional points may be recorded for the spinal
nerves of the 20 and 27 mm. embryos: (a) As in the adult, most
of the spinal nerves have two motor and one sensory roots.
They immediately penetrate a mass of mesenchyme which will
later form the neural arches. Up to this time the dorsal and
ventral rami have been shifted little, if any, caudad, through a
more rapid growth of the myotomes and skeletal elements over
the central nervous system. (b) It is evident from figure 12
that there is a gradual increase in the development of the spinal
nerves in passing cephalad. The last nerve is represented simply
by a mass of neural crest cells. The next to the last nerve has
an irregular-shaped ganglion from which a few central processes
have entered the dorso-lateral surface of the spinal cord as the
dorsal or sensory root. The second from the last nerve consists
of a spinal ganglion, a sensory root, and a single motor root
which has separated into a short dorsal ramus and a longer
ventral ramus. The third from the last spinal nerve shows
some progress over the second. Its dorsal and ventral motor
rami have increased in length and fibers are leaving the ventral
pole of the spinal ganglion to form the ventral sensory ramus;
while in the fourth from the last spinal nerve we have the
first appearance of a dorsal sensory ramus. From this point
cephalad, for a distance of several segments, there is a gradual
increase in length of all the various rami, taking place after the
following order—ventral motor ramus, dorsal motor ramus,
ventral sensory ramus and dorsal sensory ramus. It will be
seen that this order also represents the order of their embryonic
appearance. (c) Of the various ventral motor rami which will
cross the M. cordis caudalis, the second, third, and possibly the
fourth are somewhat longer than several of the preceding motor
rami. Opposite the caudal heart they bend inward to enter a
proliferation of cells (M.C.C.), which probably represents myo-
blasts for the future M. cordis caudalis. (d) A somewhat later
stage (fig. 15) shows a conspicuous dorsal and ventral growth of

the myotomes and a corresponding lengthening of the spinal nerves. The ventral motor and sensory rami have made no progress toward approaching each other.

While it is to be regretted that the actual stages are lacking when the ventral motor and sensory rami would be seen to approach and unite with each other, nevertheless, such a change must take place in the great majority of the spinal nerves somewhere in embryos between 27 and 57 mm. in length. When compared with the adult it was shown that each of the more cephalic ventral motor rami joined the next following ventral sensory ramus; that a few in the region of the cephalic end of the M. cordis caudalis probably migrated cephalad, counter to the course of the growing myotomes which would tend to carry them backward, and joined their more cephalic sensory rami; while some seven or eight of the most caudal ventral motor rami not only grew cephalad against the force of the growing myotomes, but continued to grow some distance cephalad of their corresponding sensory rami to end in the myotomes. I am unable to offer a satisfactory explanation why the motor rami of the last spinal nerves should grow past their more cephalic sensory rami to terminate in the myotomes in front, unless perchance it can be attributed to the myotomes of this particular region offering a particularly strong chemotropism for these motor rami. It cannot be due to their additional connections with the M. cordis caudalis, for a number of the motor rami behind this muscle have an identical distribution.

4. Adult and embryonic conditions in Squalus acanthias

Since my Polistotrema material did not permit of a complete analysis of the mode of union of the ventral motor and sensory rami, it seemed advisable for this and other reasons to investigate the origin of the various spinal nerves in some selachian. A dissection was made of some of the lower abdominal spinal nerves of a fetal shark at birth to obtain an adequate understanding of the various motor and sensory components of an adult spinal nerve, with which embryonic comparisons could be made. The following description is taken from this dissection.

It is evident from figure 23 that the distribution of the motor and sensory components of the spinal nerves of an adult Squalus differs in a number of details from other vertebrates. As far as could be determined each spinal nerve has but one ventral or motor root $(V.R.)$, which may take origin from several rootlets, arising from the ventro-lateral surface of the spinal cord, a little in front of the spinal ganglion of this nerve. The dorsal or sensory root fibers $(D.R.)$ upon leaving the dorsal poles of their respective ganglia, immediately enter the neural arch about on a level with the dorsal surface of the spinal cord, and without pursuing a cephalic course within the neural canal terminate in the dorso-lateral surface of the spinal cord. Immediately after leaving the neural arch a motor root separates into a dorsal and a ventral ramus. The motor ramus dorsalis $(R.D.M.)$ pursues at first a general caudal course and after crossing the lower caudal surface of its ganglion, is joined by the sensory ramus dorsalis fibers $(R.D.S.)$, which take exit from the lower caudal surface of the ganglion (instead of from the dorsal pole as in cyclostomes). The mixed ramus dorsalis, thus formed, in place of following its corresponding intermuscular septum, continues caudad opposite the neural arch for a distance of a segment, to the foramen for the motor root of the following nerve, where it bends obliquely dorsad and crosses the upper outer surface of the succeeding spinal ganglion, to assume a general dorsal caudal course along the inner surface of the second following intermuscular septum, which is one segment behind the one it would follow in most vertebrates. This peculiar disposition of the ramus dorsalis in Squalus recalls a somewhat different arrangement found in some bony fishes, where the ramus dorsalis is formed from a sensory filament of its own nerve and a motor branch from the preceding nerve. The motor ramus ventralis $(R.V.M.)$ or ventral continuation of many of the motor root fibers takes a general ventro-caudal course across the skeletal axis and, when part way across, is joined by the sensory ramus ventralis $(R.V.S.)$, which fibers arise from the ventral pole of its ganglion. The course of the mixed ramus ventralis $(R.V.)$ is then along the inner surface of the following intermuscular septum.

Notwithstanding that the early developmental stages of the spinal nerves of Squalus have been carefully worked out by Balfour, Onodi, Hoffmann, Neal, and Scammon, it has seemed best to include the early developmental history along with the later. After examining a number of early Squalus series, two graphic reconstructions were made of several spinal nerves of the lower and middle abdominal regions of a 7.5 mm. Squalus embryo. It is clear from a comparison of figure 17 with 16 that the spinal nerves of Squalus present no exception to the general rule, that a given segmental organ or structure tends to become more embryonic in passing from a cephalic to a caudal direction.

In figure 16 the spinal nerves of the lower abdominal region will be seen to be in a very embryonic condition. It is evident that the motor or effective portion of the spinal nerves show considerable advancement over the sensory or receptive portions in so far as development of fibers is concerned. Ventral or motor root fibers (*V.R.*) together with a liberal amount of cells destined to become neurilemma have grown out from the ventro-lateral surface of the spinal cord, to extend laterally as far as the myotomes and then to bend ventrally and follow the inner surface of the myotomes for a short distance as the motor rami ventrales (*R.V.M.*). In one spinal nerve a few motor ramus dorsalis fibers (*R.D.M.*) branched off from a motor root or motor ramus ventralis to extend dorsally a short distance along the inner surface of the myotomes. The sensory or receptive system is represented in this figure by a continuous band of neural crest cells (*Neu.C.*), which follows the dorso-lateral surface of the spinal cord. At regular intervals the neural crest sends out ventral extensions or proliferations of cells. They represent the anlage of the spinal ganglia and their most distal portions, the beginnings of the sensory rami ventrales (*R.V.S.*), which at first consist largely of cells. It is apparent that each of these ventral prolongations of the neural crest migrated ventrally between two motor roots, coming into closer relationship with the more cephalic one and its ventral ramus. In this region the spinal ganglion cells showed no peripheral processes, although it is possible that silver or methylene blue

preparations would have revealed central processes extending
into the spinal cord.

A reconstruction of two of the more cephalic spinal nerves
(fig. 17) shows considerable advancement of all of the spinal
nerve components. Thus far only one ventral or motor root
($V.R.$) has appeared for each nerve. It contains a relatively
large number of fibers. The motor rami ventrales ($R.V.M.$)
have grown ventrally along the inner surface of the myotomes
nearly to the level of the dorsal aorta. They are composed
mainly of fibers lined with embryonic neurilemma cells (figs.
19 and 24) which migrated out of the neural tube with the fibers.
Motor rami dorsales fibers (figs. 17 and 24, $R.D.M.$) have
branched off from all of the motor roots or motor rami ventrales
and have grown a short distance dorso-caudad between the
lower lateral surface of the spinal ganglia and the myotomes.
The sensory or receptive portion of the spinal nerves have
likewise made considerable progress over the condition shown
for the more caudal nerves. The neural crest still persists as
a continuous mass of cells extending along the dorso-lateral
surface of the spinal cord, although the dorsal border of the neural
crest will be seen to be considerably lower and that portion be-
tween the ganglia is reduced to a narrow strip of cells. Numer-
ous central and peripheral processes have grown out from the
spinal ganglion cells. The central processes leave the dorso-
median surface of the ganglia to enter the dorso-lateral surface
of the spinal cord, constituting the dorsal or sensory roots (figs.
17 and 19, $D.R.$) of the spinal nerves. Some of the peripheral
processes pass out of the ventral poles of their ganglia for short
distances into a ventral migration of neural crest cells and form
the sensory rami ventrales (figs. 17 and 19, $R.V.S.$). These
rami, which at this stage are composed almost entirely of neural
crest cells, assume at first a more caudal and median course than
the corresponding motor rami, but come in contact with the
motor rami about on a level with the central axis of the noto-
chord. Their course is then nearly ventrad along the inner sur-
face of their respective motor rami, from which they can be
readily distinguished (fig. 19, $R.V.M.$ and $R.V.S.$) by the fact

that the motor rami are composed of fibers, bordered by a few embryonic neurilemma cells; while the sensory rami, especially their distal extremities, are made up almost entirely of cells. It will be seen from an examination of figure 17 that the cells of the sensory ventral rami have extended some little distance ventrad of the fibers of the ventral motor rami and that they have accumulated in a mass opposite the dorsal aorta, which undoubtedly represents the anlage of a vertebral sympathetic ganglion. This reconstruction of the spinal nerves of a 7.5 mm. Squalus certainly supports Onodi's hypothesis that sympathetic ganglia are formed of cells migrating from the spinal ganglia. It is highly improbable that the few scattered cells on the outer surface of the ventral motor rami take any part in the formation of sympathetic ganglia. At this stage the anlage of the sympathetic ganglia will be seen to be not only median, but considerably below the level of the motor rami. The cells about the motor rami are probably concerned only in the formation of neurilemma and connective tissue coverings.

Figure 20, which is a graphic reconstruction of two spinal nerves from the lower abdominal region of a 19 mm. Squalus, presents the following points of developmental progress: (a) From a comparison with figure 17 it is evident that this advancement has been concerned largely with the increase in length of the nerve elements already formed. The motor rami dorsalis (*R.D.M.*) have crossed obliquely the outer surface of their ganglia and have grown along the inner surface of the myotomes in a general dorso-caudal direction to the region of the dorsal pole of the ganglion of each succeeding spinal nerve. The motor and sensory rami ventrales (*R.V.M.* and *R.V.S.*) have extended caudo-ventrally along the inner surface of the myotomes to the level of the caudal vein and the Wolffian duct. They have remained entirely separate as far as the lower margin of the dorsal aorta, with the exception that for a short distance in the region of the upper surface of the aorta they have come in contact with each other, but are prevented from intermingling by a layer of connective tissue cells or neurilemma. At the level of the lower border of the dorsal aorta the motor and sensory rami ventrales

join in forming short mixed rami ventrales (*R.V.*). (b) A conspicuous cluster of cells, the anlage of a vertebral or chain ganglion (figs. 20 and 34, *Sy.G.*), is present on the direct course of each sensory ramus ventralis, opposite the dorsal aorta. In figures 20 and 34 sensory fibers (*R.V.S.*) presumably having their cell bodies in a spinal ganglion will be seen leaving a sympathetic ganglion to join with the fibers of a corresponding motor nerve in forming a mixed ramus ventralis. As was stated for an earlier reconstruction (fig. 17), it is difficult to see how in Squalus many, if any, of the cells of neural tube origin found along the outer surface of the motor rami ventrales would take any part in the formation of sympathetic ganglia. In this reconstruction it is of interest to recall the case of Petromyzon, where the motor and sensory rami ventrales are always separate, in which Julian ('86) and ('87) found sympathetic cells about the dorsal aorta, at the end of the rami communicantes, which are branches from the sensory rami ventrales. From this arrangement in the ammocoetes stage it is reasonable to suppose that these sympathetic cells had their origin from the neural crest and migrated to the aorta along the course of the above mentioned nerves. It must be admitted that it is no easy task to reconcile the origin of purely excitatory or effective sympathetic cells from the neural crest, a supposedly purely receptive area. Concerning the vertebral, prevertebral, and effective peripheral ganglion cells it is conceivable that they may have migrated out into the neural crest from an effective or motor area of the neural tube, or they may have been indifferent cells, or their function may have been modified. It would seem that the principal requirement for a spinal visceral effective or motor relay is that its primary cell in the gray matter of the spinal cord should take origin from the effective or motor area of the neural tube. (c) It is clear from figure 20 that each spinal nerve at this stage has two ventral or motor roots (*V.R.*). It was not determined whether this means the formation of additional fibers in another region or the splitting up of the original root fibers into two portions. (d) A comparison with figure 17 shows that the distal portions of the dorsal and ventral rami

have been carried some distance caudad, presumably through a greater growth of the myotomes in those regions. (e) Nothing in the way of a sensory ramus dorsalis has appeared in this stage.

A more advanced stage in the development of a spinal nerve is shown in a graphic reconstruction (fig. 21) of two upper abdominal spinal nerves from a 28 mm. Squalus. It is evident from this reconstruction that a number of important changes have taken place since the previous stage, and that the arrangements of the spinal nerves in this stage have much in common with the adult previously described. All of the various rami that were described in the previous reconstruction will be found to have increased considerably in length; this is especially true for the rami ventrales, which have nearly doubled in length. The motor and sensory rami ventrales ($R.V.M.$ and $R.V.S.$) have remained separated as far as the lower surface of the aorta or a little below, where they unite in forming mixed rami ventrales ($R.V.$), which can be traced along the inner surface of the myotomes or their intermuscular septa nearly to the floor of the abdomen. Figure 35 will disclose how sharply a layer of connective tissue or neurilemma separates the motor from the sensory rami ventrales fibers in the region of the dorsal aorta. As in the previous stage there are two ventral or motor roots ($V.R.$). The mode of origin and distribution of the motor rami dorsales ($R.D.M.$) is about the same as in the previous stage, with the exception that they have extended a little farther dorsad, nearly to the level of the upper surface of the spinal cord, and more important, after crossing the spinal ganglia they are accompanied by the sensory rami dorsales fibers, which took origin from the caudal surface of their respective spinal ganglia. Most of the sections show the motor and sensory portions of a ramus dorsalis to be separated by connective tissue cells, but in places these bundles come in contact so that it is impossible to distinguish them. It is of especial significance to note that the sensory rami dorsales left the caudal end of their respective ganglia at exactly the point where a motor ramus dorsalis crossed. Also at this stage the motor and sen-

sory rami dorsales fibers traverse the inner surface of the myo-
tomes along the intermuscular septum joining the first and the
second following myotomes.

In a reconstruction of two of the lower abdominal spinal
nerves of a 32 mm. Squalus (fig. 22) it will be seen that the ar-
rangement of the nerve components have reached practically
adult conditions. The most noticeable advance over the pre-
vious stage is an increase in length of the rami dorsales and a
pronounced caudal shifting of both the dorsal and the ventral
rami. In the dorsal rami this takes place much more abruptly.
It involves a movement of both the nerves and the myotomes
for a distance of a segment, and is probably caused by a more
rapid growth of the myotomes than of the skeletal and neural
axes. Even at this late stage the motor and sensory fibers in
both the dorsal and ventral rami remain separated for some dis-
tance. Each motor ramus dorsalis assumes at first a general
caudal course upon branching from the motor ramus ventralis,
and after crossing the outer surface of its ganglion, it is accom-
panied by sensory fibers ($R.D.S.$), arising from the caudal sur-
face of the ganglion at the point where the motor nerve crosses.
The two dorsal rami continue caudad, side by side, to a point
in front of the foramen for the following motor root, then bend-
ing dorsally, they cross somewhat dorsally the outer surface
of the succeeding spinal ganglion. Soon after leaving this
ganglion the two rami join in forming a mixed ramus dorsalis
($R.D.$), which continues in a general caudo-dorsal direction
along the inner surface of the corresponding intermuscular
septum, which has apparently shifted caudad for a distance of a
segment from its original embryonic position. Throughout the
proximal course of a ramus dorsalis, where sensory and motor
nerves were described as running side by side, they will be
found to be sharply separated by connective tissue or neuri-
lemma. Figure 26, which passes through the exit of the sen-
sory ramus dorsalis from the ganglion, shows very clearly its
relationship with the corresponding motor component, while a
more distal section (fig. 25) through the two rami shows them
to be well-separated and to lie in nearly the same horizontal

plane. In like manner a motor ramus ventralis ($R.V.M.$) approaches its corresponding sensory ramus ($R.V.S.$) from in front, a little below the level of its spinal ganglion, and the two pursue a parallel and general caudo-ventral course along the inner surface of the intermuscular septum, to a level with the dorsal aorta, where they unite in forming a mixed ramus ventralis ($R.V.$). Figure 27 discloses the relationship of the motor and sensory rami ventrales in transverse section on a level with the dorsal aorta. Apparently each spinal nerve has two ventral or motor roots within the neural canal, which may take origin from one or two rootlets. Upon leaving the neural canal these roots enter the two arms of a Y-shaped foramen and emerge outside as a single root, which soon separates into a dorsal and a ventral motor ramus.

Some of the important points of the previous section may be summarized as follows: (a) In a description of a reconstruction of several of the lower abdominal spinal nerves of a 7.5 mm. Squalus it was shown that while the neural crest was engaged in the formation of spinal ganglia, motor fibers and embryonic neurilemma cells had left the ventro-lateral surface of the neural tube and had grown laterally to the inner surface of the myotomes, there to turn ventrad a short distance as motor rami ventrales. From one of these nerves a few motor ramus dorsalis fibers were given off to the inner surface of the myotomes. Meanwhile the neural crest had proliferated and segmentally arranged portions of it had migrated ventrally between the motor roots, there to bend toward the more cephalic roots and motor rami ventrales as if by some attraction. These ventral proliferations of the neural crest represent the anlage ganglia and probably the beginnings of the sensory rami ventrales. (b) In the reconstruction of a later stage, taken from more cephalic nerves of the same series, it was noted that the sensory rami ventrales had made more rapid growth than the motor rami ventrales and had extended ventrally to the level of the dorsal aorta, where at their distal ends there was an assemblage of cells, representing, doubtless, the anlage of the vertebral or chain sympathetic ganglia. For the greater part

of their course the motor rami ventrales were situated cephalic and lateral to their corresponding sensory rami; they were readily distinguishable on account of difference in structure, the motor rami consisting of fibers bordered by a few embryonic neurilemma cells, and the sensory rami were composed almost entirely of neural crest cells. A short motor ramus dorsalis had branched off from each motor ramus ventralis to pass caudo-dorsad, between its ganglion and the myotomes. (c) Each spinal nerve possessed a dorsal or sensory root, which took origin from the dorsal pole of its respective spinal ganglion. The earliest embryos disclosed but one ventral or motor root for each nerve; while the later stages had two, some of which arose from more than one rootlet. In position the motor root or roots were always more cephalad than the corresponding sensory root. (d) Later stages were concerned mainly with the prolongation of the above mentioned elements of a spinal nerve, together with a further differentiation of a vertebral sympathetic ganglion on the course of each sensory ramus ventralis, a full discussion of which was given on p. 163. (e) The sensory rami dorsales were found to be very late in appearing, and it was of especial interest to record that they did not leave the ventral poles of their ganglia as in Cyclostomes, but took origin from the caudal surfaces of their ganglia at the intersection with the corresponding motor rami. (f) It will be seen that the order of appearance of the various branches of the spinal nerves is the same as in Polistotrema, namely, motor ramus ventralis, motor ramus dorsalis, sensory ramus ventralis, and sensory ramus dorsalis. (g) Up to embryos of 32 mm., where the various components of a spinal nerve are practically the same as in the adult, the motor and sensory elements have remained separated by connective tissue or neurilemma throughout a considerable part of the proximal portion of the rami dorsales and the rami ventrales. (h) Both the rami dorsales and the rami ventrales have been carried caudad for a distance of a segment as a result, probably, of a more rapid growth of the myotomes than of the skeletal and neural axes.

5. *Arrangement in the turtle and pigeon embryos*

From an examination of a reconstruction of a 6 and an 8 mm.
pigeon (figs. 31 and 32), a transverse section through a spinal
nerve of a 10 mm. turtle (fig. 33), and from series of chicks
and pigs it is apparent that the various motor and sensory
components of a spinal nerve lie in the same vertical plane.
None of the motor elements were found to be entirely sepa-
rated from the sensory as in Polistotrema and Squalus embryos.
It is possible that this stage is skipped in the higher vertebrates
or it may have been passed through so rapidly that it was missed
in my series. The latter view is supported by the fact that,
while the motor and sensory rami come in contact, they are
separated in so far as any exchange of fibers is concerned for
some distance peripherally, as is indicated by a difference in
structure, the sensory portion consisting mainly of cells and the
motor portion chiefly of fibers. Inasmuch as a study of the
development of the spinal nerves in Amniota can be of little
assistance in solving how the motor and sensory rami unite, a
detailed description of the above mentioned reconstructions is
unwarranted. It can be mentioned, however, that the various
rami of a spinal nerve have apparently a slightly different order
of appearance from cyclostomes and selachians. It will be
seen from the 6 mm. pigeon reconstruction (fig. 31) that the
sensory rami ventrales are well-formed before there is any
trace of motor rami dorsales. The latter are shown in the 8
mm. pigeon (fig. 32) to be formed about the same time as the
sensory rami dorsales. All of the peripheral sensory fibers leave
the ventral pole of their respective ganglia to separate imme-
diately into dorsal and ventral rami.

POSSIBLE CAUSES FOR THE UNION OF THE MOTOR AND SENSORY
SPINAL RAMI IN ALL VERTEBRATES ABOVE THE CYCLOSTOMES

From the previous section it is clear that in the simplest
vertebrates, Amphioxus and Petromyzon, the motor and sen-
sory components of a spinal nerve always remain separated,
and in the higher vertebrates from selachians up the sensory
and motor elements intermingle in embryonic life, forming mixed

rami. In the higher vertebrates it was found that the motor and sensory components of a spinal nerve unite about as soon as they are formed; while in selachians they remained separate for some little time in embryonic life. Between these two extremes, where the motor and sensory nerves never unite and those where they unite in embryonic life, Polistotrema forms a most interesting link. In this representative of cyclostomes it was noted that the motor and sensory components of the most caudal nerves and the motor and sensory elements of the dorsal rami always remain separate, while the motor and sensory elements of each cephalic ventral ramus join in forming a mixed ramus ventralis.

From the fact that the union of the motor and sensory components of the rami ventrales occurs before it does in the rami dorsales in selachians, taking place before the sensory portion of the dorsal rami are formed, and that the motor and sensory portions of the dorsal rami of Polistotrema never fuse, it is fair to assume that the union of the motor and sensory elements of the rami ventrales is phylogenetically older than the union of the motor and sensory components of the rami dorsales.

The first intermingling of the motor and sensory fibers of the rami ventrales was found to be below the level of the dorsal aorta in the most primitive (caudal) region of adult Polistotrema and in Squalus embryos. Also in the higher vertebrates, where the motor and sensory rami come in contact about as soon as they are formed, there is a considerable period of time in which there is no exchange of fibers in the rami ventrales down to a level with the aorta. The same is true for the motor and sensory components of the rami dorsales of Squalus; they may come in contact in several places, but the first intermingling of fibers is in the periphery, not far from the dorsal border of the myotomes. For the majority of vertebrates the generalization will hold that the union of the motor and sensory portions of a spinal nerve occurs at first in the periphery and then takes place gradually in a central direction.

As a rule in the embryo and in the simpler vertebrates, where the motor and sensory components of a spinal nerve were sepa-

rate, the sensory rami followed the intermuscular septa of the myotomes and the motor rami penetrated the inner surface of the myotomes. In Polistotrema it was evident that the ventral motor rami joined their corresponding sensory rami, irrespective of whether the motor rami were situated cephalad or caudad of their respective sensory rami, in all cases where such union takes place. In early Squalus embryos it was shown that the ventral motor rami were formed first and that there was a tendency for the ventral prolongations of the neural crest to migrate cephalad toward their corresponding motor rami. Also the sensory fibers for the dorsal rami left the caudal surface of their respective ganglia at exactly the point of crossing of their corresponding motor rami.

In reply to the question what causes bring about the union of the original separate motor and sensory spinal nerves into a common mixed trunk, it can be said that there are probably several factors involved in producing this result, some of which are mechanical, due to the mode of development of neighboring structures, while others may be attributed to a possible chemical attraction, chemotropism.

1. Mechanical factors.

The most obvious is doubtless due to the rapid and continuous growth of the myotomes, which is much more pronounced than that of the skeletal or neural axis. The general effect of this rapid growth of the myotomes is to crowd the muscle plates backward, which is most noticeable in the more caudal segments and more pronounced in the dorsal and ventral portions than the central portion of any given muscle plate. As a result of the general shifting backward of the muscle plates the spinal nerves have been carried along with them. In this connection the following points in the development of the spinal nerves should be recalled: (a) At first the motor components of a spinal nerve are more closely connected with the myotomes than the sensory. (b) In most cases the motor rami are formed before the sensory. (c) The motor components of a spinal nerve are

ordinarily situated cephalad if their corresponding sensory
portion with which they later join in forming a mixed ramus.
Hence the more cephalic motor ramus will be exposed for a
longer period of time to this force which would tend to carry
both rami caudad, which factor might be sufficient to cause the
motor elements of the spinal nerves to approximate their corre-
sponding sensory elements. Furthermore, in the majority of
embryos, especially of the simpler vertebrates, the sensory
branches lie median to their corresponding motor branches.
Consequently the rapid growth of the myotomes on the outside
together with the increase of the connective tissue within
would tend to bring the motor and sensory portions of a nerve
nearer.

Another mechanical factor which might contribute to the
approximation of the motor and sensory branches of a spinal
nerve is the more rapid and in some cases a more continuous
growth of the skeletal than the neural axis, which would neces-
sarily carry both the motor and sensory roots caudad. Since
the motor roots are formed first and are more cephalic in posi-
tion, they would be subjected longer to this force which would
tend to carry both roots caudad, and as a result the motor roots
should approach their corresponding sensory roots.

2. Chemical attraction (chemotropism)

That there may be such a mutual attraction between the
sensory and motor components of a spinal nerve is suggested
by the following observations on the development of the spinal
nerves in various vertebrates: (a) Certain of the caudal ventral
motor rami (figs. 1, 3, 12 and 13, $R.V.M.$) in Polistotrema have
migrated cephalad, counter to the force of the growing myo-
tomes which would tend to carry them caudad, and have joined
their corresponding sensory rami ($R.V.S.$) in forming mixed
rami ventrales. (b) In the 7.5 mm. Squalus embryo it was shown
that the ventral prolongations of the neural crest, representing
the beginning of the spinal ganglia and the ventral sensory rami
(fig. 16, $Sp.G.$ and $R.V.S.$) have the appearance of being at-

tracted cephalad toward their corresponding motor rami. (c)
A later stage of Squalus (figs. 21 and 22) discloses that the dorsal
sensory rami (R.D.S.), which are formed considerably later
than the other branches of a spinal nerve, take their exit from
the caudal surface of their ganglia at exactly the point of cross-
ing of their corresponding motor rami (R.D.M.) instead of the
dorsal pole as in cyclostomes. That the dorsal sensory fibers
should be given off from this point of the ganglion rather than
elsewhere is at least suggestive of an attraction for the sensory
fibers, provided that other conditions are equal.

I am unable to explain why these previously described forces
should not be operative in Amphioxus and Petromyzon and
cause the various motor and sensory rami in these species and
the motor and sensory components of the dorsal and the caudal
ventral rami of Polistotrema to unite and form common mixed
trunks as they do in the higher vertebrates; unless in Amphioxus
and Petromyzon the relatively earlier appearance of the myo-
tomes would do away with a considerable caudal shifting of these
nerves. Also it is evident where a few of the caudal ventral
motor rami migrated cephalad across their corresponding sen-
sory rami to end in the myotomes in front, that there must be
much less attraction (chemotropism) between the sensory and
motor rami than there is between the myotomes and the motor
rami.

DISTRIBUTION AND POSSIBLE SIGNIFICANCE OF CERTAIN GAN-GLION CELLS ON THE COURSE OF THE SPINAL AND VAGUS NERVES OF POLISTOTREMA

Numerous investigators have shown that there are no well-
defined spinal ganglia in Amphioxus, but that scattered spinal
ganglion cells (figs. 28 and 29, N.C.) occur in the dorsal roots
and sensory rami from the spinal cord to the skin. From meth-
ylen blue preparations Retzius has described certain so-called
sympathetic cells in the periphery of the spinal nerves of Myxine
and these cells are figured as enclosed in a network of sympa-
thetic fibers. Julin ('86) and ('87) found a well-defined vertebral
sympathetic system about the aorta in 15 and 18 mm. Petro-

myzon. The vertebral ganglia were said to be in connection
with the ventral sensory rami by rami communicantes. John-
ston ('05) and ('08) described and figured a vertebral sympa-
thetic trunk in the branchial region of Petromyzon, which takes
origin from the facial nerve and has connections with the vagus.
This nerve is composed of fine fibers and said to have sympa-
thetic nerve cells in the gill region, the peripheral processes of
which probably supply the neighboring blood vessels, lymph
sinuses and other visceral surfaces. Johnston did not find the
vertebral sympathetic ganglia as described by Julin, but found
one, and only one, peripheral ganglion cell in close relationship
with a ventral spinal nerve root. Numerous peripheral ganglion
cells were described and figured in the head region, some of which
were multipolar, but the majority were bipolar. They were
in relationship to the V, VII, IX, and X nerves. No vertebral
sympathetic ganglia and cord have been described for the
myxinoids. A continuation of the vagus nerves to the viscera
is represented by J. Müller and Cole as a sympathetic trunk in
Myxine. The relationship of the vertebral sympathetic sys-
tem to the spinal nerves is too well known in the higher verte-
brates to need mentioning here.

1. Peripheral spinal nerve cells

As was pointed out earlier, nerve cells isolated and in clusters
appear peripherally in many of the spinal nerves. In the re-
construction of the two most cephalic spinal nerves (fig. 6) no
nerve cells were found other than the true spinal ganglion cells.
A spinal nerve in the pharynx region (fig. 5) contained a single
peripheral nerve cell (N.C.) situated in the ramus ventralis
close to the ventral border of the myotomes.

In the reconstruction of the spinal nerves of the tail region of
the 20 cm. series (fig. 1) there is a single peripheral nerve cell
(N.C.) located in the ramus ventralis passing between the last
two mucous sacs. In the second spinal nerve there is a nerve
cell (N.C.) in the dorsal sensory ramus at the base of a dorsal fin
ray. No peripheral nerve cells were found in the third, fourth

and fifth nerves.   There is a nerve cell (*N.C.*) on a caudal branch
of the sixth dorsal sensory ramus a little above the median
dorsal cartilaginous bar, midway between two dorsal fin rays.
No peripheral nerve cells were found in connection with the
seventh and eighth spinal nerves.   The dorsal sensory ramus
of the ninth nerve has a cluster of three peripheral nerve cells
opposite the base of a dorsal fin ray, and the caudal branch of
the dorsal sensory ramus of the tenth nerve has two cells oppo-
site the median dorsal bar and one cell some distance out in
the dorsal fin.   Numerous scattered nerve cells appear in a
a plexus of sensory nerves that enter the last two spinal ganglia.
These cells are more abundant in the dorsal, than in the caudal
or ventral nerves.   As was noted previously, there is one more
sensory nerve (sensory ramus dorsalis) on the opposite or left
side of this specimen.   This nerve is shown in figure 2 as having
no well-defined spinal ganglion, but it has a number of isolated
nerve cells (*N.C.*) scattered along its course; some six or more
are outside the neural arch and one within it, suggesting some-
what the arrangement found in Amphioxus.

A reconstruction (fig. 4) of two spinal nerves from the region
of the posterior extremity of the caudal heart from another adult
Polistotrema series shows a number of scattered nerve cells
about the dorsal sensory rami in the neighborhood of the median
dorsal bar.   They are more numerous on the first ramus than
on the second.   In addition to the above mentioned cells, sev-
eral nerve cells are clustered about a cephalic branch of the first
dorsal sensory nerve; which branch runs in close proximity to
a blood vessel in the connective tissue of the dorsal fin.   No
nerve cells were found in the ventral sensory rami of either of
these nerves.   Attention should be directed to the fact that the
cells in the dorsal pole of the spinal ganglia of these nerves
(fig. 4, *Sp.G.*) are very diffuse and scattered as compared with
the cells of the central and ventral portions of the ganglia.
This arrangement of cells in the dorsal pole of the ganglia indi-
cates that the above mentioned peripheral nerve cells in the
dorsal sensory rami must have migrated peripherally from the
neural crest, suggesting a tendency in certain spinal nerves to

repeat in part the condition found in more primitive forms, as for example Amphioxus.

In a transverse section (fig. 10) taken several segments behind the above reconstruction a considerable number of nerve cells (*N.C.*), enough to constitute a ganglion, are located on the sensory ramus dorsalis, immediately dorsal and lateral to the myotomes. In these haematoxylin preparations the peripheral nerve cells did not differ from the ordinary spinal ganglion cells.

A reconstruction of seven spinal nerves in the caudal heart region of an 85 mm. Polistotrema embryo (fig. 3) shows a cluster of nerve cells (*N.C.*) on the peripheral course of the first, second, third and fifth dorsal sensory rami, adjacent to the median dorsal bar, and the second dorsal sensory ramus has in addition a nerve cell in the dorsal fin region. The ventral rami of these nerves, in contrast, possess only one peripheral nerve cell, which is situated in the last ventral sensory ramus opposite the notochord.

The following generalizations can be made for certain peripheral ganglion cells found along the course of the spinal nerves of Polistotrema. (a) Nerve cells appear isolated or in clusters along the peripheral course of many spinal nerves (b) It is uncommon to find a peripheral nerve cell in a ventral sensory ramus or a mixed ramus ventralis. Out of twenty-four typical rami ventrales reconstructed, if the last two nerves are eliminated, but three peripheral nerve cells were found, and no nerve contained more than one cell. One of these cells was situated opposite the notochord and the other two were about on a level with the lower border of the myotomes. (c) None of the cephalic sensory rami dorsales reconstructed possessed any peripheral nerve cells; while in the caudal region they are common, nevertheless over one-half have no cells. (d) In the region of the caudal heart the most common place for the peripheral nerve cells in the dorsal sensory rami is opposite the median dorsal bar; they frequently occur above the dorsal border of the myotomes. (e) Scattered nerve cells are found everywhere in the plexus of nerves ending in the last two spinal ganglia. Their distribution suggests the arrangement found in

Amphioxus. (f) From ordinary haematoxylin preparations the peripheral nerve cells have the appearance of being bipolar, resembling in every particular the true spinal ganglion cells. (g) The distribution of these peripheral ganglion cells in the adult and late embryo, and the fact that the most dorsal cells of a spinal ganglion are more diffuse than the central or ventral cells suggest that the peripheral cells had migrated from the original neural crest.

The writer would regard the arrangement of the scattered peripheral nerve cells in the caudal sensory nerves, the isolated and clustered peripheral cells in some of the more cephalic nerves of Polistotrema as representing a tendency to repeat the scattered disposition of the spinal ganglion cells found in the simpler vertebrates of which Amphioxus is a type. This is supported by the fact that in the simplest or most generalized part of the nervous system of Polistotrema, namely, in the extreme caudal portion, the peripheral nerve cells are abundant throughout the course of the sensory rami, while they are collected in small masses further cephalad, and still further cephalad in the abdominal region, where the central nervous system becomes most specialized, only an occasional isolated nerve cell is to be found along the course of some of the sensory nerves. This scattered arrangement of the nerve cells throughout the spinal nerves of Amphioxus and some of the nerves of Polistotrema is doubtless very old phylogenetically, dating back, possibly, to a still more diffuse arrangement of nerve cells found in some of the invertebrates.

The opposing view, which receives less support, would regard these peripheral nerve cells in the spinal nerves as the anlage of the sympathetic system. If this conjecture is true, the peripheral migration of the cells should be more pronounced in the trunk region than in the tail region of Polistotrema, and would take place in the ventral rami rather than in the dorsal; while, as a matter of fact, this migration of nerve cells takes place mainly in the dorsal rami in Polistotrema. It is very doubtful if a relayed visceral or sympathetic system occurs in the trunk region of Amphioxus and Polistotrema, although continuous visceral

fibers probably carry on the function of a sympathetic system to the peripheral blood vessels and glands. The true spinal-sympathetic system of the higher vertebrates may arise as a survival and further modification of this primitive migration of the neural crest cells, or the neural crest may later in phylogeny assume a similar but an entirely independent migratory process along very different channels.

2. Vagus nerve cells

An examination of a reconstruction of the vagus-glossopharyngeal nerve trunk immediately after it leaves the skull discloses several nerve cells (fig. 6, N.C.) scattered through it. These cells have a tendency to be collected immediately cephalad of the branching off of the glossopharyngeal nerve. Transverse sections through this region show no grouping of the nerve fibers into large bundles as is characteristic for lower levels. Nerve cells appear in all parts of this common trunk, but no section had more than two cells and there were no cells in the majority of the sections. A few cells (figs. 6 and 9, N.C.) were found in the glossopharyngeal nerve at the point of its separation from the vagus. Their arrangement and abundance are about the same as in the common trunk.

It will be seen from transverse sections (figs. 7 and 8) and a reconstruction (fig. 5, X.) of the vagus nerve in the region of the caudal extremity of the mandibular retractor muscles that the nerve fibers have been separated into four bundles. Isolated nerve cells are scattered throughout the three most dorsal bundles, while these cells have accumulated in sufficient numbers in the ventral bundle to suggest an elongated ganglion. From one or two cells in the cephalic and caudal sections of the ventral bundle, these cells increase in number centrally until some ten or fourteen cells will be seen in the most central sections (fig. 8).

It is apparent then that all of the bundles of the vagus and glossopharyngeal nerves contain receptive or sensory fibers, unless perchance some of these cells represent effective sympathetic relays. Likewise all of these bundles may contain ef-

fective or motor fibers. It is also apparent from figure 5 that branches from the ventral bundle of the vagus innervate the immediate region. Dorsal branches, rami pharyngei or oeso-phagei (fig. 5, *R.Oes.*) pass dorsally at first along the outer surface of the M. constrictor pharyngis, then bend caudally to follow the dorso-lateral surface of the oesophagus or pharynx. Ventral branches (fig. 5, *R.M.C.P.* (*1*)) leave the ventral bundle to supply the posterior division of the M. constrictor pharyngis and the connective tissue outside. No connections were noted between the spinal nerves and the vagus as has been described by Johnston ('05) for Petromyzon.

From ordinary haematoxylin preparations the nerve cells in the vagus nerve have every appearance of being bipolar, having probably one peripheral and one central process. Although no silver or methylen blue preparations were available, the writer is unable to see any reason for regarding these cells as sympathetic cells. Apparently we have in the vagus and glosso-pharyngeal nerves in Polistotrema the same tendency for the nerve cells of the neural crest to migrate peripherally as in the spinal nerves. This may represent a partial repetition of a more primitive condition, which possibly dates back to the diffuse condition of nerve cells found in some invertebrates. Parts of this system likely survive in the higher vertebrates to become differentiated into the sympathetic system, while other cells are collected in the head region as the cranial ganglia.

SENSORY AND MOTOR INNERVATION OF THE M. CORDIS CAUDALIS

From an examination of figures 1, 3, 4, and 12 it is evident that the nerve supply for the pulsating muscle for the caudal heart, the M. cordis caudalis, is from certain of the neighboring spinal nerves. It will be seen from these reconstructions that more than one nerve takes part in supplying this muscle, that there is considerable variation in the arrangement and branching of these nerves, resulting in some irregularities in the mode of innervation of the M. cordis caudalis.

In figure 1 the motor and sensory components of at least five spinal nerves pass between the myotomes and the M. cordis

caudalis. The first three differ in several respects from the last two; they unite in forming mixed rami ventrales and doubtless take part in the motor and sensory supply of the M. cordis caudalis. Soon after this junction takes place two branches from the first and a cephalic branch from each of the second and third nerves pass ventrally close to the surface of the M. cordis caudalis. Each of these nerves divides into a cephalic and a caudal branch. These branches unite with each other in forming a continuous longitudinal nerve, which lies close to the cephalic ventro-lateral surface of the M. cordis caudalis, and from which several branches ($R.M.C.C.$) enter the muscle. This longitudinal nerve has connections also with the first ramus ventralis in front of the M. cordis caudalis. It is drawn mainly in black in figure 1 as if it were a motor nerve, but it doubtless contains sensory fibers as well, which probably supply some eight or more muscle spindles ($M.S.$), situated on some of the large muscle bundles ($L.M.B.$) belonging to this muscle. The motor and sensory ventral rami of the last two spinal nerves passing between the myotomes and the M. cordis caudalis not only remain separate throughout, but follow the contour of the myotomes rather than the M. cordis caudalis and apparently take no part in the innervation of the latter. The motor ramus ventralis of the first spinal nerve behind the M. cordis caudalis is of especial interest in this specimen because it gives off a conspicuous branch ($R.M.C.C.$), which traverses nearly the entire length of the inner surface of the M. cordis caudalis and probably furnishes a large part of its motor supply.

Some variation occurs in the distribution of the spinal nerves to the M. cordis caudalis in the 85 mm. embryo (fig. 3) from the previous description of the 20 cm. specimen (fig. 1). There is a possibility of at least an equal number of spinal nerves taking part in its innervation. The ventral motor and sensory rami of each of the spinal nerves destined to pass between the myotomes and the M. cordis caudalis remain separate throughout in the 85 mm. embryo, with the exception to be noted for the first nerve. In this nerve the motor and sensory ventral rami unite at the level of the caudal artery and form a mixed

ramus ventralis. Branches from the first ramus ventralis and the second ventral motor ramus join in forming a short longitudinal nerve, which is situated close to the cephalic ventrolateral surface of the M. cordis caudalis, and from which branches (*R.M.C.C.*) are given off to the muscle. It is likely that this longitudinal nerve receives sensory fibers from the first ramus ventralis, which may supply muscle spindles that are probably present in the M. cordis caudalis, but which were not differentiated with the stain used. In like manner branches from the third and fourth ventral motor rami formed a more caudal longitudinal nerve situated close to the lateral surface of the M. cordis caudalis. The fifth ventral motor ramus is continued cephalad for some distance, at first along the outer surface and then the inner surface of the M. cordis caudalis, apparently furnishing the muscle with a large part of its motor supply. This nerve is directly comparable with a similar nerve described for the 20 cm. specimen. The sixth ventral motor ramus ends in an isolated portion of the M. cordis caudalis, which at this stage has not joined the main mass.

Enough of the M. cordis caudalis (*M.C.C.*) has been reconstructed from another adult Polistotrema (fig. 4) to show that there is no conspicuous cephalic branch from one of the last ventral motor rami destined to supply a large portion of the M. cordis caudalis as was described for reconstructions 1 and 3. The next to the last ventral motor ramus passing between the myotomes and the M. cordis caudalis sends off a short caudal branch (fig. 4, *R.M.C.C.*), which follows the outer surface of the M. cordis caudalis for a short distance and apparently innervates it.

In a reconstruction of the caudal heart region of a 20 mm. Polistotrema embryo (fig. 12), where the M. cordis caudalis is represented by a mass of myoblast derived from the embryonic myotomes, two or three of the ventral motor rami, which are longer than the others, have turned medially and entered the M. cordis caudalis myoblast as if attracted by it.

Muscle spindles

As has been noted previously, there are eight or more muscle or neuro-muscle spindles (fig. 1, *M.S.*) in the ventro-cephalic end of the M. cordis caudalis of the 20 cm. Polistotrema, immediately outside the ventral veno-lymphatic trunk. This part of the muscle is composed mainly of large muscle bundles similar to those of the myotomes. Further cephalad there is a suggestion of muscle spindles in certain of the constrictor muscles of the mucous sacs, and a study of the specialized mandibular muscles might reveal their presence. This series happened to be deeply and favorably stained for bringing out the spindle placques so that the non-appearance of muscle spindles in the other more lightly stained series probably signifies undifferentiation rather than absence. Unfortunately silver and methylen blue preparations were not available for this study.

Muscle sense endings have been described for most vertebrates, but so far as known Polistotrema (Bdellostoma) is the lowest vertebrate in which muscle spindles have been found. Huber and DeWitt ('00) quote Pansini as finding neuro-tendinous end-organs in the bony fish Hippocampus and in a selachian. Johnston ('08) has described free endings in the intermuscular septa in connection with the dorsal spinal nerves of Petromyzon. If a cerebellum is absent in Polistotrema, as is usually maintained, muscle sense must be correlated entirely in the spinal cord, brain stem, and cerebral hemispheres.

Ordinarily the muscle spindles of the caudal heart muscle in Polistotrema are enclosed in a lamellated connective tissue sheath or capsule, which may be poorly developed or even absent in some cases. These spindles may occur centrally on the muscle fibers, but more often they are near their tendinous attachments. Portions of the spiral or annular nerve placque are usually present in longitudinal sections of these muscle spindles. A section through the cephalic end of the M. cordis caudalis of the 20 cm. Polistotrema series (fig. 14) shows three muscle spindles. No nervous elements are visible in the uppermost spindle. This spindle is composed of three muscle fibers (*M.F.*) surrounded by a well-developed lamellated connective

tissue capsule. In the middle spindle which happens to be cut
longitudinally, three muscle fibers and a portion of the spiral or
annular placque (*Pl.*) are represented in figure 14 as they appeared
in the section, while the connective tissue capsule (*Cap.*) is
seen to be poorly developed. The lowermost spindle is cut
transversely through its distal end. Its tendinous bundle
(*Ten.*) took the haematoxylin stain strongly and is enveloped
in a conspicuous lamellated connective tissue sheath.

REMARKABLE SIZE OF CERTAIN MOTOR FIBERS IN THE SPINAL NERVES

Certain structures, which were at first taken to be peripheral
ganglion cells, appeared in transverse section of the rami ven-
trales of the Polistotrema from which figure 5 was reconstructed.
Upon closer examination no nuclei were found in these struc-
tures, and they are evidently the axones of certain large motor
cells in the spinal cord. In order that a direct comparison of
the size of these fibers might be made with the largest fibers of
other nerves, figure 18 was constructed, using a magnification
of 100 diameters in each case. The above mentioned colossal
fibers appear in transverse (fig. 18, *B.*), from which it will be seen
that these fibers are enormous when compared to the largest fibers
of the vagus and glossopharnygeal nerves (fig. 18, *A. and C.*).
In figure 18 a direct comparison can also be made of the size of
the largest fibers (*D.*) from the ramus ventralis of the spinal
nerve reconstructed in figure 4 with the much smaller sensory
fibers (*E.*) from the sensory ramus dorsalis of the same nerve

In turning to the literature on this subject it was of especial
interest to find that Johnston ('08) had called attention to the
large size of the few fibers found in the ventral motor rami of
the spinal nerves of Petromyzon The enormous caliber of
these nerve fibers is attributed by Johnston to the fact that,
since there are relatively very few fibers in a ventral motor
ramus, each fiber must necessarily supply a great number of
muscle fibers.

What significance then can be attached to the enormous
caliber of the motor spinal nerve fibers in Polistotrema? Appar-

ently Polistotrema agrees with Petromyzon in that the size of
the motor fibers is in direct proportion to the number of muscle
fibers each must supply.   Also the following observations point
to the fact that some of the large motor fibers are the axones of
the giant Müllerian cells of the spinal cord: (1) The series from
which reconstruction 5 was made was stained rather deeply
with iron hæmatoxylin to bring out the nerve fibers in the cen-
tral nervous system.   As a result both the large motor fibers
of the spinal nerves and the giant or Müllerian fibers of the
central nervous system were stained a much deeper brown than
the sensory axones.   (2) Upon examining sections of different
levels of the spinal cord it was found that the number of giant or
Müllerian cells and fibers became greatly reduced in the tail
region in direct proportion apparently to the reduction of the
body musculature.   In a region behind the caudal heart there
were so few Müllerian fibers that it was possible to make a series
of reconstructional drawings of a given cell and its fiber.   The
cell selected was situated in the ventral border of the flattened
gray matter about equidistant from the central canal and the
lateral surface of the spinal cord, in a region which is undoubt-
edly somatic motor or effective.   This cell had at least six very
large paired processes.   Two lateral processes, evidently den-
drites, were followed for short distances dorsally and ventrally
into the gray matter.   Two ventral dendrites traversed the
ventral white matter to the ventral surface of the cord.   Of the
two median processes, a dendrite passed through the ventral
white matter nearly to the center of the cord, while the remain-
ing process or neurite assumed a general caudal course in the
white matter, about midway between the central canal and the
lateral surface of the cord.   Throughout its course through 48
sections of 15 microns thickness it became gradually reduced
in caliber and assumed a more ventral and lateral position.
When last seen it was a little median of some ventral root fibers,
which it probably joined.   In tracing out this fiber a second
giant cell was observed.   Its position was more dorsal than the
first, being located in the border zone of the dorsal gray and white
matter.

SUMMARY AND CONCLUSIONS

The more important points of this paper may be summarized as follows:

1. Concerning the manner of distribution of the sensory and motor components of the spinal nerves, Polistotrema furnishes an interesting intergradation between the simple arrangement found in Amphioxus and Petromyzon, where the sensory and motor components of the spinal nerves were distributed as separate rami, and the more specialized condition found in the higher vertebrates, were these components joined in forming mixed nerves. In Polistotrema all of the dorsal and all of the most caudal ventral motor and sensory spinal nerve fibers were distributed in separate motor and sensory rami, while the ventral motor and sensory components of all but the extreme caudal spinal nerves united in forming mixed rami ventrales. A few of the last spinal nerves presented some irregularities in the way of branching.

2. For a considerable period in embryonic life the motor and sensory fibers of all the spinal nerves of Polistotrema and Squalus passed to the periphery in separate motor and sensory rami. The writer agrees with Onodi that the vertebral sympathetic ganglion cells of Squalus are derived entirely from neural crest cells, which migrated along the ventral sensory rami at a time when they were separated from their corresponding motor rami. The few cells which passed out of the neural tube with the ventral root fibers are apparently concerned only with the formation of neurilemma.

3. In rather late embryos of Polistotrema and Squalus there are always two ventral roots for each spinal nerve, which receive fibers from several rootlets. In Polistotrema these roots emerge from the membranous neural arch through separate foramina.

4. There is considerable variation in different vertebrates as to the place of exit of the central and peripheral processes of the spinal ganglion cells. In most vertebrates the dorsal root fibers leave from the dorsal pole of their respective ganglia,

while in Polistotrema each dorsal root takes exit from the median side of its ganglion not far from the center. From the text it is clear that all of the ventral sensory rami fibers in all vertebrates leave their ganglia by way of the ventral pole, that the dorsal rami fibers in Polistotrema leave from the dorsal pole, that the dorsal rami fibers of Squalus leave from the caudal surface of their ganglia, and in the higher vertebrates the dorsal rami leave with the ventral rami fibers through the ventral pole of their ganglia. It appears, then, that there may have been a gradual shifting of the point of exit of the dorsal rami fibers from the dorsal surface of the ganglia in simple vertebrates to the ventral surface of the ganglia in the higher vertebrates, and that this change in the place of exit of the fibers took place around the caudal surface of the ganglia rather than around the cephalic surface.

5. The order of appearance of the various spinal nerve rami of Polistotrema and Squalus is as follows: motor ramus ventralis, motor ramus dorsalis, sensory ramus ventralis, and sensory ramus dorsalis. The last mentioned appears considerably later than the others in Squalus, not until after the motor ramus dorsalis had grown caudad across the lateral surface of its corresponding ganglion.

6. Two interesting observations were recorded in connection with the development of the spinal nerves in Squalus. First, a graphic reconstruction of several spinal nerves of an early Squalus embryo demonstrated that the ventral expansions of the spinal ganglia, representing the anlage of the ventral sensory rami and the vertebral sympathetic system, in passing between the motor roots and the ventral motor rami, approached the more cephalic motor components as if attracted by them. Second, a sensory ramus dorsalis always arises from the caudal surface of its ganglion at exactly the point of crossing of the corresponding motor ramus, with which it later forms a mixed ramus.

7. While there appears to be no exchange of fibers in my early turtle and pigeon embryos, the motor and sensory components of the spinal nerves were in no instance completely separated by

supporting tissue. It is possible that such a stage exists and is passed over so rapidly that additional material would be required to demonstrate it.

8. In all embryos where the motor and sensory elements of a spinal nerve formed mixed nerves, this union begins peripherally and proceeds centrally. In Polistotrema the motor components always join the sensory, irrespective of the position of the nerves.

9. The most obvious of the mechanical factors in the growth of the embryo which might contribute to the approximation of the motor and sensory spinal nerves is the more rapid and longer continued growth of the myotomes over the skeletal and neural axes. Since the motor components of an embryonic spinal nerve are situated in front of the sensory and are formed earlier, they would be subjected longer to the same force of the general shifting backward of the myotomes, and would in consequence be carried further caudad than the sensory nerves.

10. The possibility of the existence of a chemical attraction (chemotropism) between the embryonic motor and sensory spinal nerves was suggested from two observations. First, the reconstruction of several of the caudal spinal nerves of the 7.5 mm. Squalus embryo demonstrated that the ventral migration of the spinal ganglion cells and fibers to form the ventral sensory rami and the vertebral sympathetic system, assumed a cephalic direction in passing between the corresponding motor components, as if they were attracted by the more cephalic motor rami. Second, the dorsal sensory rami fibers of Squalus did not grow out of the spinal ganglia until some little time after the corresponding dorsal motor rami fibers had migrated caudally across the lateral surface of their ganglia, and when they finally appeared, it was from the caudal surface of their ganglia at the point of crossing of the dorsal motor rami, with which they later join in forming mixed rami dorsales.

11. Isolated or clustered nerve cells were found along the course of certain of the sensory and mixed spinal rami of Polistotrema. In the extreme caudal sensory nerves these cells were sufficiently numerous and scattered throughout the peripheral

course of the nerves to recall the arrangement found in Amphi-
oxus. In the region of the caudal heart clusters of these cells
were present in the dorsal sensory rami between the myotomes
and the median dorsal cartilaginous bar or at the point of branch-
ing of the nerve above the myotomes. Elsewhere not more than
one cell was found along the course of a sensory or a mixed spinal
nerve. These isolated cells were situated almost anywhere on
the main stem or branch. They appeared more frequently on
the dorsal rami than on the ventral, and very few of the abdomi-
nal or thoracic spinal rami possessed a peripheral nerve cell.
A tendency was noted for some of the spinal ganglion cells to
migrate a short distance into the nerve trunks, especially into
the dorsal rami. The peripheral nerve cells were apparently
bipolar, resembling in every particular the nerve cells of the
spinal ganglia.

12. There is a similar distribution of peripheral ganglion cells
along the course of the vagus and glossopharyngeal nerves. At
the level of the caudal extremity of the mandibular muscles
these cells were sufficiently numerous in the vagus nerve to
constitute an elongated ganglion.

13. The writer would regard the above mentioned peripheral
nerve cells, especially those of the spinal nerves, as representing
vestigeal ganglion cells of neural crest origin, which have mi-
grated peripherally along the nerve fibers, repeating in part a
more diffuse arrangement found in more primitive vertebrates
(Amphioxus). This view seems more tenable than to regard
them as the anlage of the sympathetic system; for the reason
that the peripheral spinal nerve cells are most numerous in the
extreme caudal nerves, more numerous in the dorsal than in the
ventral rami, very few appearing in the abdominal or thoracic
nerves, and in addition the cells are apparently bipolar, resem-
bling the structure and relationships of the ordinary spinal
ganglion cells. The vertebral and peripheral sympathetic sys-
tem of the higher vertebrates may arise from a further modi-
fication of this primitive migration process or it may have an
entirely independent but similar origin.

14. The motor and sensory innervation of the M. cordis caudalis is from branches of certain of the neighboring ventral spinal rami. Two reconstructions showed a large cephalic branch from one of the last ventral motor nerves crossing the M. cordis caudalis or from the last nerve behind it, as passing along the inner surface of the M. cordis caudalis for some distance. It doubtless furnished a large part of the motor supply for this muscle.

15. Some seven or eight muscle spindles were found in connection with some very large muscle fibers in the ventro-cephalic portion of the M. cordis caudalis. Most of these spindles were enclosed in a well-developed lamellated connective tissue capsule, and were apparently supplied from branches of the first ventral spinal rami that cross the muscle.

16. Certain very large axones were seen in transverse section of the rami ventralis of Polistotrema. They were supposed to be axones of the giant cells of the spinal cord.

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EXPLANATION OF THE FIGURES

All figures are from drawings of graphic reconstructions, transverse sections or dissections. The graphic reconstructions were made after a method given in an earlier publication (Quart. Jour. Micro. Sci., vol. 59, p. 352-3, 1913). The outlines for the transverse sections were drawn with the aid of an Edinger-Leitz projection apparatus. In general the motor nerves are represented as solid black areas, the sensory nerves, spinal ganglia, and veno-lymphatics are drawn in outline. The central nervous system is cross-barred. Arteries are finely stippled on two sides and the veins are filled in with circular lines. Cartilage is coarsely stippled and muscles are shown as fine lines longitudinally or as fine dots transversely. Mucous sacs are portrayed as a mass of small circles and nerve cells may be represented similarly or they may be filled in with solid black. In a number of instances the dorsal and ventral borders of the myotomes are designated by a dotted line, consisting of alternate long and short lines. A-B in the reconstruction drawings denotes the base or projection line from which all structures in the reconstructional drawings were plotted. In the first re-constructional drawing of every reconstruction it represented a horizontal line passing along the ventral surface of the notochord. When a given structure assumes a dotted outline upon arriving at a second structure or organ it signi-fies that the first structure passes deeper to or within the second structure.

PLATE 1

1   Graphic reconstruction of the caudal spinal nerves of a 20 cm. Polistotrema (Bdellostoma) series as seen from the left side, but which in reality should be from the right side but for an error in making the reconstruction.   It shows the complete distribution of the motor and sensory components of the spinal nerves in the tail region and the relationship of the motor and sensory components to each other in each nerve.   It portrays also the irregularities of the last three nerves, the innervation of the M. cordis caudalis, the presence of isolated or clustered peripheral nerve cells along the course of some of the sensory and mixed nerves and the existence of seven or eight muscle spindles in the ventro-cephalic end of the M. cordis caudalis. $\times$ 14.

ABBREVIATIONS

C.A., caudal artery

C.H., caudal heart

C.V., caudal vein

D.Myo., dorsal border of myotomes

D.R., dorsal root (radix posterior)

D.S., dorsal spine

L.M.B., large muscle bundles in the M. cordis caudalis

M.C.C., M. cordis caudalis

M.D.B., median dorsal cartilaginous bar

M.S., muscle spindle

M.T.C., M. transversus caudalis

Muc.S., mucous or slime sac

Nc., notochord

N.C., nerve cell

R.Cut.M., ramus cutaneus medius

R.Cut.S., ramus cutaneus superior

R.D.M., ramus dorsalis or posterior (motor)

R.D.S., ramus dorsalis or posterior (sensory)

R.M.C.C., motor rami for M. cordis caudalis

R.V.M., ramus ventralis or anterior (motor)

R.V.S., ramus ventralis or anterior (sensory)

Sp.Cd., spinal cord

Sp.G., spinal ganglion

V.Myo., ventral border of myotomes

V.R. (1), cephalic ventral or anterior root

V.R.(2), caudal ventral or anterior root

V.T., ventral veno-lymphatic trunk

PLATE 1

SPINAL NERVES IN VERTEBRATES
[WILLIAM F. ALLEN
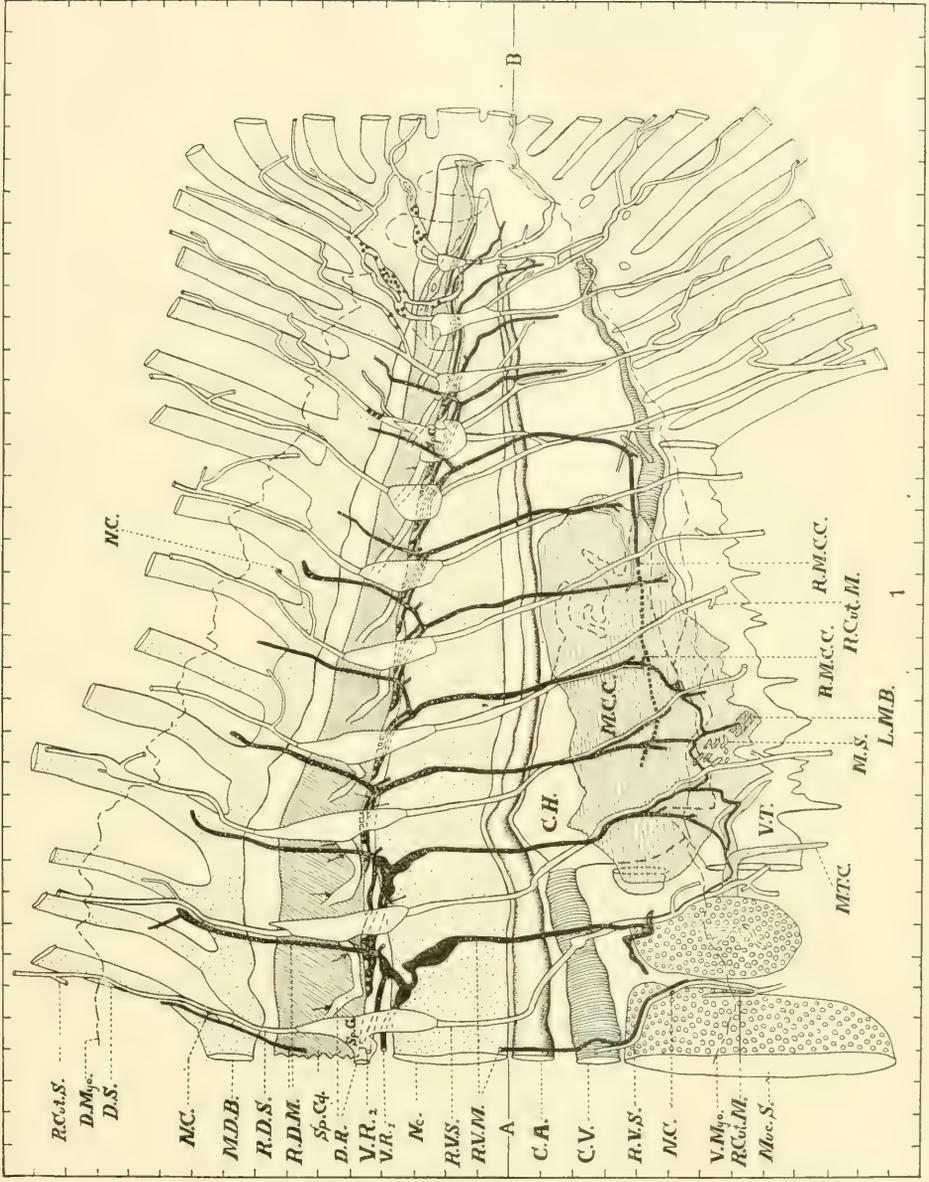
PLATE 2

2 Graphic reconstruction of the last spinal nerve on the opposite or left side of the same Polistotrema series as figure 1. The space between the two dotted lines denotes the interval traversed in passing through the membranous neural arch. This nerve presents a number of interesting variations. It has no ventral sensory ramus or corresponding motor rami. Its ganglion cells are sufficiently scattered, one of which is found within the neural canal, to suggest the arrangement found in Amphioxus. × 40.

3 Graphic reconstruction of the spinal nerves in the region of the caudal heart from an 85 mm. Polistotrema embryo, as seen from the left side. It is evident that the general arrangement of the motor and sensory components is about the same as in figure 1. Observe that many of the dorsal sensory rami have clusters of peripheral ganglion cells opposite the median dorsal cartilaginous bar. Also the last ventral sensory ramus has an isolated nerve cell opposite the notochord and the second dorsal sensory ramus has an isolated ganglion cell at the point of branching above the myotomes. × 20.

ABBREVIATIONS

C.A., caudal artery
C.H., caudal heart
C.V., caudal vein
D.Myo., dorsal border of myotomes
D.R., dorsal root (radix posterior)
D.S., dorsal spine
M.C.C., M. cordis caudalis
M.D.B., median dorsal cartilaginous bar
Muc.S., mucous or slime sac
M.T.C., M. transversus caudalis
Nc., notochord
N.C., nerve cell

R.D.M., ramus dorsalis or posterior (motor)
R.D.S., ramus dorsalis or posterior (sensory)
R.M.C.C., motor rami for M. cordis caudalis
R.V.M., ramus ventralis or anterior (motor)·
R.V.S., ramus ventralis or anterior (sensory)
Sp.Cd., spinal cord
Sp.G., spinal ganglion
V.R., ventral root (radix anterior)
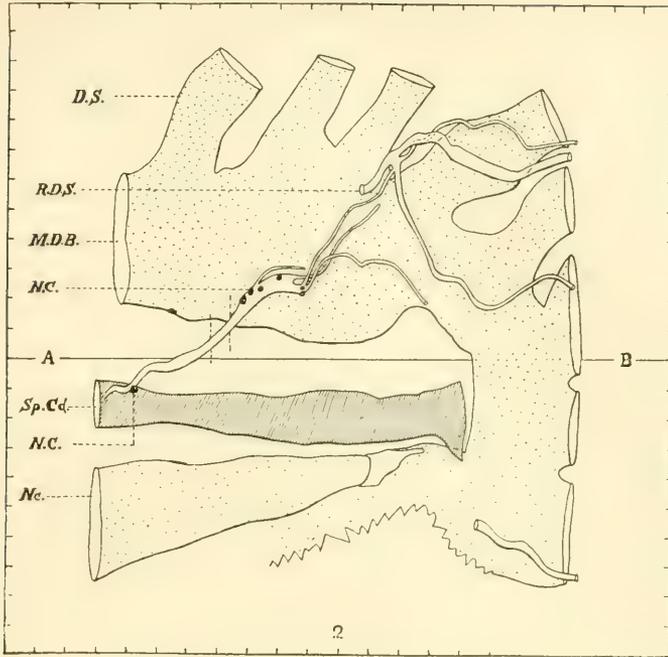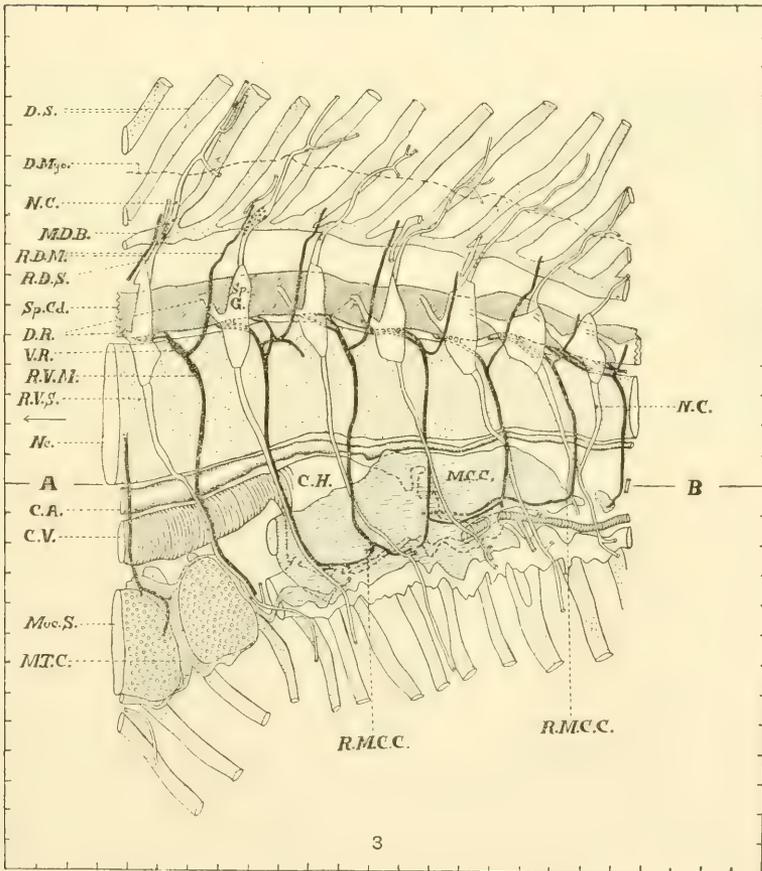
4   Graphic reconstruction of two spinal nerves in the region of the posterior extremity of the M. cordis caudalis from a series of a small adult Polistotrema. Observe that the motor and sensory components of both the dorsal and ventral spinal rami are distributed as separate nerves; that no conspicuous branch from the first ventral motor ramus behind the M. cordis caudalis is given off to supply the inner surface of this muscle as is shown in figures 1 and 3; that there is a cluster of peripheral ganglion cells about both dorsal sensory rami in the neighborhood of the median dorsal cartilaginous bar, but no peripheral nerve cells were found in either of the ventral sensory rami; and that there is a marked thinning out of the nerve cells in the dorsal pole of the ganglia, indicating a tendency for the cells to migrate peripherally.   × 50.

ABBREVIATIONS

C.A., caudal artery

C.V., caudal vein

D.Myo., dorsal border of myotomes

D.R., dorsal root (radix posterior)

D.S., dorsal spine

M.C.C., M. cordis caudalis

M.D.B., median dorsal cartilaginous bar

Nc., notochord

N.C., nerve cell

R.D.M., ramus dorsalis or posterior (motor)

R.D.S., ramus dorsalis or posterior (sensory)

R.M.C.C., motor rami for M. cordis caudalis

R.V.M., ramus ventralis or anterior (motor)

R.V.S., ramus ventralis or anterior (sensory)

Sp.Cd., spinal cord

Sp.G., spinal ganglion

V.Myo., ventral border of myotomes

V.R. (*1*), cephalic ventral or anterior root

V.R. (*2*), caudal ventral or anterior root

4

PLATE 4

5   Graphic reconstruction of a spinal nerve and the vagus trunk from an adult Polistotrema series in the neighborhood of the caudal extremity of the retractor mandibulae muscles.   It is clear from this figure: 1) That the dorsal motor and sensory nerves are separate.   2) The ventral motor nerve joins the ventral sensory nerve opposite the notochord to form a mixed ramus ventralis.   3) An isolated peripheral ganglion cell appears in the ramus ventralis about on a level with the ventral border of the myotomes.   4) The vagus nerve is here divided into three or four large bundles, and since all of these bundles possess nerve cells they all probably contain sensory or receptive fibers.   5) These cells are sufficiently numerous in the ventral bundle to form an elongated ganglion about a segment long, and any section through it would reveal from one or two to fourteen cells.   6) All of the fibers to the oesophagus, both sensory and motor, come from this ventral bundle. $\times$ 11.

ABBREVIATIONS

D.Myo., dorsal border of myotomes
D.R., dorsal root (radix posterior)
M.C.P. (*1*), posterior division of the M. constrictor pharyngis
Nc., notochord
N.C., nerve cell
Oes., oesophagus or pharynx
R.D.M., ramus dorsalis or posterior (motor)
R.D.S., ramus dorsalis or posterior (sensory)
R.M.C.P. (*1*), vagus branches to posterior division of the M. constrictor pharyngis
R.Oes., vagus branches to oesophagus

or pharynx
R.V., ramus ventralis or anterior
R.V.M., ramus ventralis or anterior (motor)
R.V.S., ramus ventralis or anterior (sensory)
Sp.Cd., spinal cord
Sp.G., spinal ganglion
V.Myo., ventral border of myotomes
V.R. (*1*), cephalic ventral or anterior root
V.R. (*2*), caudal ventral or anterior root
X., vagus nerve

PLATE 5 ·

6   Graphic reconstruction of a portion of two of the most cephalic spinal nerves from an adult Polistotrema series.   This region is in the same segment as the second branchial arch and immediately behind the auditory capsule.   The cephalic end of the specimen is at the left.   As in the previous reconstruction, the dorsal sensory rami are separate from the motor rami, while the ventral

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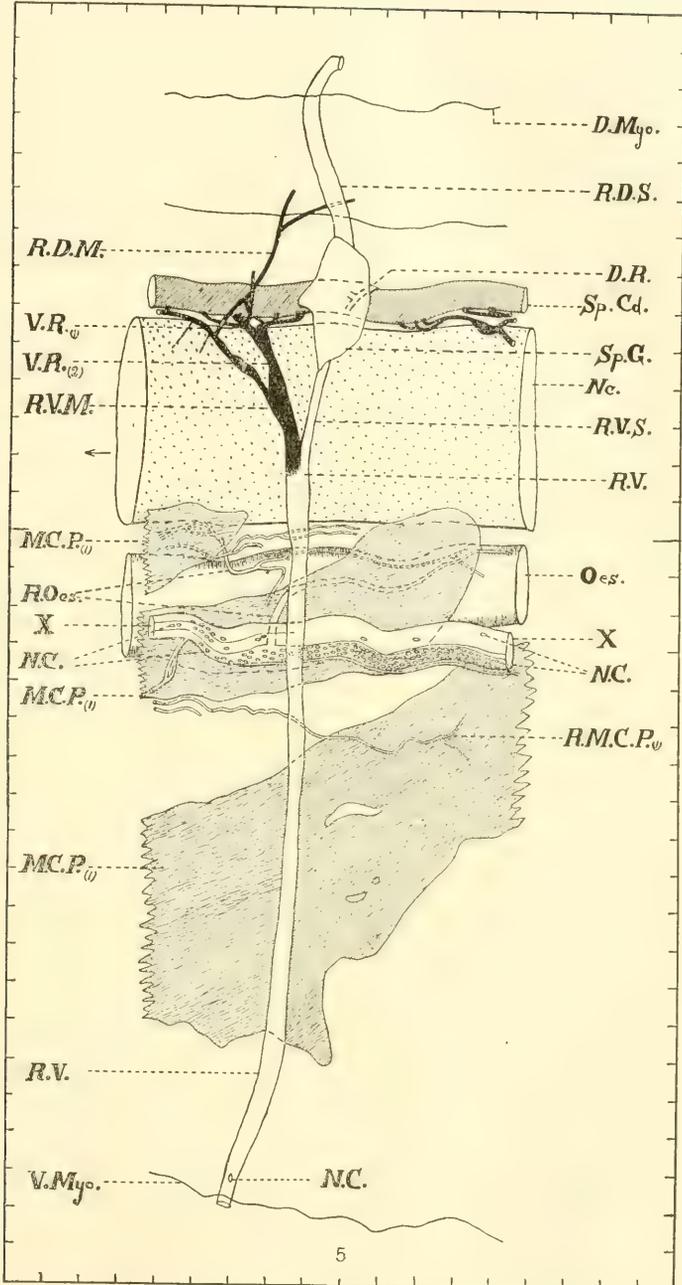
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motor rami join the corresponding sensory rami from in front, forming mixed rami ventrales. The dorsal and ventral roots have apparently been carried cephalad instead of caudad in this region, through a more continuous growth of the myotomes than of the skeletal elements. No peripheral nerve cells were found in either of the spinal nerves, but scattered ganglion cells are present in the vagus-glossopharyngeal trunk and in the glossopharyngeal at the point of its separation from the vagus. × 16.5.

7 . Transverse section through the cephalic end of the same vagus nerve as was reconstructed in figure 5. Observe that the nerve is divided into four bundles, three of which possess ganglion cells. × 50.

8 Similar transverse section to figure 7, but taken from about the central portion of the vagus nerve in figure 5. It consequently passes through the center of the elongated ganglion of the ventral bundle, which shows thirteen ganglion cells in this section. There is also one nerve cell in the median dorsal bundle. × 50.

9 Cephalic transverse section through the vagus and glossopharnygeal nerves at the point of separation of the glossopharnygeal, taken from one of the caudal sections used in preparing reconstruction 6. Observe the presence of a ganglion cell in the glossopharnygeal and the absence of large bundles of nerve fibers characteristic of the more caudal sections. × 50.

10 Portion of a transverse section through the caudal heart region of an adult Polistotrema. Introduced to show a cluster of peripheral ganglion cells found along the course of the dorsal sensory ramus at the point of branching a little above the myotomes. × 25.

11 Enlargement of the peripheral nerve cell shown in figure 5. × 50.

<div align="center">ABBREVIATIONS</div>

Ao., aorta
Br.A. (2), second branchial arch
D.Myo., dorsal border of myotomes
D.R., dorsal root (radix posterior)
D.S., dorsal spine
End., endoneurium
Epi., epineurium
M.& S.F., motor and sensory fibers
M.C.P., anterior division of the M. constrictor pharyngis
M.C.Q.S., M. copulo-quadratus superficialis
M.D.B., median dorsal cartilaginous bar
M.V.Q., M. velo-quadratus
Myo., myotomes or M. parietalis
N.B., nerve bundle
Nc., notochord
N.C., nerve cell

Per., perineurium
R.D.M., ramus dorsalis or posterior (motor)
R.D.S., ramus dorsalis or posterior (sensory)
R.V., ramus ventralis or anterior
R.V.M., ramus ventralis or anterior (motor)
R.V.S., ramus ventralis or anterior (sensory)
Sp.Cd., spinal cord
Sp.G., spinal ganglion
V.N.B., ventral nerve bundle of X
V.R., ventral root (radix anterior)
V.R. (1), cephalic ventral or anterior root
IX., glossopharyngeal nerve
X., vagus nerve
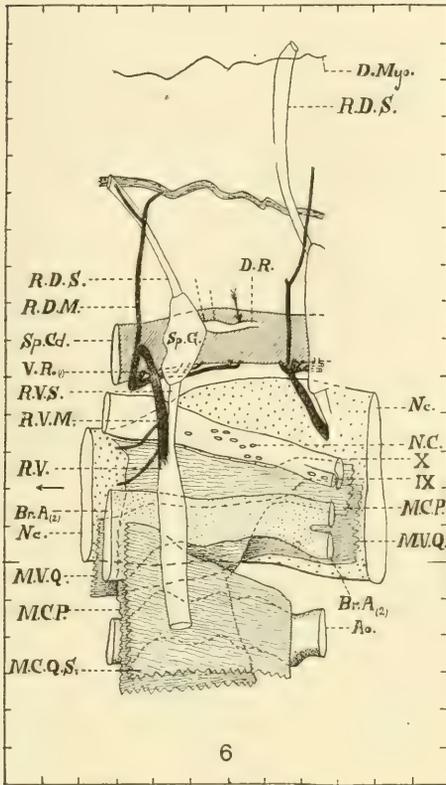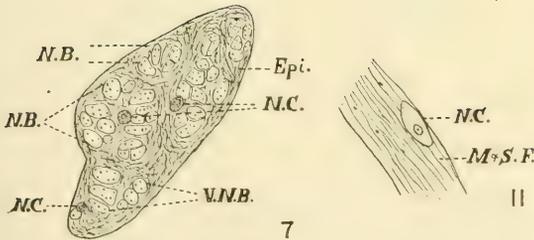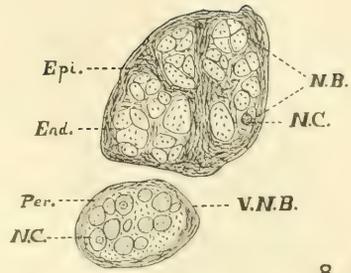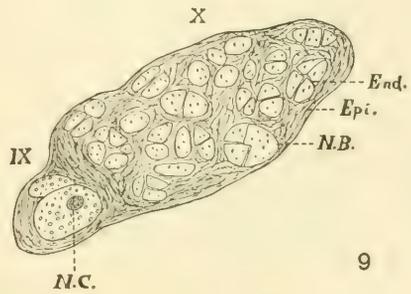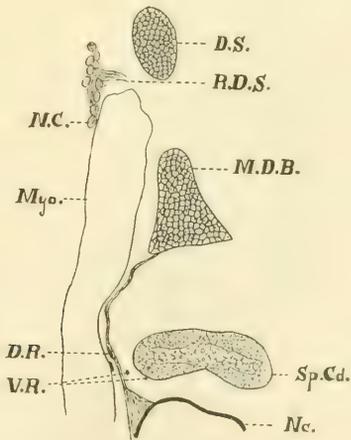
PLATE 6

12 To the right, graphic reconstruction of the spinal nerves from the region of the caudal heart to the caudal fin of a 20 mm. Polistotrema embryo. To the left, graphic reconstruction of two spinal nerves several segments in front of the caudal heart of the same embryo. Note, 1) In every instance where the motor and sensory rami have appeared they are well-separated. 2) The extreme caudal nerves are in a very early embryonic stage. The last one consists of an irregular mass of neural crest, and there is a gradual increase in the development of the spinal nerves in passing forward, indicating that the order of appearance of the various parts of a spinal nerve is as follows,—ventral motor ramus, dorsal motor ramus, ventral sensory ramus, dorsal sensory ramus. 3) Of the various spinal nerves which will later cross the M. cordis caudalis, the second, third, and fourth are much longer and have penetrated a mass of myoblast destined to form the M. cordis caudalis. × 50.

13 From a dissection of several of the spinal nerves of an adult Polistotrema immediately in front of the M. cordis caudalis. This dissection shows how a ventral sensory nerve by dividing and each branch receiving a motor ramus from separate nerves may bring about the change of the caudal rami ventrales receiving their motor components from the rear instead of from in front. × 2.

14 Portion of a transverse section through the cephalic end of the M. cordis caudalis belonging to the series reconstructed in figure 1, showing two muscle spindles in transverse section and one in longitudinal section. A portion of the spiral placque is visible in the longitudinal spindle and both transverse spindles show well-developed lamellated connective tissue capsules. × 100.

ABBREVIATIONS

A.R., anal fin ray
B.V., blood vessel
C.A., caudal artery
Cap., muscle spindle or tendinous capsule
C.V., caudal vein
D.Myo., dorsal border of myotomes
D.R., dorsal root (radix posterior)
M.C.C., M. cordis caudalis
M.F., muscle fibers
Muc.S., mucous or slime sac
Nc., notochord
Neu.C., neural crest
Pl., annular or spiral placques
R.D.M., ramus dorsalis or posterior (motor)

R.D.S., ramus dorsalis or posterior (sensory)
R.V., ramus ventralis or anterior
R.V.M., ramus ventralis or anterior (motor)
R.V.S., ramus ventralis or anterior (sensory)
Sp.Cd., spinal cord
Sp.G., spinal ganglion
Ten., tendon
V.Myo., ventral border of myotomes
V.R., ventral root (radix anterior)
V.R. (1), cephalic ventral or anterior root
V.R. (2), caudal ventral or anterior root

PLATE 7

15 Graphic reconstruction of two spinal nerves from a region a little behind the anus of a 27 mm. Polistotrema embryo. It will be seen that the motor and

PLATE 6

SPINAL NERVES IN VERTEBRATES
WILLIAM F. ALLEN
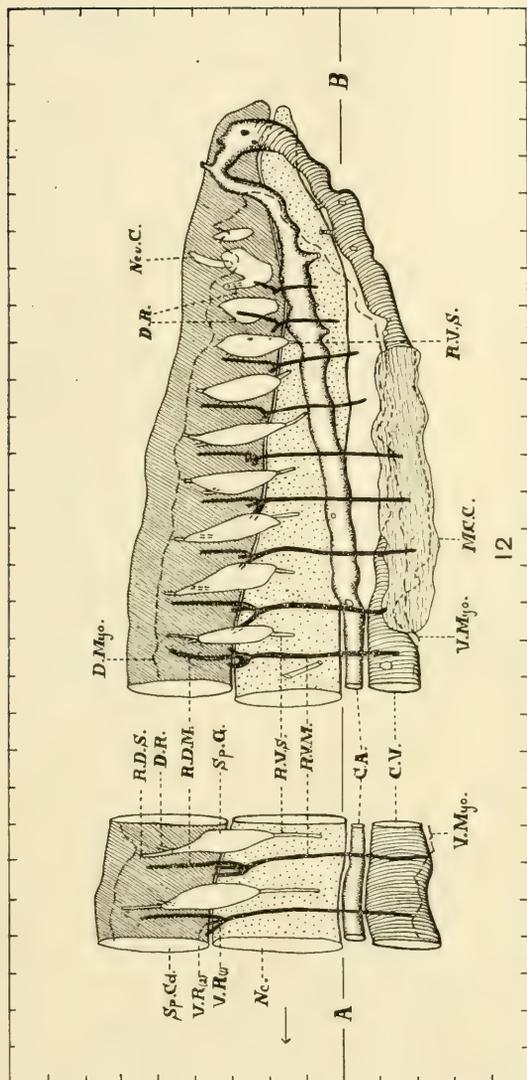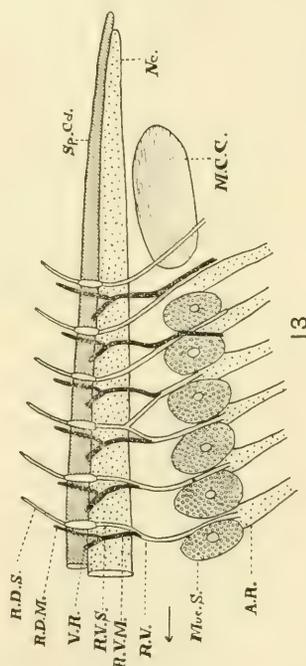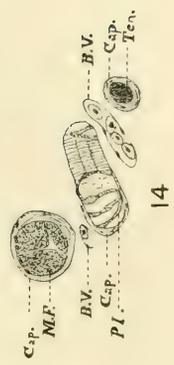
sensory fibers are distributed in separate nerves as in 20 mm. embryo, but that the motor nerves have been carried further caudad and are nearer their respective sensory rami. × 50.

16 Graphic reconstruction of an early developmental stage of several of the lower abdominal spinal nerves from a 7.5 mm. Squalus acanthias embryo. Observe that the motor roots have grown outward and downward for a short distance as ventral motor rami, and in one instance there is the beginning of a dorsal motor ramus. Ventral prolongations of the neural crest, representing the anlage of the spinal ganglia and the sensory ventral spinal rami, in passing ventrally between the motor components are bent slightly toward the cephalic motor nerves. × 50.

17 Graphic reconstruction of two more cephalic spinal nerves of the same embryo as figure 6. Note the increased growth of the ventral sensory and motor rami, the two being readily distinguished by the abundance of cells in the sensory rami. Observe the vertebral sympathetic ganglia, appearing as swellings at the end of the ventral sensory rami, and the presence of short dorsal motor rami. × 50.

18 A, B and C transverse sections through some of the largest fibers of the vagus, cephalic ventral spinal, and glossopharyngeal nerves. D and E are longitudinal sections through some of the largest fibers of a mixed ramus ventralis and a dorsal sensory ramus of a spinal nerve. × 100.

19 Transverse section of a 7.5 mm. Squalus acanthias embryo, passing through the motor and sensory components of a spinal nerve. Note the position of the ventral spinal rami and the greater number of cells in the sensory ramus. × 83.

20 Graphic reconstruction of two spinal nerves in the lower abdominal region of a 19 mm. Squalus acanthias embryo. Observe a notable increase in length of all nerves over the corresponding nerves shown in figure 17. The dorsal motor rami have grown caudally across the lateral surface of their spinal ganglia. The ventral motor and sensory rami have joined opposite the aorta in forming mixed rami ventrales. Sympathetic ganglia have appeared on the ventral sensory rami as the result of a migration of some of the neural crest cells. No dorsal motor rami have appeared thus far. × 50.

ABBREVIATIONS

Ao., aorta

Car.V., cardinal vein

C.V., caudal vein

D.Myo., dorsal border of myotomes

D.R., dorsal root (radix posterior)

M.T.C., M. transversus caudalis

Muc.S., mucous or slime sac

Nc., notochord

Neu.C., neural crest

R.D.M., ramus dorsalis or posterior (motor)

R.D.S., ramus dorsalis or posterior (sensory)

R.V., ramus ventralis or anterior

R.V.M., ramus ventralis or anterior (motor)

R.V.S., ramus ventralis or anterior (sensory)

Sp.Cd., spinal cord

Sp.G., spinal ganglion

Sy.G., sympathetic ganglion

V.R., ventral root (radix anterior)

V.R. (1), cephalic ventral or anterior root

V.R. (2), caudal ventral or anterior root

W.D., Wolffian or mesonephric duct

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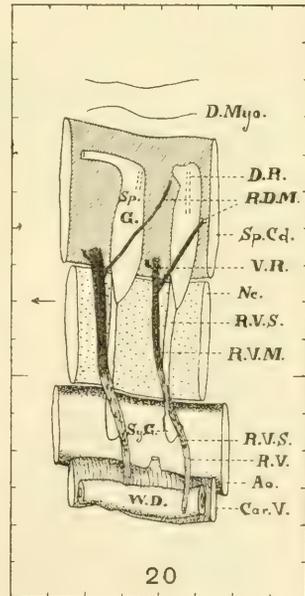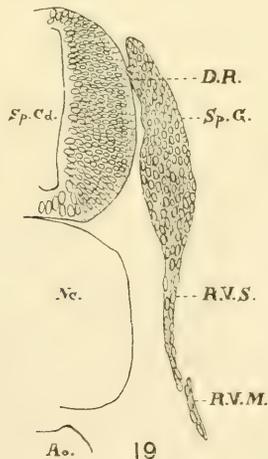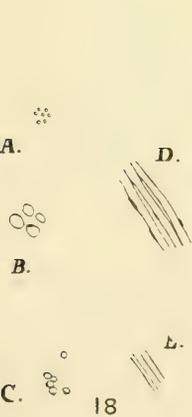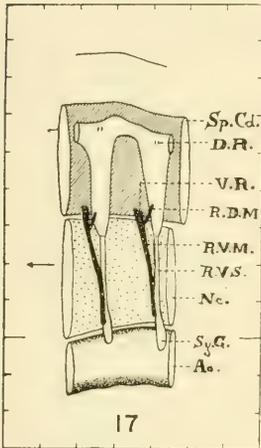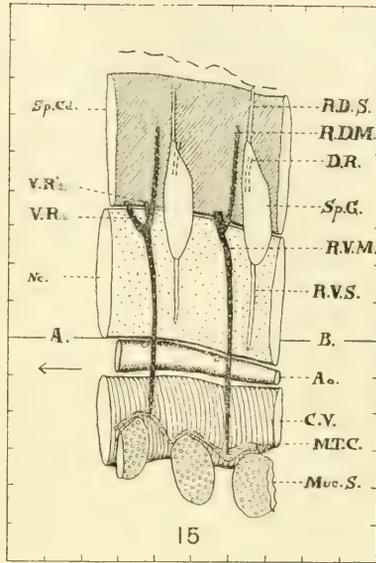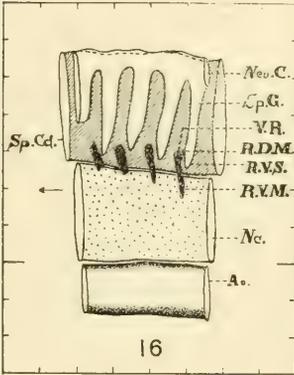
PLATE 8

21 Graphic reconstruction of two abdominal spinal nerves from a 28 mm. Squalus acanthias embryo. Note the appearance of the dorsal sensory rami fibers arising from the caudal surface of their respective ganglia at the point of crossing of the dorsal motor rami. They follow the dorsal motor fibers, but for the greater part of their course do not intermingle with them. Likewise the ventral sensory and motor rami run side by side to a little below the level of the aorta, where they intermingle and form mixed rami ventrales. It will be seen that the rami ventrales are much larger and longer than the rami dorsales, due to a much greater development of the myotomes ventrally. $\times$ 25.

22 Graphic reconstruction of two of the spinal nerves from the lower abdominal region of a 32 mm. Squalus acanthias embryo, which has attained nearly adult conditions in so far as the distribution of the spinal nerves is concerned. Observe, as in figure 21, that the dorsal sensory fibers take exit from the caudal surface of their ganglia at exactly the point of crossing of the dorsal motor rami. It will be seen that the dorsal motor and sensory rami have been carried caudally for a distance of a segment to cross the lateral surface of the following ganglia, where they unite in forming mixed rami dorsales, which traverse the intermuscular septa one segment behind the one followed by the corresponding rami ventrales. As in the 28 mm. embryo, the motor and sensory rami ventrales run side by side to a little below the level of the aorta, where they join in forming mixed rami ventrales. $\times$ 50.

23 From a dissection of the lower abdominal spinal nerves of a small adult Squalus acanthias from the left side. It is clear that the arrangement of motor and sensory spinal nerve components is practically the same as was described for the 32 mm. embryo. $\times$ 4.

24 Transverse section through the middle of the abdomen of a 7.5 mm. Squalus acanthias embryo, passing through the ventral root, and showing extent and structure of the dorsal and ventral motor nerves. $\times$ 83.

25 From a peripheral section of the more cephalic dorsal motor and sensory rami shown in figure 22. Observe that the nerves are well separated. $\times$ 165.

26 Similar section of the same nerves as figure 25, but taken at exit of the sensory fibers from the spinal ganglion. $\times$ 165.

27 Transverse section through the more cephalic ventral motor and sensory rami shown in figure 22. $\times$ 165.

Ao., aorta

D.Myo., dorsal border of myotomes

D.R., dorsal root (radix posterior)

Nc., notochord

Neu., neurilemma or connective tissue cells

R.D., ramus dorsalis or posterior

R.D.M., ramus dorsalis or posterior (motor)

R.D.S., ramus dorsalis or posterior (sensory)

R.V., ramus ventralis or anterior

R.V.M., ramus ventralis or anterior (motor)

R.V.S., ramus ventralis or anterior (sensory)

Sp.Cd., spinal cord

Sp.G., spinal ganglion

V.Myo., ventral border of myotomes

V.R., ventral root (radix anterior)

V.R. (1), cephalic ventral or anterior root

V.R. (2), caudal ventral or anterior root
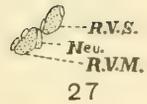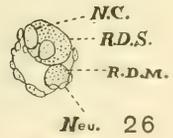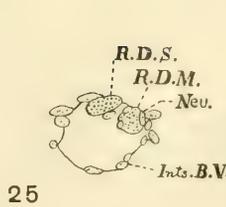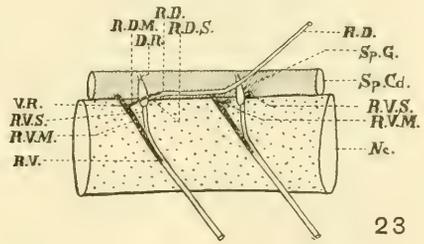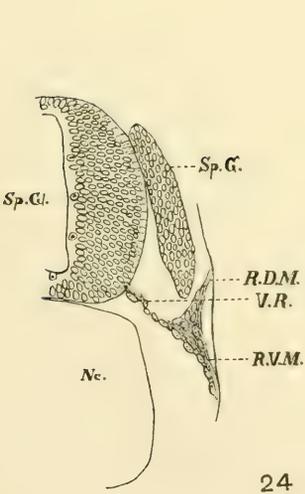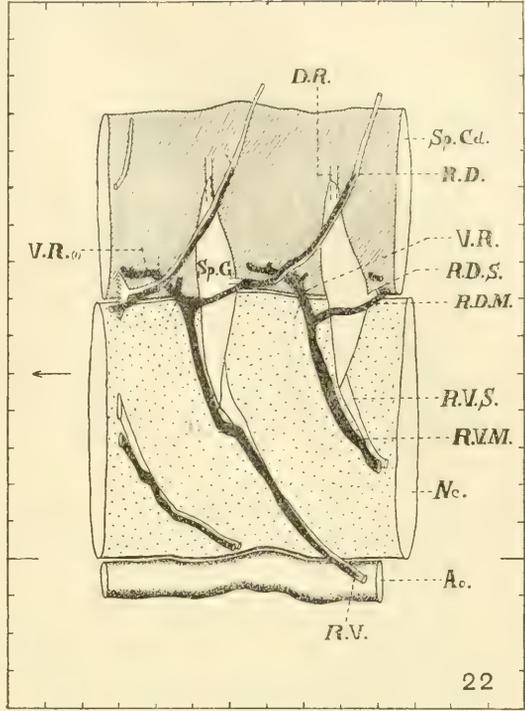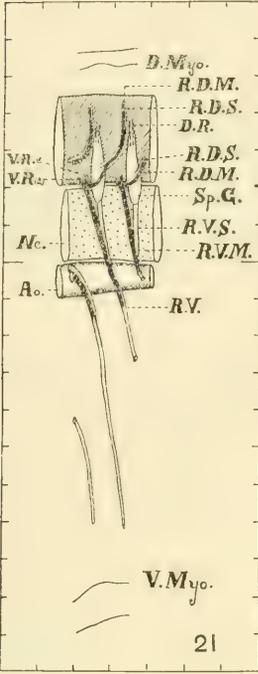
PLATE 9

28   Transverse section passing through the dorsal root and ramus ventralis from an adult Amphioxus.   Note the ganglion cells scattered through the dorsal root, ramus ventralis and dorso-lateral portion of the spinal cord.   × 50.

29   Graphic reconstruction of two spinal nerves from an adult Amphioxus. Observe that the motor and sensory components are widely separated and that the ganglion cells are scattered through the sensory rami and the dorsal root. The light area immediately outside the motor rami, bordered by a dotted line, I take to be fibrous connective tissue.   × 50.

30   Diagrammatic transverse section of the motor and sensory components of a spinal nerve of one of the higher vertebrates.

31   Graphic reconstruction of an abdominal spinal nerve from a 6 mm. pigeon. Observe the presence of rami ventrales, which have the appearance at first sight of being mixed nerves, but which upon closer examination of their finer structure are shown to have separate motor and sensory bundles running side by side. × 50.

32   Graphic reconstruction of two abdominal spinal nerves from an 8 mm. pigeon embryo.   Note the presence of short rami dorsales.   Both the rami dorsales and rami ventrales have the appearance of being mixed nerves, but an examination of their finer structure shows the motor and sensory fibers of the rami dorsales to be separate throughout and the motor and sensory fibers of the rami ventrales do not intermingle before the level of the aorta is reached.   × 25.

33   Abdominal transverse section of a 10 mm. turtle embryo passing through the ramus ventralis of a spinal nerve.   Observe the difference in structure of the motor and sensory components.   × 50.

34   Transverse section of a 19 mm. Squalus acanthias embryo, showing the point of union of the motor and sensory nerves to form a mixed ramus ventralis a little below the vertebral sympathetic ganglion.   × 163.

35   Shows the general appearance of the motor and sensory portions of the ramus ventralis at the level of the aorta in a transverse section through the abdomen of a 19 mm. Squalus acanthias embryo.   Note that the motor and sensory fibers are separated by a layer of connective tissue or neurolemma cells.   × 163.

a., inner border of myotomes
Ao., aorta
b., outer border of myotomes
C.T., connective tissue or neurolemma cells?
D.R., dorsal root (radix posterior)
Myo., myotomes
Nc., notochord
N.C., nerve cell
R.C., ramus communicans
R.D., ramus dorsalis or posterior
R.D.M., ramus dorsalis or posterior (motor)

R.D.S., ramus dorsalis or posterior (sensory)
R.V., ramus ventralis or anterior
R.V.M., ramus ventralis or anterior (motor)
R.V.S., ramus ventralis or anterior (sensory)
Sp.Cd., spinal cord
Sp.G., spinal ganglion
Sy.G., sympathetic ganglion
V.R., ventral root (radix anterior)
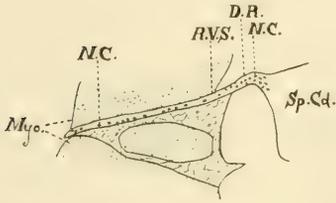
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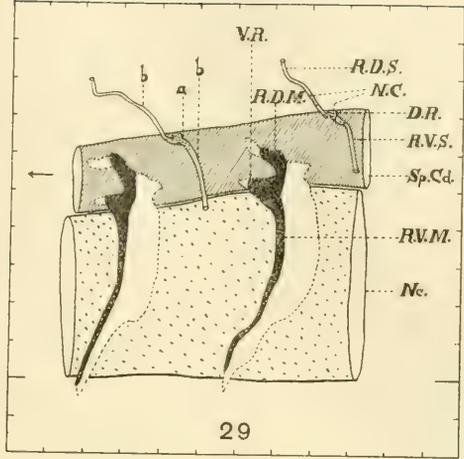
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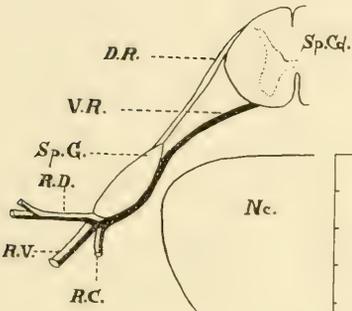
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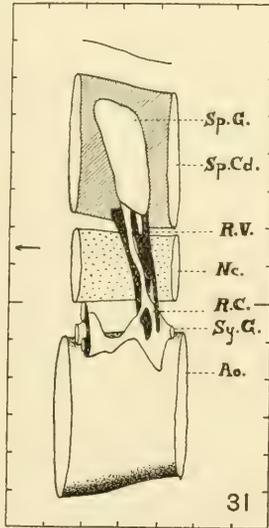
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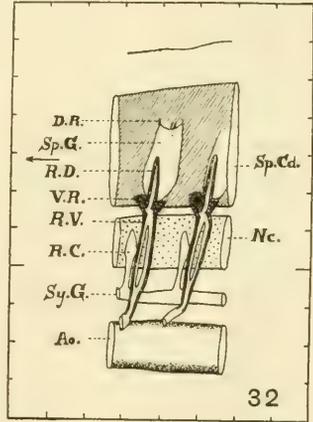
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THE INTERNAL STRUCTURE OF THE MIDBRAIN AND THALAMUS OF NECTURUS

C. JUDSON HERRICK

From the Anatomical Laboratory of The University of Chicago

SIXTY–EIGHT FIGURES

CONTENTS

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1. INTRODUCTION

In attacking the problem of the origin and biological significance of the cerebral cortex comparative neurologists have hitherto generally directed their attention primarily to the cortex itself and its supposed primordia in the lowest vertebrates. As a result of these studies it is becoming increasingly evident that the key to this difficult question is to be sought in the subcortical centers of the primitive types, that is, in the 'old brain' (palaeencephalon of Edinger, segmental apparatus of Adolf Meyer), which attains its definitive pattern as a mechanism for reflexes and instincts prior in evolutionary history to the emergence of the true cortex (neencephalon, or suprasegmental apparatus of the forebrain). It is important, therefore, that the exact pattern of these primitive sensori-motor mechanisms of the forebrain be determined in advance; for here are to be sought the primary physiological factors through whose interaction the functional pattern of the cerebral hemisphere has been elaborated.

As we pass from the lower mammals to the reptiles, the true cerebral cortex shrinks abruptly to relatively small dimensions; in reptiles, however, the subcortical centers of the cerebral hemisphere (striatum complex and olfactory apparatus) are relatively enormously developed. Again, as we pass from the reptiles to the amphibians, the true cortex (i.e., the superficial gray layer of the cerebral hemispheres) disappears entirely, a small trace of it being present in the primordial hippocampal area of the frog, and none at all in urodeles. The striatum complex also is greatly diminished, and the olfactory apparatus remains as the dominant functional system of the cerebral hemisphere. In all types of fishes the dominance of the olfactory apparatus and the reduction of the striatum complex ('somatic area' of Johnston) are still more pronounced.

The complex mesencephalic and diencephalic structures of fishes are chiefly intrinsic apparatuses, i.e., correlation centers developed *in situ* for the performance of the most complex correlations of which these animals are capable; in the reptiles, birds and mammals, however, the increasing complexity of the thalamic centers is directly related with the elaboration of the mechanisms of the cerebral hemisphere. These parts of the thalamus, therefore, constitute here a cortical dependency in the sense of von Monakow, and are termed by Edinger the neothalamus by reason of their functional relationship with the neopallium.

In the urodele Amphibia the midbrain and thalamus are in a relatively generalized form, lacking the extreme specialization of the higher fishes, on the one hand, and of the amniote vertebrates, on the other hand. The recent Amphibia themselves are, it is true, somewhat aberrant with reference to the main line of vertebrate descent from fishes to mammals. Nevertheless in the consideration of the morphogenesis of the cerebral hemispheres these forms are very instructive, for here the picture is uncomplicated by the great neencephalic systems and the primitive apparatus of the brain stem is not elaborately specialized in any direction.

The Amphibia, therefore, appear to be the critical types, among existing vertebrates, for the study of the transformations of functional pattern which were effected when the nonolfactory components gradually passed from a subordinate to a dominant position in the architecture of the cerebral hemisphere. Within this group it is possible to follow the history of this process from generalized and very primitive forms up to the relations in the adult frog, where most of the chief subdivisions of the mammalian hemisphere can be recognized. This group possesses the further advantage that the tadpoles begin to function at a very immature stage, whose functional pattern and anatomical organization are being exhaustively investigated by Coghill. The result is that we are able to follow, in the higher amphibian species, the transformation of the cerebral mechanisms from the simplest pattern in the youngest larva to the rather highly elaborated form of the adult in a series of developmental stages each of which is in active function. This offers us very much more favorable material for the embryological study of these patterns than in higher vertebrate types whose embryos acquire functional capacity at relatively much later ages.

Unfortunately, the reflex mechanisms of the Amphibia are still very imperfectly known. I have, accordingly, devoted myself to an analysis of these systems in larval and adult urodeles. This has necessitated an investigation of their nervous systems in their entirety, from the peripheral end-organs to the highest correlation centers; for the latter can be understood only after the precise functional significance of every fiber tract which enters them has been determined.

The peripheral nerve components of a number of amphibian species have been carefully described by Strong, Coghill, Norris, and others. Kingsbury ('95) gave an admirable description of some of the central connections of these components in Necturus, and in 1914 I added further details of the functional analysis of the medulla oblongata in Amblystoma. I published also in 1914 some observations on the structure and connections of the cerebellum of lower urodeles, particularly Necturus.

In the present contribution this analysis is continued into the midbrain and its connections, using Necturus maculatus as the type. Here is included, then, as complete an analysis as the material at hand permits of the midbrain of Necturus. The structure and connections of the diencephalon are included only in so far (with few exceptions) as these are necessary for an understanding of the mesencephalic connections. A fuller discussion of the amphibian diencephalon I hope to present in a subsequent paper.

The general structure of the brain of Necturus has been very clearly described in the work of Kingsbury ('95), to which reference has already been made, and the chief fiber tracts are there illustrated, so far as they can be shown by the Weigert method. Osborn ('87 and '88) had previously described some features of this brain with good figures, Miller in 1900 published two figures of a wax model, Johnston in several works (e.g., '05, '06) has made references to it, Warren in 1905 described the development of the pineal region and gave a figure of the adult brain, and von Kupffer ('06) described the brain of a 24 mm. embryo. In 1911 McKibben published a drawing of the ventricular surface after section in the sagittal plane and a series of sketches of cross sections through the forebrain illustrating the general relations, together with a detailed description of the nervus terminalis. The same author in 1913 described the eye-muscle nerves with accurate figures of dorsal and ventral views of the brain after fixation *in situ* in the cranium. Norris and Buckley ('11) briefly described the cranial nerves of Necturus and mentioned the previous literature on the subject, and in another short paper Norris ('11) discussed the phylogenetic rank of Necturus. Röthig in 1911 published a brief account of the myelinated fiber tracts of the cerebral hemispheres with an excellent series of figures of cross sections, which add some details to the earlier account of Kingsbury, and in 1912 he contributed some further information on the internal structure of this brain and of that of other urodeles.

In this descriptive paper we shall not undertake a summary of the contents of the papers just cited or of the literature on the

urodele brain in general, including among others the papers of
Burckhardt ('91) on Triton and Ichthyophis, Mrs. Gage ('93)
on Diemyctylus, Hirsch-Tabor ('08) on Proteus, and Bindewald
('14) on Amblystoma.

Material and methods

This contribution is based upon the study of an extensive
series of brains of adult Necturus maculosus (Raf.) prepared
by various methods by Dr. Paul S. McKibben, to whom I am
greatly indebted for generously placing his entire collection at
my disposal.   This collection includes, among others, the fol-
lowing series of histological preparations:

3 series by Weigert's method (transverse, sagittal and horizontal).
3 series by Mallory's method for connective tissue (one transverse and two
      horizontal).
2 series by Heidenhain's iron hematoxylin and orange G (horizontal).
1 series by Ehrlich's hematoxylin (horizontal).
1 series by toluidin blue (transverse).
1 series by carmine and Lyons blue (transverse).
4 series by vom Rath's method (transverse, sagittal and horizontal).
9 series by Ramón y Cajal's method (transverse, sagittal and horizontal).
40 series by Golgi's method (transverse, sagittal and horizontal).
1 series by the Cox-Golgi sublimate method (horizontal).

The Weigert material was first fixed in a formalin-bichromate
mixture, followed by Müller's fluid for several days, then em-
bedded in paraffin and stained by the original Weigert procedure
with remordanting in section with copper acetate.   The destain-
ing was carried to the point where the myelinated fibers are
clearly differentiated and yet sufficient color is left in the back-
ground to render many of the unmyelinated fiber tracts and
the cell nuclei clearly recognizable.

II. GENERAL MORPHOLOGY

The brain of Necturus is simple from both the morphological
and the histological standpoints; and, though in some respects
perhaps degenerate, the general type is clearly very primitive.

The mesencephalon and diencephalon form a continuous tube
with rather thin walls and extensive ventricular cavities, open-

ing backward into the fourth ventricle and forward into the unpaired telencephalic ventricle in front of the optic chiasma.

The mesencephalon

The mesencephalic ventricle is widely dilated both dorso-ventrally and laterally, with the widest part somewhat above the middle (figs. 7 to 14 and 63). As in vertebrates generally, it is composed of a dorsal tectum mesencephali and a ventral pedunculus cerebri. There is no external boundary between these. The internal structure indicates that the widest part of the ventricle does not represent the position of the embryonic sulcus limitans separating the dorsal sensory from the ventral motor lamina, this boundary lying somewhat ventrally of that level.

The approximate boundary between the mesencephalon and the diencephalon is marked externally by a shallow transverse groove extending from the dorsal to the ventral surface at the level where the hypothalamus becomes free from the cerebral peduncle. Internally the upper limit of the mesencephalon is commonly described as marked by the rostral border of the posterior commissure dorsally and of the tuberculum posterius ventrally.

The *tuberculum posterius* (figs. 62, 63, 64, *tub.p.*) is the sharp bend in the brain floor where the rostral end of the cerebral peduncle turns ventralward to join the dorsal part of the hypothalamus.

The caudal boundary of the mesencephalon is marked dorsally by the decussatio veli, containing the cerebellar commissures and the decussation of the IV nerves (fig. 62). On the lateral surface it is indicated by the constriction of the isthmus (fissura isthmi), behind which on the dorso-lateral aspect are the en-largements formed by the small cerebellum and the very large auricular lobes of the medulla oblongata. The ventral part of the midbrain (cerebral peduncle) extends caudalward less than half as far as does the dorsal part or tectum.

In the ventro-medial plane the caudal boundary of the cere-bral peduncle is indicated by a well defined pit, the *forea isthmi*

(figs. 11, 46, 47, 62, 63, 64, *f.i.*), which lies at about the transverse level of the roots of the III nerves. Large blood vessels enter the brain floor at this point. Here the ventral commissure system is interrupted and the gray layer extends to the ventral surface of the brain, as was noted by Kingsbury ('95, p. 163), who termed this depression the mesencephalic pit. This landmark was first noted by Stieda ('75, p. 294), who described it in the axolotl (Amblystoma mexicanum) as a slight but evident constriction of the pedunculus cerebri which marks the boundary between the pars peduncularis and the medulla oblongata. Burckhardt ('91, p. 379) noted its presence in Ichthyophis and numerous other vertebrates, and called it the 'Mittelhirngrenze.' His ('92, p. 355) again refers to it under the name 'Isthmusgrube,' and this designation in its Latin form is here adopted.

In adult Necturus the fovea isthmi lies so far forward as to raise the question whether it really marks the caudal border of the pedunculus cerebri, as the authors just cited have maintained. The figures published by these authors, however, show that in early developmental stages of vertebrates in general it lies relatively farther caudad.

Embryological stages of Necturus not being available, I have examined the relations of this landmark in a series of wax models of the brain of Amblystoma tigrinum from the 10 mm. larva to the adult. In the adult the fovea is not as conspicuous as in Necturus; nevertheless its position is quite evident in essentially the same relations when the more compact form of the Amblystoma brain is taken into account. In the 10 mm. larva, on account of the great mesencephalic flexure, the tuberculum posterius lies in the same transverse plane as the recessus posterior mesencephali (see p. 224) and the posterior commissure lies far forward, so that the fibers of the descending limb of this commissure, to reach their termination above the tuberculum posterius, must take a course nearly horizontal with reference to the body axis. From the fovea isthmi a strong sulcus, which I shall call the *sulcus isthmi* (figs. 63, 64, *s.is.*), runs dorsalward to end in the recessus posterior mesencephali. The eminentia subcerebellaris tegmenti (see p. 225)

lies caudad of this sulcus and thus reaches forward to the fovea isthmi.

In older larvae, as the mesencephalic flexure is straightened out, this sulcus comes to be directed caudad as well as dorsad from the fovea isthmi and it becomes very deep and narrow. Its relations to the eminentia subcerebellaris tegmenti in a 38 mm. larva are shown in figure 2 of my paper ('14 a, p. 391). Its rostral end reaches the fovea isthmi. In all half grown larvae the ventral end of the strong external fissura isthmi or isthmic constriction is bent sharply rostrad to meet the ventral surface at a point a short distance caudad of the superficial origin of the III nerve, i.e., at about the level of the fovea isthmi. The external fissura isthmi, therefore, takes a course which closely parallels the ventricular sulcus isthmi, the two constrictions marking a thin line in the wall of the brain tube which separates the thicker cerebral peduncle in front from the still more massive eminentia subcerebellaris tegmenti behind, the latter appearing as a distinct eminence on both the external and the ventricular surfaces of the brain (see figs. 2 and 3 of my paper, '14 a).

In adult Amblystoma the relations of the isthmic fissure and sulcus are the same, though not so sharply defined as in the larvae, and the eminentia subcerebellaris tegmenti is much elongated. In adult Necturus the sulcus isthmi is a distinct, though shallow, ependymal groove (figs. 63, 64, *s.is.*), extending dorso-caudad from the fovea isthmi, and the eminentia subcerebellaris tegmenti is still more elongated.

From these relations it is clear that the eminentia subcerebellaris tegmenti lies in the rhombencephalon and the fovea isthmi marks, as Stieda supposed, the caudal boundary of the pedunculus cerebri on the ventral surface. It follows that in Necturus the floor of the midbrain is limited to the short distance between the tuberculum posterius and the fovea isthmi.

The complex decussations in the floor of the midbrain are commonly referred to by comparative neurologists as the ansulate commissure, but since this term as usually applied includes also the tegmental decussations below the fovea isthmi

it will be avoided here. The decussations below the fovea will
be called the ventral tegmental commissure, and those above
the fovea will be called the commissure of the tuberculum pos-
terius on account of their proximity to that landmark. This
latter commissure is a very complex system and includes, among
other elements, postinfundibular hypothalamic fibers of the de-
cussatio hypothalamica posterior. The interpeduncular nucleus
ventrally of the commissure of the tuberculum posterius is
not well developed, but caudad of the fovea isthmi the cor-
responding nucleus is very extensive, this region receiving most
of the terminals of the tractus habenulo-peduncularis (fascicu-
lus retroflexus of Meynert).

Extending from the tuberculum posterius forward and dorsal-
ward on each lateral wall of the brain is a ventricular eminence
of considerable extent which extends as far forward as the
caudal end of the pars ventralis thalami from which it is sep-
arated by a sharp sulcus. This eminence is termed the *nucleus
of the tuberculum posterius* (figs. 7, 8, 9, 23, 24, 29, 30, 47, 59,
63, 64, *nuc.tub.p.*). The nucleus of the posterior commissure
and fasciculus longitudinalis medialis lies partly within the
dorsal part of this eminence, but mostly farther dorsally (see
p. 271 and figs. 8, 23, 60, *nuc.com.post.*).

At the caudal end of the tectum mesencephali there is a
broad dorso-lateral eminence formed by the *nucleus posterior
tecti* (figs. 14, 52, 53, *nuc.p.t.*). In larval urodeles generally
there is in this region a lateral dilation of the ventricle, the
recessus posterior mesencephali. This is illustrated in a 24 mm.
embryo of Necturus by Kupffer ('06, fig. 187; for these rela-
tions in larval Amblystoma see my paper, '14 a, p. 353 and figs.
1 to 6). In adult Necturus the recess is contracted except
under the extreme caudal end of the tectum, where the roof is
thin and non-nervous, forming a small velum medullare anterius
(fig. 62, *v.m.a.*).

As we have already seen, the cerebral peduncle is bounded
caudalward by the isthmic fissure externally and the isthmic
sulcus internally. Below this constriction is a highly dif-
ferentiated region which in my former paper ('14 a, p. 353)

I have termed the *eminentia subcerebellaris tegmenti*. It lies ventrally of the cerebellum and nucleus posterior tecti and is directly continuous below with the general motor tegmentum of the medulla oblongata. It is an important relay center where many of the descending systems of the motor tegmentum are interrupted by synapses.

The diencephalon

The boundary between the diencephalon and the mesencephalon we have already discussed (p. 221). The rostral boundary of the diencephalon may be defined, following Johnston, by a plane passing from the site of the embryonic velum transversum to the optic chiasma. The exact location of this plane in the adult brain is not easily determined. The extensive unevaginated telencephalon medium of urodeles includes the preoptic nucleus, anterior commissure, lamina terminalis, lamina supraneuroporica (Johnston), paraphysis, and, according to Johnston, the chiasma ridge. The remainder of the telencephalon is represented in two lateral evaginations, the cerebral hemispheres. I have discussed the morphological subdivision of the amphibian cerebral hemisphere and its relation to the diencephalon in general in a previous contribution ('10), to which the reader is referred for an explanation of some of the terms here employed.

The floor of the diencephalon (figs. 62, 63, 64) contains the massive chiasma ridge (or a part of it), with the slender optic chiasma and the very extensive system of postoptic commissures. Farther caudad the wide infundibulum and hypophysis extend far backward under the midbrain and rostral end of the medulla oblongata.

The roof of the diencephalon is membranous in front, the postvelar arch of Minot (dorsal sac of others). Then follow the commissura habenularum (superior commissure), recessus pinealis, and pars intercalaris with its contained commissura tecti diencephali (figs. 62, 63, 64). The epiphysis, as described by Warren ('05), evaginates from the recessus pinealis (epiphyseal arch) in early embryonic stages and its cavity early

loses its connection with that of the third ventricle. The sub-
commissural organ of the midbrain roof (see p. 281) is continued
forward through most of the length of the pars intercalaris
diencephali, almost to the recessus pinealis.

Kupffer ('06, p. 174), on embryological grounds, divides the
dorsal portion of the diencephalon of Necturus into two trans-
verse regions. The more anterior of these (parencephalon,
Nebenhirn) includes the habenular bodies, dorsal sac, and
epiphysis; the more posterior (synencephalon, Schalthirn) in-
cludes the region between the epiphysis and the posterior com-
missure. The more ventral parts of the diencephalon he calls
the hypencephalon.

Johnston ('09, p. 489), in describing the development of
Amblystoma, recognizes two neuromeres in the diencephalon.
The first is largely consumed in the formation of the optic
vesicles; the second gives rise to most of the other diencephalic
structures. There is no agreement, however, among embry-
ologists regarding the metameric subdivision of either the dien-
cephalon or the mesencephalon; and any scheme of subdivision
of these regions based on primitive metamerism is as yet very
precarious.

In my opinion the attempt to elaborate a final analysis and
subdivision of the diencephalon of vertebrates on the basis of
present knowledge is premature. The immediate need is for
a precise analysis of the adult functional relations in a series of
representative species; this pattern should then be read back-
ward in the ontogeny as far as possible. Only after this has
been done are we in a position to separate the palingenetic
from the cenogenetic factors in the problem.

In this communication I shall follow the topographic sub-
division of the diencephalon which was outlined in my paper
on the morphology of the forebrain ('10, p. 431). The limits
of these subdivisions are not clearly marked on the external
surface of the brain, but on the ventricular surface they are
very evident. The sculpturing of the ventricular surface of
the mesencephalon and diencephalon of Necturus is illustrated
in figure 5 of McKibben's paper on the nervus terminalis ('11)

and by the series of cross sections shown in his figures 7 to 18, where the terminology is that of my 1910 paper. In figure 6 of McKibben's paper the sulcus *s.shab.* probably separates two lobules of the habenula and the true sulcus subhabenularis is the one marked *s.d.* Compare figures 63 and 64 of the present paper, the cross sections, figures 1 to 14, and page 229 beyond.

Figure 62 illustrates a sagittal section through the brain of Necturus drawn from a series of Weigert sections. The sections were not cut exactly parallel to the sagittal plane, which was reconstructed by superposing camera outlines on tracing paper of all of the sections which pass through this plane.

On the basis of the sagittal series from which figure 62 was drawn a graphic reconstruction was made to show the relief of the lateral walls of the diencephalic and mesencephalic ventricles. Camera outlines of the sections were drawn on tracing paper and the locations of the sulci as projected upon the median plane were determined by the superposition of these tracings (fig. 64). Figure 63 illustrates the ventricular sculpturing of this specimen as reconstructed in this way. This graphic reconstruction by flat projection was controlled by comparison with figure 5 of McKibben's paper ('11) and with a series of five additional gross sagittal sections which Dr. McKibben made in the spring of 1917 and very kindly placed at my disposal. These were prepared according to the directions published by McKibben ('13, p. 155) which ensure a minimum of distortion.

Measurements were made from the seven specimens above referred to (six gross specimens and one microscopic), which included, (a) the distance in the median plane from the tuberculum posterius to the dorso-caudal border of the commissura hippocampi, (b) from the dorsal to the ventral surface of the brain midway between the habenular and posterior commissures, and (c) the total length from the cerebellar commissure to the rostral end of the cerebral hemispheres. These measurements are given in the accompanying table, in which the measurements A, B, and C are as defined immediately above.

All of these measurements were made on the gross specimens except that of series CIII, made from microscopic preparations.

Measurements in millimeters of seven Necturus brains

SPECIMEN	FIXATION	A	B	C
McKibben, 1911, figure 5.......	Formalin-Zenker	3.46	2.58	
McKibben, CIII...............	Formalin-bichromate	2.58	2.33	11.0
Series of 1917 (1)...............	Formalin-Zenker	3.2	3.0	12.3
Series of 1917 (2)...............	Formalin-Zenker	2.9	2.9	11.7
Series of 1917 (3)...............	Formalin-Zenker	3.1	2.5	12.2
Series of 1917 (4)...............	Ammonium molybdate and formalin	2.9	2.2	11.8
Series of 1917 (5)...............	Ammonium molybdate and formalin	2.6	2.7	10.8
Average...................		2.96	2.6	11.6

The variations in these measurements are naturally large, but it is evident that the proportions of the reconstruction here shown in figure 63 fall within the limits of variation shown by the gross specimens. In this figure the distance (a) is below the average and in McKibben's figure 5 ('11) this distance is above the average. These variations are doubtless in part due to distortion in preparation, but probably in life the variations are very large.

The ventricular surfaces of specimens 1, 2, and 3 of McKibben's series of 1917 were carefully studied under the stereobinocular microscope and the drawing, figure 63, was controlled in all details from these specimens. This figure, therefore, represents a composite of the data derived from sagittal microscopic sections and the gross appearance of six half brains, the outline being based in the first instance on the microscopic sections.

In this pattern we recognize a number of massive thickenings of the lateral wall of the brain tube separated by sharp ventricular sulci which mark lines where the wall is thinner. The thickenings in general represent centers of greater proliferation of nervous elements in the embryo and regions of greater functional activity in the adult. Their pattern is in large measure

functionally determined, as will appear from the following consideration of the related fiber tracts.

The diencephalon of Necturus includes the following parts, whose boundaries are clearly defined by the ventricular sulci (figs. 63, 64): (1) the epithalamus, including the epiphysis, the habenula and dorsal sac in front of the recessus pinealis and attachment of the epiphyseal stalk, and the pars intercalaris lying between the epiphysis and the posterior commissure; (2) the pars dorsalis thalami, bounded dorsally by the sulcus subhabenularis or sulcus dorsalis thalami or both and ventrally by the sulcus medius thalami; (3) the pars ventralis thalami, bounded dorsally by the sulcus medius thalami and ventrally by the sulcus ventralis thalami; (4) the hypothalamus, bounded above by the pars ventralis thalami (see further on p. 231). The hypothalamus is continued directly forward across the chiasma ridge into the enormous preoptic nucleus, which is regarded as belonging in the telencephalon.

The details of the pattern of the ventricular sculpturing described above are somewhat variable in different specimens of Necturus and still more so when different species of urodeles are compared; but in a general way the pattern is characteristic of all Amphibia. In these animals the habenula lies far forward and it is large and somewhat lobulated. The sulcus dorsalis may be a single groove separating the epithalamus from the pars dorsalis thalami, or, as in Necturus and Amblystoma, it may be separated into two parts, (1) a sulcus subhabenularis following the ventral and caudal borders of the habenula and behind the latter curving dorsalward to enter the recessus pinealis, and (2) a partially or completely detached sulcus dorsalis extending backward from the recessus pinealis under the pars intercalaris diencephali. The details of the arrangement of these sulci are variable and probably of no great morphological significance, much depending upon the relative size and position of the habenular and posthabenular portions of the epithalamus. Kupffer many years ago ('93, p. 61) commented upon the great extent of the posthabenular region (pars intercalaris, or Schalthirn) in Amphibia as compared with other vertebrates

and the consequent position of the habenula relatively farther forward in these forms. In general, the sulcus subhabenularis forms the ventral boundary of the habenula and the sulcus dorsalis thalami that of the posthabenular region when the latter is extensive.

The posthabenular region (synencephalon of Kupffer, '06) has been well named by Gaupp ('99, p. 70) *pars intercalaris diencephali,* and the commissure contained within it ('99, p. 92) the *commissura tecti diencephali.* In the early embryos of many vertebrates the pars intercalaris is large; but its fate in most types other than the Amphibia is obscure.

The *pars dorsalis thalami* is relatively small in Necturus and in urodeles generally. It is larger in the frog and in Amniota it is still larger, comprising the great sensory nuclei of the thalamus (nucleus sensitivus of Ramón y Cajal). The *pars ventralis thalami* exhibits the converse evolutionary history, diminishing from its relatively large proportions in Amphibia to insignificance in higher forms.

At the rostral end of the thalamus there is a strong eminence which projects into the ventricle immediately behind the interventricular foramen and is termed the *eminentia thalami* (figs. 41, 42, 43, 48, 63, 64, *em.th.*). Lying somewhat dorsad and caudad of the eminentia thalami is a much smaller and less prominent eminence which marks the rostral end of the pars dorsalis thalami. As we shall see beyond (p. 244), this is the nucleus of the *pars optica thalami* (figs. 42 to 45, 48, 63, 64, 65, 68, *nuc.p.o.th.*). Laterally of this in the white layer is a superficial area of characteristic dense neuropil which in some preparations produces a slight eminence on the pial surface of the thalamus; it is here termed the pars optica thalami, for here are the actual synapses between fibers of the optic tract and thalamic neurons (fig. 1, 2, 25, 26, 40, 42 to 45, 48, 54, 56, 57, *p.o.th.*).

The *hypothalamus* and chiasma ridge are separated from the preoptic nucleus by an oblique ventricular sulcus which runs forward and ventralward in front of the chiasma ridge to end in the deep preoptic recess (figs. 63, 64). This I interpret

as a persistent portion of the anterior end of the embryonic sulcus limitans.

The hypothalamus of Necturus may be divided on topographic grounds into two general regions, which I shall term the pars dorsalis and the pars ventralis. The *pars dorsalis hypothalami* is a massive ridge through which the hypothalamus is connected with the pars ventralis thalami in front and the nucleus of the tuberculum posterius in the floor of the midbrain (figs. 7, 8, 9, 29, 30, 57, 63, 64, *p.d.hyth.*). It is comparable with the 'Haubenwulst' of Gaupp's description of the hypothalamus of the frog ('99, p. 78). The *pars ventralis hypothalami* is a more extensive thickening extending backward from the chiasma ridge in the floor and lateral walls of the infundibulum. At its rostral end the region occupied by the fibers of the postoptic commissure in the extensive chiasma ridge is the urodele equivalent of the pars subchiasmatica of the lobus infundibularis of Gaupp ('99, p. 71). Farther caudad the pars ventralis is enlarged on both sides of the median plane to form the infundibular lobes (figs. 7, 8, 9, 29, 30, 32, 57, 63, 64, *p.v. hyth.*).

The hypothalamus is in very intimate relation with the preoptic nucleus in front and with the pars ventralis thalami above. The rostral part of the pars ventralis thalami is separated from the preoptic nucleus by the very deep sulcus ventralis, but this sulcus is interrupted above the chiasma ridge (fig. 63) by a slender connecting bridge between the pars ventralis thalami and the pars ventralis hypothalami. Immediately caudad of this bridge a deep sulcus separates the dorsal and ventral parts of the hypothalamus. The ventral part of the caudal end of the pars ventralis thalami is broadly continuous with the pars dorsalis hypothalami. The boundary between them is marked, however, by a very narrow and shallow transverse sulcus which was clearly seen in the microscopic preparations and in some, but not all, of the gross preparations upon which figure 63 is based. This may be a remnant of the embryonic sulcus limitans. The dorsal part of the caudal end of the pars ventralis thalami is similarly related with the nucleus of the tuberculum

posterius, though here the boundary between the two structures is more clearly marked by a very broad depression, which may also represent a portion of the sulcus limitans.

The floor of the wide infundibulum in the mid-ventral plane is thin but nervous, containing a large tract of unmyelinated nerve fibers related with the pars infundibularis of the hypophysis. The roof of the infundibulum is also thin but nervous, containing a large number of unmyelinated nerve fibers which ramify throughout the saccus vasculosus. This is a convoluted epithelial structure which forms the more posterior part of the roof of the infundibulum (fig. 62, sac.v.). Attached to the caudal end of the infundibulum is the very large glandular part of the hypophysis (fig. 62, hyp.g.).

In this article we shall not attempt a systematic exposition of the functional connections of the diencephalon. Its mesencephalic connections will be described and other diencephalic tracts will be mentioned only incidentally or not at all.

General histology

The walls of urodele brains are seen in section to be sharply divided into a deep stratum griseum or ventricular gray, and a superficial stratum album. In ordinary histological preparations the cells of the ventricular gray appear to be similar, save for the presence of occasional groups of larger cells and for an obscure lamination in some parts of the brain.

In Necturus neither the walls of the brain tube as a whole nor their two layers are of uniform thickness throughout. The thickening of the wall may be due to the presence in the stratum album of long fiber tracts connecting remote parts, as in the cerebral peduncle, or to a more active functional differentiation locally. In the latter case the stratum griseum will be thickened as a result of an increase in the number of cell bodies of the contained neurons, and this thickening in general first takes the form of a projection into the ventricle rather than outward into the stratum album. The eminences thus formed on the ventricular surfaces of the brain are, therefore, of great value

in determining the regions of highest functional importance. Their positions and functional connections are tolerably constant in different brains of the same species, but their relative development varies widely in different urodeles.

The dendrites of the neurons whose cell bodies lie in the ventricular gray layer arborize chiefly in the stratum album, where they effect synaptic connections with their appropriate systems of fibers. These connections are for the most part made within a diffuse neuropil whose analysis is extremely difficult on account of the lack of structural landmarks defining the functionally differentiated regions. The more highly developed functional regions are, however, characterized by a denser neuropil in the stratum album, shown by Golgi preparations to be composed of an intricate felt-work of dendrites and axonal terminal arborizations. Such regions are sometimes so sharply circumscribed and dense as to form true glomeruli, and they may even form low eminences on the outer surface of the brain. Many such regions of neuropil can be identified as functional equivalents of special groups of neurons ('nuclei') of higher brains, though in Necturus the corresponding cell bodies may be located in quite remote parts of the gray layer. A single neuron may send dendrites to more than one such region, thus receiving two or more functionally distinct kinds of excitation.

There are very few cell bodies of any kind in the stratum album. Figures 5 to 14 illustrate the arrangement of these cell bodies in the midbrain and some of them are shown as impregnated by the Golgi method in figures 24, 32, 33, 35 (m.n.). Their dendrites tend to be arranged tangentially.

The stratum album contains ependymal fibers (figs. 34, 43), various myelinated and unmyelinated fiber tracts, dendrites and axons of the cells of the stratum griseum, and the several areas of specially differentiated neuropil referred to above. These structures are not arranged in definite laminae, nor are the fiber tracts in general gathered into distinct bundles as definitely as in most other vertebrates, so that their analysis offers many difficulties. The number of myelinated fibers is

smaller in Necturus than in most other Amphibia, and for this
reason the more fundamental systems of myelinated tracts
can be followed here notwithstanding their diffuse formation.
On the other hand, the analysis of the much more extensive
unmyelinated systems is a problem requiring the most careful
study of many different kinds of histological preparations, the
silver impregnation methods of Golgi and Ramón y Cajal having
been chiefly relied upon in this research.

With this general orientation in mind, we shall now take up
the consideration of the fiber tracts and the functional connec-
tions of the parts just enumerated. Notwithstanding the great
mass of histological material examined and the variety of tech-
nique employed, the results of this study are still very incom-
plete and many obscure points remain for future research.

III. THE PERIPHERAL CONNECTIONS OF THE MIDBRAIN AND THALAMUS

These regions of the brain are connected with the periphery
by the III and IV nerves, the mesencephalic root of the V nerve,
the nervus terminalis, and the parietal nerve (fig. 65); they are
related to the retina through the so-called optic nerve and optic
tracts, but since the retina is really a part of the brain these
tracts belong morphologically with the lemniscus systems and
will be described in the next section.

The eye-muscle nerves of Necturus have been described by
Kingsbury ('95) and by McKibben ('13). The mesencephalic
root of the V nerve has been described by Osborn ('88), Kings-
bury ('95), Johnston ('05), Norris ('13), and Herrick ('14).
Some further details regarding this root in larval Amblystoma
are included in my paper on the medulla oblongata of Ambly-
stoma ('14 a, p. 361). The central connections of the nervus
terminalis of Necturus have been described by McKibben ('11).
To these descriptions I have little to add.

1. The oculomotor nerve

The superficial origin of the III nerve lies at the transverse
level of the fovea isthmi (p. 221). The heavily myelinated

root fibers arise in two or three fascicles, of which the larger comes from the neurons of the central gray at about the same dorso-ventral level as the superficial origin of the root (fig. 11). These neurons are somewhat larger than the surrounding cells of the central gray, though the limits of the nucleus are not sharply defined. There is a more ventral fascicle of root fibers which connects with the neurons of the floor of the fovea isthmi. Some of these fibers appear to come from the nucleus of the opposite side. This medial ventral nucleus is probably the nucleus of Edinger-Westphal, giving rise to visceral efferent fibers.

2. *The trochlear nerve*

The IV nerve immediately distally of its crossing in the decussatio veli contains about 20 very coarse and heavily myelinated fibers. Their peripheral relations have been described by McKibben ('13, p. 158), who also describes the relations of the IV nerve near its decussation with coarse myelinated fibers of the tectum mesencephali and also with myelinated fibers distributed peripherally to the choroid plexus of the fourth ventricle. The latter have been previously noted by Kingsbury ('95, p. 146).

The internal course of the IV nerve is very difficult to follow, for its fibers on the proximal side of their decussation are scattered and mingled with those of the mesencephalic root of the V nerve, which are also coarse and heavily myelinated. In Weigert preparations cut in the sagittal plane they can be followed from the decussation downward and forward in the deepest layer of the stratum album, accompanying the finer fibers of the tecto-peduncular system, to a region caudad of the nucleus of the III nerve, but the exact location of their nucleus of origin has not been demonstrated.

3. *The mesencephalic root of the V nerve*

The cells of the nucleus of this root (nucleus magnocellularis tecti) are widely distributed throughout the entire tectum mesencephali, especially caudad, and their axons, which are

coarse and heavily myelinated, lie in the deepest layer of the stratum album (figs. 9 to 14, *r.V.mes.*). This nucleus and root can be readily identified in both Weigert and Cajal preparations.

4. The nervus terminalis

The central course of the nervus terminalis of Necturus has been described by McKibben ('11), and I have confirmed his description in every particular. These unmyelinated fibers enter the brain at the ventral border of the olfactory bulb and pass backward in many slender fascicles in a very superficial position along the ventral border of the cerebral hemisphere and ventro-lateral border of the diencephalon, as far as the tuberculum posterius in the cerebral peduncle. Some of these fascicles decussate in the anterior commissure, in the postoptic commissure, and in the commissure of the tuberculum posterius. The mesencephalic fibers apparently end in the immediate vicinity of the commissure of the tuberculum posterius.

5. The parietal nerve

The epiphysis of Necturus has been described by Kingsbury ('95, p. 160) and its development has been studied by Warren ('05). It is a hollow epithelial vesicle, whose cavity presents numerous diverticula formed by incomplete epithelial septa. In early developmental stages this cavity becomes completely separated from the diencephalic ventricle by the occlusion of the cavity of the epiphyseal stalk.

The parietal nerve of Necturus was seen by Kingsbury, who writes ('95, p, 161): "Two or three myelinic nerve fibers, on each side, were found to pass to the ectal surface of the brain and disappear after turning mesad beneath the epiphysis. They came from the mesencephal to which they could be traced caudad."

To this description I can add some further details without, however, reaching a satisfactory interpretation. The number of myelinated nerve fibers connected with the epiphysis in our

specimens is much greater than the two or three seen by Kingsbury. They enter the epiphysis from each side at its attachment to the epithalamus and then turn dorsalward through the epiphyseal stalk and spread out in the walls of the vesicle.

The study of the peripheral distribution of these fibers is rendered very difficult by the presence in the epiphyseal epithelium of granules of a lipoid substance which stains in our Weigert preparations with exactly the same color as the myelin sheaths. This material is intracellular and is very abundant in the epithelium of the pineal vesicle and less so in the dorsal sac and paraphysis. In the epiphysis it takes various forms as seen in Weigert preparations—sometimes small deep blue granules scattered throughout the cytoplasm, sometimes crowded crescentic masses of deeply stained granules closely applied to the nucleus, and sometimes isolated larger deep blue granules. The latter form is in some cases difficult to distinguish from the myelinated nerve fibers. The nerve fibers are, however, intercellular and under high powers of the microscope can usually be recognized. They appear to be distributed throughout all parts of the pineal vesicle.

In view of the presence in the pineal sac of Sphenodon (Dendy, '10) of nerve cells, which are said to give rise to the right pineal nerve, I have looked for evidence of nerve cells in the epiphysis of Necturus, but without success. Accordingly, I am unable to say whether these nerves are afferent or efferent with reference to the brain.

The course of the parietal nerve will first be described as seen in horizontal sections by the Weigert method. There are three imperfectly separable roots of the parietal nerve, (1) a rostral root connected with the brain in front of the habenular commissure and observed on only one side; (2) a middle root, larger than the other two and connected with the brain behind the habenular commissure; and (3) a caudal root, distally associated with the second root but proximally turning caudad near the mid-plane to intersect the fibers of the commissura tecti diencephali. The fibers of these three roots (or part of them) unite within the brain ventrally of the pars inter-

calaris diencephali and accompany the tractus habenulo-
peduncularis, or fasciculus retroflexus of Meynert.

The epiphysis envelopes the commissura habenularum dorsal-
ly and nerve fibers leave the epiphysis, not only at its attach-
ment behind the commissure, but also in smaller numbers
farther rostrally. The last was observed only on one side, the
fibers of this rostral root passing from the epiphysis into the
rostral border of the commissura habenularum, thence laterally
and slightly rostrad to the rostral surface of the habenula, where
they turn ventrad. At lower levels these fibers turn ventrad
and join the larger second root. The larger bundle of fibers
forming the second or middle root of the parietal nerve is com-
posed of very coarse deeply staining myelinated fibers and
passes from the epiphyseal stalk into the habenula immediately
caudally of the habenular commissure, then descending through
the substance of the habenula. At the level where the tractus
habenulo-peduncularis leaves the caudal border of the habenula
these fibers accompany it and are immediately joined by the
fibers of the first root.

The commissura tecti diencephali is extended forward through-
out the whole length of the pars intercalaris of the diencephalon
and thus comes almost into contact with the place of attach-
ment of the pineal stalk. The fibers of the third root of the
parietal nerve pass backward crossing at right angles the fibers
of the commissura tecti but none of them were seen to decussate
in the commissure. It is probable that none of the fibers of
the parietal nerve decussate in either the commissura habenu-
larum or the commissura tecti diencephali.

The fibers of the parietal nerve can be followed ventralward
accompanying those of the tractus habenulo-peduncularis (which
is wholly unmyelinated in Necturus) across the massive emi-
nence which underlies the pars intercalaris of the roof (Schalt-
stück) and to which I shall give the same name (figs. 63, 64,
65 *p.i.th.*). Some of these fibers may end in the habenula and
others in the pars intercalaris, but others certainly turn ventral-
ly with the tractus habenulo-peduncularis. Their ultimate
destination has not been determined, for they become mingled

with other myelinated fibers belonging to the tractus thalamo-peduncularis dorsalis which come from the pars dorsalis thalami.

In the sagittal series stained by Weigert's method the same relations were found as in the horizontal series, including the small first root entering the brain in front of the habenular commissure on one side. Fibers of the parietal nerve near the median plane are mingled with those of both the commissura habenularum and the commissura tecti diencephali, but none of them decussate in these commissures. At the dorsal surface of the brain the parietal nerve is represented by several fascicles each containing about six heavily myelinated fibers, which farther ventrally converge to join the unmyelinated fibers of the tractus habenulo-peduncularis. They can be definitely followed ventralward for about half the length of the latter tract, and probably reach the cerebral peduncle, though this cannot be positively stated.

In the transverse Weigert series also these connections of the parietal nerve are confirmed (figs. 1 to 7, *n.par.*). The rostral root was not demonstrated, but the middle root passes downward and joins the tractus habenulo-peduncularis, as in the horizontal and sagittal series. It is joined farther caudally by the third root in the same way. No decussating fibers were seen. In this series also the nerve can be traced about half of the distance between the habenula and the interpeduncular nucleus.

From these incomplete observations it appears that the parietal nerve of Necturus is composed of a small number of heavily myelinated fibers running between the epithelium of the pineal vesicle and a point dorsally of the tuberculum posterius near the boundary between the pars ventralis thalami and the pedunculus cerebri or some point still farther ventrally. There is no evidence of nerve cells or of any active sensory function in the pineal vesicle. The nerve fibers here demonstrated are, therefore, probably not functionally equivalent with those of the unmyelinated parietal nerve described by Dendy in Sphenodon. From their connection with the motor lamina of the brain tube it is suggested that they are probably efferent in function

Gaupp ('99, p. 95) is also inclined to question the homology of the parietal nerve of the frog with that described for reptiles.

IV. THE FIBER TRACTS

1. The lemniscus systems

The term lemniscus (or fillet or laqueus) was originally applied to certain sensory tracts of second or higher orders terminating in the thalamus. In the interest of uniformity of terminology there is a tendency among recent writers to enlarge the concept to include all of the sensory tracts of this group. Thus we have various spinal and bulbar lemniscus systems, each of these tracts being defined by its functional connections. The olfactory tracts are excluded, because they have no direct connections with the thalamus proper; but the so-called optic nerves should be included.

In fishes these lemniscus systems connect primarily with the tectum mesencephali, and the thalamic connections are for the most part effected by tracts of a still higher order after a synapse in the tectum (tecto-thalamic tracts). In the tailed amphibians the tectal connections are still the dominant features of these systems, but some fibers of most of the lemniscus tracts continue forward under the tectum to connect directly with the thalamus, and the latter type of connection becomes increasingly important in higher vertebrates. Accordingly, in vertebrates in general, it is convenient to include with the lemniscus tracts the tectal, as well as the thalamic fibers.

In Necturus I have recognized the following members of the lemniscus complex: (1) the optic lemniscus (tractus opticus), (2) the spinal lemniscus (tractus spino-tectalis et thalamicus), (3) the acoustico-lateral lemniscus (fasciculus lateralis of Mayser in fishes, lateral lemniscus of mammals), (4) tractus bulbo-tectalis (of uncertain significance), (5) the visceral lemniscus (secondary vagus bundle of Mayser in fishes, ascending visceral tract). These will next be described.

2. The optic tracts

The fibers of the optic nerves of Necturus are entirely unmyelinated and can therefore readily be distinguished from the myelinated fibers of the postoptic commissure complex with which they are mingled in the chiasma ridge. Moreover they are readily impregnated in preparations prepared by the methods of Golgi and Ramón y Cájal, so that one would expect to be able to gain a tolerably complete knowledge of their central connections. The relations about the optic chiasma are, however, peculiarly difficult and many points remain obscure.

As has long been known, the so-called optic nerve of adult Necturus retains the embryonic character of a hollow epithelial tube whose lumen communicates with the ventral part of the pre-optic recess. The number of fibers in the nerve is relatively small and as the nerve approaches the optic chiasma these fibers are accumulated chiefly in the caudo-ventral wall of the epithelial tube (fig. 57). All of these fibers decussate in the optic chiasma.

In the frog and some other lower vertebrates, connections of the optic nerve have been described with other parts of the brain than the optic tectum of the midbrain. Wlassak ('93) describes three optic tracts in the frog, the axial, marginal, and basal bundles. The marginal bundle is the chief tract and maintains a superficial position until it terminates in the more superficial layers of the stratum medullare superficiale of the tectum. The axial bundle is of coarser fibers, which decussate in the chiasma farther dorsally than the other optic tracts. They pass across the face of the thalamus at a deeper level than those of the marginal bundle, traversing both the corpus geniculatum thalami and its nucleus anterior superior (see below, p. 243), and terminate in the deeper layers of the stratum medullare superficiale of the tectum. These fibers appear to be those described by Bellonci ('88) as fibrae ansulatae. The basal bundle decussates farthest ventral and caudal in the chiasma and its fibers pass backward to a ventral nucleus in the cerebral peduncle lying rostrally of the oculomotor nucleus.

In Necturus I have found these three parts of the optic tract substantially as described by Wlassak, though all are unmyelinated both before and after their decussation. The axial bundle is composed of coarser unmyelinated fibers than the others and in the nerve immediately distally of the chiasma these fibers occupy the dorsal part of the cross section. Upon entering the brain they turn sharply caudad and dorsad (figs. 1, 15 to 19, 25, 65 *tr.op.ax.*) and decussate in the dorso-rostral part of the chiasma ridge. Immediately after their decussation these fibers spread out and pass dorsad and caudad throughout the deeper parts of the stratum album above the chiasma ridge, some of them penetrating the lateral forebrain tract (figs. 1, 18, 19, 65, *tr.op.ax.x.*). They can be followed as far dorsally as the sulcus ventralis thalami; beyond this level they are so scattered that it is impossible to distinguish them from the fibers of the postoptic commissure with which they are mingled (see further, p. 244).

The basal bundle of the optic tract is of considerable size; its fibers decussate in the ventral part of the chiasma ridge in company with those of the marginal bundle. After their crossing they turn abruptly caudad and dorsad along the extreme lateral surface of the hypothalamus and end on the ventro-lateral surface of the cerebral peduncle among the dendrites of the neurons of the cerebral peduncle rostrally of the nucleus of the III nerve. These dendrites pass to the lateral surface of the brain and then forward and participate in the formation of a dense neuropil which I term the *area lateralis tegmenti.* The contorted fibers of the basal optic tract end freely in this dense neuropil. They do not extend farther caudad than the level of the III nerve (figs. 52 to 55, 65, *tr.op.b.*). I find this area of neuropil in larval Amblystoma and within it free terminals of fibers derived from the chiasma ridge. In these larvae also I have observed free endings of fibers of similar appearance coming into the same neuropil from behind, probably fibers of the ascending visceral tract (p. 250, cf. also p. 295).

The fibers here designated as basal optic tract undoubtedly correspond with the 'basal optic bundle' of Wlassak's account

of the frog. That they are actually optic fibers entering the peripheral optic nerve is not definitely shown by our preparations, although this is certainly the appearance. At their decussation the fibers of the optic tracts are in intimate association with those of the tractus tecto-thalamicus et hypothalamicus anterior, most of whose fibers are also unmyelinated (see p. 256). In our material it is impossible to be certain that the fibers of the so-called basal optic bundle do not come from the opposite tectum opticum after decussation in the postoptic commissure.

The marginal bundle of the optic tract receives the largest part of the peripheral optic fibers, these fibers crossing in the most ventral and rostral part of the chiasma ridge. After their decussation they pass directly lateralward and dorsalward across the lateral aspect of the preoptic nucleus and thalamus to enter the rostral end of the roof of the midbrain at its dorsomedial border. Throughout the whole of their course within the brain these fibers are strictly superficial (figs. 1 to 12, 16, 17, 25, 28, 36 to 41, 44, 45, 46, 48, 52 to 55, 57, 58, 65, *tr.op.*). The part of the tectum mesencephali reached by these optic fibers will be termed the *colliculus superior*, though there are no external evidences of its precise limits.

In the frog Gaupp ('99, p. 79) describes in the caudal two-thirds of the thalamus a division of the stratum album into a stratum medullare profundum and a stratum medullare superficiale with a gray mass, the corpus geniculatum, between. The stratum medullare superficiale is composed chiefly of fibers of the optic tracts. Correlated with the reduction of the optic tracts in Necturus, there is no obvious division of the stratum album into deep and superficial fiber layers, nor is the corpus geniculatum thalami of Gaupp especially differentiated.

In the anterior third of the thalamus of the frog, in addition to the corpus geniculatum thalami and more dorsally and superficially of it, is a smaller circumscribed area of neuropil in the stratum album, the nucleus anterior superior corporis geniculati thalami of Bellonci ('88, p. 10). This area of neuropil is well differentiated in Necturus (figs. 1, 2, *p.o.th.*). As the

marginal optic tract passed dorsalward across the rostral end
of the pars dorsalis thalami, it traverses this area superficially
and sends numerous collaterals into it (fig. 54, *p.o.th.*).   There
are no complete neurons in this area, which is simply an entangle-
ment of the richly arborized dendrites of neurons whose cell
bodies lie in the underlying gray layer, together with terminal
arborizations from collaterals of the fibers of the optic tract
and other types of fibers (figs. 25, 26, 40, 42 to 45, 48, 54, 56,
57, 65, *p.o.th.*).   The character of these other types of fibers is
not altogether clear.   They lie at deeper levels than those of
the marginal optic tract and exhibit free endings of a different
morphological type from the collaterals from the latter tract
(figs. 26, 56, 57, *p.o.th.*).   They may be derived either from the
axial optic tract or from the tractus tecto-thalamicus et hypo-
thalamicus cruciatus by way of the postoptic commissure (p.
256).   This area of neuropil contains synaptic connections
between the fibers of the optic tract and neurons of the thala-
mus, and is, therefore, functionally comparable with the optic
centers of the mammalian thalamus in spite of its anomalous
position near the rostral end of the thalamus.   Since this area
seems to constitute the only optic receptive center in the
thalamus, I shall term it simply the optic part of the thalamus
(*pars optica thalami*).

This neuropil of the pars optica thalami is reached by den-
drites from rather widely separated parts of the stratum griseum
of the thalamus, including the pars dorsalis farther caudad and
the pars ventralis; but there is one group of cell bodies producing
a slight ventricular eminence between the eminentia thalami,
the pars ventralis thalami, and the pars dorsalis thalami whose
dendrites seem to be especially related to the ventral part of
this area, which has already been referred to (p. 230) as the
nucleus of the pars optica thalami (figs. 42 to 45, 48, 63, 64,
65, 67, 68, *nuc.p.o.th.*).

At the level of the posterior commissure the marginal optic
tract enters the midbrain roof, passes inward toward the mid-
dorsal line, and distributes by widely spread free arborizations
of its fibers throughout the dorso-medial part of the tectum

mesencephali. This area of distribution is the true tectum opticum, or colliculus superior; apparently it does not extend backward to the caudal end of the tectum (figs. 9 to 12, 21 to 24, 32, 33, 36, 37, 65, 67 *col.sup.*).

3. *The spinal lemniscus*

This tract has been referred to in my paper on the cerebellum of Necturus ('14, pp. 7, 11) under the name tractus spino-tectalis and more fully described in larval Amblystoma ('14 a, p. 374) with the same designation. In Necturus, as in larval Amblystoma, fibers ascend in the lateral funiculus of the spinal cord and traverse the entire length of the medulla oblongata as a large fascicle containing spino-bulbar, spino-cerebellar, spino-tectal, and spino-thalamic fibers. The fibers of this system which ascend beyond the isthmus (i.e., the tractus spino-tectalis and tractus spino-thalamicus) are here termed spinal lemniscus.

For the relations of the spinal lemniscus in the medulla oblongata of Necturus and Amblystoma, see the papers last cited. In the region of the auricular lobes just caudad of the isthmus the spinal lemniscus fibers are intimately associated with those of the tractus thalamo-peduncularis cruciatus which descends from the postoptic commissure (see p. 259). At the level of the isthmus the spinal lemniscus fibers ascend sharply and take a position as a series of small fascicles at the boundary between the gray and white layers immediately ventrally of the fibers of the mesencephalic V root and dorso-medially of the much larger acoustico-lateral lemniscus (figs. 6 to 14, *tr.sp.t.* and *tr.sp.th.*). In this relation they continue forward throughout the entire length of the tectum, most of them ending in its caudo-lateral part (colliculus inferior, figs. 21, 24, 32, 33, 35, 56, *tr.sp.t.*); but a smaller number clearly continue forward to terminate in the caudal part of the pars dorsalis thalami (figs. 6, 66, *tr.sp.th.*). These fibers are coarser than those of the bulbar lemniscus systems and can readily be distinguished from them.

As I have already mentioned in the case of Amblystoma larva ('14 a, p. 376), the spino-bulbar, spino-cerebellar, spino-tectal, and spino-thalamic fibers of urodeles appear to belong to a single primitive ascending system which distributes nervous impulses throughout the entire length of the brain stem. Many of the terminals ending in all of these levels are collaterals from the long tectal and thalamic fibers; this applies even to the spino-cerebellar fibers, which form one of the most clearly differentiated members of the system.

4. *The acoustico-lateral lemniscus*

This is a relatively compact tract of small but heavily myelinated fibers which arise throughout the entire extent of the acoustico-lateral area of the oblongata. It is the equivalent of the fasciculus lateralis of Mayser ('81) in teleosts, and in a more general sense of the lateral lemniscus of mammals. Its fibers immediately after leaving their cells of origin decussate in the ventral commissure of the medulla oblongata as internal arcuate fibers and then turn forward in the middle depths of the stratum album. In the caudal part of the oblongata this tract lies not far from the mid-ventral plane; farther forward it moves lateralward, increasing in size, and at the isthmus turns abruptly dorsalward to enter the tectum mesencephali. Its bulbar course in Necturus has been well described and figured by Kingsbury ('95, p. 192), and in the upper levels of the oblongata it is figured in my paper on the cerebellum ('14, figs. 4 to 11). I have described the origin and spinal portion of this tract in larval Amblystoma under the name tractus octavo-tectalis et thalamicus ('14 a, p. 369).

In the midbrain this lemniscus tract ascends along the ventral border of the tectum through the intermediate level of the stratum album (figs. 7 to 14, 21, 24, 32, 33, 35, 39, 41, 49, 53 to 56, 66, *lm.*). Most of its fibers terminate in a diffuse neuropil in the more lateral and caudal parts of the tectum, i.e., in the portion of the roof which I have compared with the colliculus inferior of mammals (figs. 39, 53 to 56, *lm.*). Many terminals

enter also the optic part of the tectum, or colliculus superior (figs. 21, 24, 39, *lm.*). A smaller number of its fibers continues forward to terminate in the caudal part of the pars dorsalis thalami (fig. 66, *lm.*).

5. *Tractus bulbo-tectalis*

Under this name I have described in larval Amblystoma ('14 a, p. 372) a companion tract to the acoustico-lateral lemniscus which follows the ventral border of the latter tract throughout the medulla oblongata. A portion of its course in the upper part of the oblongata is figured in my former paper ('14, figs. 4 to 9, *tr.b.t.*). In the isthmus region it turns dorsalward along the lateral side of the acoustico-lateral lemniscus and its fibers end by free arborizations in the nucleus posterior tecti. The origin of these fibers is imperfectly known. In larval Amblystoma some of its axons have been seen to arise from neurons related to both the spinal V tract and the fasciculus solitarius of the opposite side of the medulla oblongata, and it is provisionally regarded as an imperfectly differentiated crossed secondary ascending trigeminal tract, probably with visceral connections also.

In this contribution I am able to add nothing further regarding the origin and significance of this tract. In the upper levels of the medulla oblongata of Necturus it is easily recognized in the same position as in Amblystoma, and its feebly myelinated fibers distribute in the same way in the tectum. Golgi preparations show these fibers ending in wide arborizations throughout the stratum album of the nucleus posterior tecti (figs. 14, 50 to 53, 66, *tr.b.t.*). The area of distribution of these fibers is a rather large and poorly defined region of neuropil filled with the coarse and contorted dendrites of the underlying neurons and the widely branched fine terminals of the bulbar tract (figs. 36, 37, 50 to 53, 66, *nuc.p.t.*). This nucleus gives rise to a strong component of the tractus tecto-thalamicus et hypothalamicus cruciatus posterior for the postoptic commissure complex (p. 257), and also to the superficial tractus tecto-peduncularis posterior (p. 254).

In the frog (Gaupp, '99, p. 50) there is a large and very dense nucleus behind the tectum at the level of the decussation of the IV nerves, which is termed the 'ganglion isthmi.' Since the term ganglion should be reserved in vertebrate neurology for collections of nerve cells outside the central nervous system, I shall term this structure the *nucleus isthmi*. The nucleus posterior tecti of Necturus may represent the nucleus isthmi in a far less compact and integrated form than in the frog, though in the present state of our knowledge this homology should not be accepted without reserve. Bellonci ('88, p. 26) regards the nucleus isthmi of the frog (which is the nucleus magnus of Reissner, '64) as the true corpus posterius, or colliculus inferior—an untenable homology, as has been pointed out by Gaupp ('99, p. 62).

6. The visceral lemniscus

In larval Amblystoma I described ('14 a, p. 373) an uncrossed secondary visceral tract comparable with the secondary vagus bundle of Mayser ('81) and with my ascending secondary gustatory tract ('05) in teleosts. Its fibers arise from neurons of the nucleus of the fasciculus solitarius and terminate in an area of neuropil in the isthmus region which occupies the angle between the auricular lobe and the midbrain ('14 a, fig. 5, *tr.v.a.*). This neuropil was regarded as the representative of the teleostean 'Rindenknoten' of Mayser, or superior secondary gustatory nucleus of my paper published in 1905. Associated with it is a group of enlarged neurons ('14 a, fig. 52).

The neurons which constitute the secondary visceral nucleus ('Rindenknoten') can be recognized in our sections of adult Necturus as a lateral extension of the central gray in the angle between the body of the cerebellum and the midbrain. They lie immediately dorsally of the eminentia subcerebellaris tegmenti and therefore dorsally of the locus of the sulcus limitans. This extension of the ventricular gray toward the surface of the brain in the isthmus region must not be confused with the superficial gray matter at the rostral end of the auricular lobe

which closes in the anterior diverticulum of the recessus lateralis ('14, pp. 2, 3 and fig. 22) and lies farther laterally and morphologically (though not anatomically) farther caudad. Ventrally of this nucleus and laterally of the dorsal part of the eminentia subcerebellaris tegmenti are a few scattered neurons in the stratum album, two of which are illustrated in figure 18 of my former paper ('14). These apparently do not belong to the secondary visceral nucleus, though their lateral dendrites arborize within the secondary visceral tract near its termination and probably receive nervous impulses from it.

In larval Amblystoma, where the recessus posterior of the tectum mesencephali is wide and deep (see '14 a, p. 353 and fig. 2, *r.p.m.,*) this nucleus lies in the posterior wall of this evagination in front of the isthmus; it belongs, therefore, in the midbrain rather than in the medulla oblongata.

In Necturus the secondary visceral tract or visceral lemniscus is wholly unmyelinated and, as in larval Amblystoma ('14 a, figs. 5, 6, 52, 54), it ascends from the medulla oblongata ventrally of the tractus spino-cerebellaris and dorsally and laterally of the acoustico-lateral lemniscus and tractus bulbo-tectalis. It does not appear in Weigert sections, though its locus can be readily identified by comparison with the Cajal and Golgi sections (fig. 14, *tr.v.a.*). The fibers of this tract in our Golgi preparations are very slender and unusually varicose. At the level of the isthmus they lie at the extreme ventro-lateral surface of the brain in the angle between the auricular lobe and the midbrain. Here the tract divides into two parts, some of the fibers continuing forward ventro-laterally of the eminentia subcerebellaris tegmenti and others turning abruptly dorsally to reach their nucleus. The latter fibers cross the tractus bulbo-tectalis superficially and then turn medialward to arborize in the secondary visceral nucleus (figs. 50 to 54, 66, *tr.v.a.* and *nuc.vis.s.*).

We have no complete impregnations of the neurons of the secondary visceral nucleus or of the tertiary visceral tract arising from it. Dendrites and axons of some of these cells are seen in figure 50, *nuc.vis.s.* In Cajal and Golgi sections a slen-

der unmyelinated tract is seen directed forward, lateralward and ventralward from the nucleus (figs. 50, 66, *tr.v.t.*). This is the tertiary visceral tract. It pierces the tractus bulbo-tectalis to reach the ventro-lateral surface, where it joins the forward extension of the secondary visceral tract already mentioned. The combined secondary and tertiary visceral bundle continues forward to the region laterally of the nucleus of the III nerve. Immediately rostral to this region is the dense superficial neuropil termed area lateralis tegmenti, which receives the supposed basal optic tract (p. 242) and the fibers of the superficial tractus thalamo-peduncularis dorsalis derived from the pars intercalaris thalami (p. 264). These visceral fibers may terminate in this neuropil or they may effect other connections farther rostrally (see p. 284).

In my account of the cerebellum of Necturus ('14, p. 8) I described a connection between the hypothalamus and the cerebellum which was provisionally called the tractus mamillo-cerebellaris ('14, fig. 19, *tr.m.cb.*) and was regarded as a special portion of a diffuse hypothalamo-tegmental system. It now appears that the tract there described includes the combined secondary and tertiary visceral tract, with perhaps mamillo-tegmental fibers mingled with them. My study of this region is still incomplete.

No commissural fibers have been traced from the secondary visceral tract or its nucleus in the isthmus into the decussatio veli, as in fishes, though a few fibers of this sort may occur.

7. Tractus tecto-cerebellaris

The connections between the cerebellum and the midbrain are not condensed into definite tracts and their study is very difficult. By analogy with other vertebrates it is probable, as I have before suggested ('14, pp. 6, 9), that tecto-cerebellar fibers are present in the confused collection of fibers bordering the rostral border of the cerebellum, but I have not been able to demonstrate them separately.

8. Brachium conjunctivum

These fibers are unmyelinated. Their origin in Necturus I have previously described ('14, p. 8). They are axons of neurons in the ventral part of the body of the cerebellum and floor of the lateral recess ('14, figs. 4, 18, 19); these neurons are, therefore, to be compared with the dentate nucleus of mammals. The fibers of the brachium conjunctivum are directed forward and inward in rather compact formation in immediate contact with the stratum griseum. In the region of the eminentia sub-cerebellaris tegmenti they drop ventralward and decussate in the ventral commissure as the most dorsal fibers of this complex decussation.

I have no clear Golgi impregnations of the further course of these fibers and they take a very pale color in Cajal preparations. In horizontal Cajal preparations they can be followed, mingled with other pale fibers derived from the tectum and the motor tegmentum under the cerebellum, to their decussation at a level near the fovea isthmi. Their course beyond the decussation is more obscure.

9. The dorsal commissures

A complex series of dorsal commissural and decussating fibers extends from the habenular bodies back to the decussatio veli at the level of the cerebellum without interruption except in the regions of the pineal recess and recessus posterior mesencephali. The first of these is the habenular commissure, or commissura superior, which will not be further considered in this communication. Then follow (1) the commissura tecti diencephali, (2) the commissura posterior, and (3) the commissura tecti mesencephali.

1) The commissura tecti diencephali. This includes the myelinated and unmyelinated fibers which cross through the roof of the pars intercalaris diencephali (Schaltstück) between the pineal recess and the posterior commissure. Part of the myelinated fibers appear to be truly commissural between the adjacent centers in the pars intercalaris. Others are decussating

fibers of the tractus tecto-habenularis cruciatus (see p. 261 and figs. 5, 6, 28, 37, 62, *com.t.d.*).

2) *The commissura posterior.* This includes a very compact mass of coarse and heavily myelinated fibers crossing at the rostral end of the tectum mesencephali. It is continuous in front with the commissura tecti diencephali and behind with the commissura tecti mesencephali. Fibers enter it from the rostral part of the tectum (tectum opticum or colliculus superior). Some of these are probably true commissural fibers; others after their decussation turn abruptly ventralward and slightly caudalward keeping close to the gray layer. These end in the large ventricular eminence dorsally of the tuberculum posterius which contains large neurons of the nucleus of the posterior commissure (figs. 7, 8, 29, 38, 39, 41, 59, 62, 63, 64, 68, *com.post.*). I have not been able to separate these large neurons into distinct nuclei of the posterior commissure and nucleus of the fasciculus longitudinalis medialis, the dendrites of these cells being related to the commissural fibers and their axons entering the longitudinal fasciculus (p. 271). Fibers of the commissura posterior doubtless also effect direct synaptic connection with dendrites of the nucleus of the III nerve, though this has not been directly observed.

3) *The commissura tecti mesencephali.* This is a continuation of the posterior commissure backward throughout the length ·of the tectum. It is a thin sheet of myelinated fibers whose connections have not been definitely observed. Some are probably true commissural fibers; others are doubtless tecto-peduncular fibers (figs. 9 to 14, 62, *com.t.m.*).

In Necturus the tectum mesencephali seems to be the most important correlation center for afferent somatic sensory impulses. From it efferent tracts diverge in all directions, for the most part in rather diffuse formation. Some of the more definite collections of these fibers are enumerated below.

10. *Tractus tecto-bulbaris*

These descending myelinated fibers arise from all parts of the tectum, especially the region of the colliculus inferior, and ac-

cumulate on the ventro-lateral border of the motor tegmentum below the fovea isthmi, a large proportion of them first decussating in the underlying ventral commissure. They continue into the ventral tracts of the medulla oblongata, and some of them may extend farther into the spinal cord as a tractus tecto-spinalis. In larval Amblystoma I have figured their course through the medulla oblongata ('14 a, figs. 4 to 17), as they descend near the median plane ventrally of the fasciculus longitudinalis medialis. The relations of the tractus tecto-bulbaris rectus et cruciatus are in Necturus essentially the same as in fishes (figs. 11 to 14, 21, 24, 68, *tr.t.b.*).

11. *Tractus tecto-peduncularis*

Fibers of the same type (myelinated and unmyelinated) as the tractus tecto-bulbaris arise from the entire length of the tectum and pass ventralward to reach the underlying motor tegmentum. These are for the most part directed rostrad as well as ventrad and are, therefore, readily distinguished from those of the tractus tecto-bulbaris (fig. 68, *tr.t.ped.*). They are arranged in superficial, deep, and intermediate groups.

The superficial group (*tractus tecto-peduncularis superficialis*) arise from the entire length of the tectum and form the most superficial fibers of the stratum album (figs. 10, 11, *tr. t.ped.s.*). Having reached a point dorsally and caudally of the superficial origin of the III nerve, these fibers turn inward, still directed ventralward, and effect various connections with the motor centers of the tegmentum, partly of the same side and partly after decussation in the ventral commissure. Some reach the nuclei of the commissura posterior and III nerve of the same side. Some appear to enter the fasciculus medialis longitudinalis directly without decussation. Others cross in the ventral commissure, some immediately rostrally of the fovea isthmi and some diverging around this recess close to the ventricle to decussate below it. After decussation these fibers may effect connection with all of the motor nuclei mentioned above, while others turn caudad and dorsad to reach the neurons of the

tegmentum farther caudad, and still others appear to enter the
fasciculus longitudinalis medialis by means of which they may
effect connection with the motor nuclei of the medulla oblongata.

A separate slip of this system arises from the nucleus posterior
tecti, passes ventralward in the region of the isthmus, and then
turns forward.   This is shown in figures 51 to 55 (*tr.t.ped.
post.*), and may be designated *tractus tecto-peduncularis posterior.*
It probably provides a pathway from the nucleus posterior tecti
to the nuclei of the III and IV nerves.

At the contact between the stratum album and the stratum
griseum of the midbrain there is a thin sheet of fibers, chiefly
unmyelinated, which are directed chiefly downward and for-
ward to the vicinity of the III and IV nuclei.   The fibers of
the brachium conjunctivum parallel this system immediately
caudad.   The fibers of this system arise from all parts of the
tectum and may be called *tractus tecto-peduncularis profundus*
(figs. 9 to 14, 21, 31, 32, 33, *tr.t.ped.p.*).   Some end in the teg-
mentum of the same side and others first decussate in the ven-
tral commissure.

There is a third system of tecto-peduncular fibers which may
be termed the *tractus tecto-peduncularis intermedius.*   These
fibers, most of which are unmyelinated, arise from the neuropil
of the caudal part of the tectum and descend into the under-
lying tegmentum at intermediate depths of the stratum album.
They form thin sheets of fibers separating the longitudinal
tracts of the tegmentum, where some ascend and some descend.
So far as observed they are all uncrossed (fig. 13, *tr.t.ped.i.*).
The unmyelinated elements of this system are not clearly im-
pregnated in our preparations.   Most of them are probably
dendrites of neurons of the motor tegmentum, reaching upward
to receive nervous impulses directly from the sensory tracts in
the tectum, and some are known to be dendrites of neurons of
the tectum reaching downward to arborize among the dorsal
tegmental fascicles.

In addition to the tecto-peduncular systems already de-
scribed (i.e., fibers passing from the tectum to the motor teg-
mentum of the same or the opposite side), fibers connected

with all parts of the tectum mesencephali are directed ventral-
ward and forward into the diencephalon to reach the postoptic
commissure in the chiasma ridge. These will next be consid-
ered. (For a summary and discussion of the components of
the postoptic commissure, see page 276.)

12. *The tractus tecto-thalamicus et hypothalamicus cruciatus*

This is the largest group of fibers in the postoptic commissure
complex. Its fibers are related to the tectum mesencephali
for its entire length, and apparently they comprise several
components of different functional significance, depending upon
the part of the tectum with which they are related. These
fibers in Necturus I divide into two chief groups which may be
termed the anterior and posterior parts of the system, the pars
anterior being related to the optic part of the tectum (col-
liculus superior) and the pars posterior to the non-optic part of
the tectum (colliculus inferior).

1) Pars anterior. There are a very few coarse myelinated
fibers related to the most dorsal part of the neuropil which re-
ceives terminals of the optic tract. They accompany the mar-
ginal optic tract almost to the optic chiasma, where they separate
and decussate somewhat farther dorsally and caudally than
the optic fibers. They form the most rostral member of the
postoptic commissure complex in the chiasma ridge (figs. 2 to
11, *tr.t.th.h.c.a.*). Their farther course is not altogether clear.
They may include true commissural fibers between the optic
tecta, but most of the fibers (which are few in number but very
coarse and heavily myelinated and therefore distinguishable
from all others in the postoptic commissure) appear to connect
with the lateral parts of the hypothalamus and adjacent motor
tegmentum. The coarsest of these fibers, which decussate
farther caudad than the others, can be followed after their de-
cussation along the extreme lateral surface of the hypothalamus
as far back as the tuberculum posterius.

A very few similar heavily myelinated fibers are described
by Hirsch-Tabor ('08, p. 724 and fig. 1, *C*) in Proteus, where

they are said to connect with the ventral regions of the mid-
brain roof. Since the optic tracts are entirely absent in Pro-
teus and are greatly reduced and unmyelinated in Necturus,
it seems improbable that these coarse heavily myelinated fibers
are related to the optic apparatus. They probably are in no
way functionally related with the associated unmyelinated
fibers to be described immediately. In this connection it
should be remembered that the area here termed colliculus su-
perior has numerous other functional connections in addition to
the optic tracts, e.g., it receives fibers from the spinal and acous-
tico-lateral lemnisci and from the mesencephalic V root.

Golgi sections show that there is a large number of unmyelina-
ted fibers associated with the coarse myelinated fibers last
described and, before their decussation, following the same
course, that is, in close association with the marginal optic
tract (figs. 17, 37, 54, 55, 57, 67, *tr.t.th.h.c.a.*). After their
decussation these fibers are associated with fibers derived from
the hypothalamus and with the basal optic tract (see p. 242)
and they cannot easily be followed. In one sagittal series by
the Golgi method, in which the marginal optic tract is entirely
unimpregnated, this tract as it descends from the tectum is
well impregnated (fig. 56, *tr.t.th.h.c.a.*), and slightly detached
from this fascicle are scattered fibers apparently belonging to
the same system which end by free arborizations in the pars
optica thalami (fig. 56, *tr.th.h.c.a.x.*). These may be interpreted
as fibers of this tract arising in the tectum and ending in the
thalamus after decussation in the postoptic commissure, though
they may have other significance (see p. 244).

2. *Pars posterior.* This tract comprises the most compact and
most easily followed component of the postoptic commissure
complex. Its fibers are related to that portion of the tectum
mesencephali which receives lemniscus fibers from the spinal
cord and oblongata. Its myelinated fibers are of small size
but have dense myelin sheaths, so that they stain intensely in
Weigert preparations. This rather compact tract in the more
dorsal part of its course is mingled with fibers of the acous-
tico-lateral lemniscus as these spread out to their termini in

the tectum. In following its course from the tectum it is seen to run forward in the intermediate level of the stratum album ventrally of the neuropil related to the optic tract, or optic tectum (figs. 7 to 10, *tr.t.th.h.c.p.*), and then similarly across the caudo-lateral face of the thalamus (figs. 3 to 6) mingled with fibers of the tractus tecto-thalamicus rectus (fig. 56). Finally it turns abruptly ventralward to decussate near the rostral border of the chiasma ridge (fig. 67). It runs parallel with the optic tract, but farther caudad and at a somewhat deeper level.

Mingled with the myelinated fibers just described are many without myelin sheaths (figs. 18, 19, 20, 27 to 30, 36, 37, 38, 41, 42, 43, 46, 52 to 58, 62, 67, *tr.t.th.h.c.p.*). Golgi sections show that many of these arise as axons of neurons of the tectum. There are, however, some free endings in the tectum which belong to this system (fig. 37). I conclude that the tract is chiefly efferent with reference to the tectum; the free endings may indicate that mingled with the efferent fibers are others which are truly commissural between the two tecta.

From the nucleus posterior tecti strong fascicles of both myelinated and unmyelinated fibers enter this system (fig. 37). In Golgi preparations of Necturus and larval Amblystoma there is in this region a densely massed group of neurons whose dendrites reach the lateral surface and there turn forward among the fibers of this tract. Slender axons of the postoptic commissure system enter this dendritic neuropil, but their exact relations with these neurons have not been determined.

As the fibers of this pars posterior cross the caudal end of the pars ventralis thalami before their decussation, they give off numerous fine contorted collaterals which form an intricate neuropil within which dendrites from neurons of the pars ventralis thalami ramify (fig. 27). Thick contorted dendrites of these neurons also turn ventrad within this tract for long distances. These fibers after their decussation cannot be distinguished with perfect certainty from other components of the postoptic commissure. Apparently they spread out both rostrad and caudad of the decussation into the pars ventralis thalami,

the pars dorsalis hypothalami and the nucleus of the tuberculum posterius.

Studies of the course of this tract in larval and adult Amblystoma support this conclusion. Here I have seen collaterals from these fibers both before and after their decussation ending among dendrites of the pars ventralis thalami in a dense neuropil. Dendrites from the pars dorsalis hypothalami and from the nucleus of the tuberculum posterius are similarly related to these fibers.

The caudal end of the pars ventralis thalami and the pars dorsalis hypothalami appear to be the chief places where this tract terminates. We therefore term it provisionally the tractus tecto-thalamicus et hypothalamicus cruciatus posterior, though some of its fibers reach farther back to terminate in the cerebral peduncle also. This tract evidently is not related to the optic part of the tectum (colliculus superior) but only to the part of the tectum which corresponds with the mammalian colliculus inferior.

I conclude that the tractus tecto-thalamicus et hypothalamicus cruciatus is a complex system, within which I have recognized two chief subdivisions related respectively with the urodele equivalents of the mammalian superior and inferior colliculi. These are termed the anterior and posterior parts of the system. Each of these parts distributes its fibers diffusely to the pars ventralis thalami, to the lateral parts of the hypothalamus, and to the nucleus of the tuberculum posterius in the cerebral peduncle. The anterior part of this system in Necturus contains very few myelinated fibers and a much larger number of unmyelinated fibers. The myelinated fibers after decussation reach the lateral parts of the hypothalamus and adjacent motor tegmentum. The unmyelinated fibers cannot be so easily followed; they appear to be distributed after decussation in part to the pars optica thalami, and in part to regions farther ventrally. The posterior, or nonoptic part, is derived from the colliculus inferior and contains numerous myelinated and unmyelinated fibers which connect, both before and after decussation, with the pars ventralis thalami, and after decussation

with the pars dorsalis hypothalami and the nucleus of the tuber-
culum posterius.   True commissural fibers from the tectum of
one side to that of the opposite side may be present in both parts
of this system.

13. Tractus thalamo-hypothalamicus et peduncularis cruciatus

This complex system of fibers forms a second very important
component of the postoptic commissure (figs. 1 to 7, 20, 25, 27,
28, 55, 57, 58, 62, 67, 68 *tr.th.h.p.c.* and *tr.th.h.p.c.x.*).   Its
fibers, which are both myelinated and unmyelinated, arise from
the pars dorsalis thalami and pass ventralward laterally of the
lateral forebrain bundle.   They leave the thalamus in company
with the unmyelinated tractus thalamo-frontalis anterior, but
in much more diffuse formation (fig. 55).   The unmyelinated
fibers at their decussation are rather widely scattered through
the chiasma ridge.   The myelinated fibers decussate in the
dorsal part of the postoptic commissure complex dorso-caudally
of the decussation of the tractus tecto-thalamicus et hypothal-
amicus cruciatus posterior (fig. 62), and after their crossing
these fibers ascend at a deeper level than the descending fibers,
penetrating the lateral forebrain bundle and then turning
abruptly caudad into the pars ventralis thalami and the cere-
bral peduncle dorsally of the latter bundle.

Some fibers of this system which are more heavily myelinated
than any others decussate in the caudal part of the postoptic
commissure and then form a compact fascicle which ascends in
the deepest level of the stratum album (figs. 4 to 7, 67, 68, *tr.th.
h.p.c.x.*) and turns caudad along the ventral border of the lateral
forebrain bundle.   The fibers of this crossed thalamo-pedun-
cular tract (*tractus thalamo-peduncularis cruciatus*), mingled with
the longer fibers of the lateral forebrain bundle, form the most
dorsal fasciculi of the motor tegmentum.   In larval Amblystoma
I have followed ('14 a, figs. 4 to 9, *tr.th.b.*) the tractus thalamo-
peduncularis cruciatus (there called tractus thalamo-bulbaris) as
far back as the superficial origin of the V cranial nerve.

Unmyelinated fibers of this system are especially numerous in the more caudal part of the postoptic commissure and these fibers turn caudad into the motor tegmentum of the cerebral peduncle in a very superficial position.

The fibers of this system which arise from the pars dorsalis thalami and after decussation end in the hypothalamus (*tractus thalamo-hypothalamicus cruciatus*) reach both the dorsal and the ventral parts of the hypothalamus. Their area of distribution is more medial than that of the hypothalamic fibers of the tractus tecto-thalamicus et hypothalamicus cruciatus. None of them extend backward into the caudal or free part of the pars ventralis hypothalami, but they reach all dorso-ventral levels close behind the postoptic commissure from the extreme ventral surface to the level of the tuberculum posterius.

The fibers of the tractus thalamo-hypothalamicus cruciatus decussate in the more caudal part of the postoptic commissure. Here the texture of the commissural complex is much more open than farther forward, where the decussating fibers cross in thick masses of straight fibers with no considerable amount of neuropil between them. In this more caudal part of the commissure Golgi sections show that the decussating fibers run singly, not in fascicles, they are contorted but not varicose, and they are embedded in an open neuropil formed in part by dendrites from all adjacent parts of the brain (figs. 20, 27, 28). Some of these dendrites come forward from the ventral part of the hypothalamus, some arise from neurons of the dorsal part of the hypothalamus, and some come even from the pars ventralis thalami farther dorsally. The nucleus of the tractus pallii lies embedded in the caudal part of this neuropil (figs. 5, 6. 67, *tr.pal.* and *nuc.tr.pal.*).

14. Tractus tecto-thalamicus rectus

From the rostral part of the tectum mesencephali, and perhaps also from its entire extent, a diffuse collection of myelinated and unmyelinated fibers passes between the tectum and the thalamus. These fibers lie at an intermediate level in the stratum album deeper than the thalamic fibers of the acoustico-

lateral lemniscus (*lm.*) and those of the tractus tecto-thalamicus et hypothalamicus cruciatus. They sweep forward across the lateral aspect of the thalamus in diffuse formation dorsally and rostrally of the two tracts last mentioned and appear to end without decussation in both the pars dorsalis and the pars ventralis thalami, chiefly in the former (figs. 5 to 8, 20, 28, 37 to 40, 55, 56, 66, *tr.t.th.r.*).

15. Tractus tecto-habenularis

Horizontal sections show a compact strand of unmyelinated fibers passing close to the mid-dorsal line between the rostral end of the tectum mesencephali and the habenula. The cell bodies from which these fibers arise are not impregnated in our preparations. Weigert sections show that there are some myelinated fibers among them.

Associated with these fibers is a small number of myelinated and unmyelinated fibers which pass in diffuse formation or in dense fascicles through the commissura tecti diencephali and are termed *tractus tecto-habenularis cruciatus*. The unmyelinated component of this tract is seen at its decussation in the commissura tecti in figure 28 (*tr.t.hab.c.*), and this bundle can be followed in the series of sections forward into the habenula (fig. 25, *tr.t.hab.c.*). In horizontal Golgi sections some fibers of this system are seen to arborize freely in the pars intercalaris diencephali and here to send collateral branches into the commissura tecti diencephali (fig. 37, *tr.t.hab.*, on the right side). This arrangement suggests that the fibers pictured arise in the habenula and terminate in the pars intercalaris and tectum; but the same series of sections shows farther ventrally fibers of this tract extending from the tectum forward through the whole length of the pars intercalaris and ending by richly branched free arborizations in the habenula (fig. 38, *tr.t.hab.*), suggesting their origin from the tectum.

16. Tractus preoptico-intercalaris

The pars intercalaris diencephali is related with the nucleus preopticus by a slender tract of unmyelinated fibers which will next be described. In a single series of transverse Golgi sections almost the entire course of this peculiar tract can be followed with perfect distinctness. It appears first at the level of the optic chiasma as three slender fascicles in the stratum album of the nucleus preopticus (fig. 15, *tr.po.i.*) and can be followed in successive sections caudad and dorsad to its termination by free endings in the pars intercalaris (figs. 16 to 20). The origin of these fibers from their cell bodies has not been observed, but in a transverse section of a different specimen a single wisp of similar fibers is found leaving the stratum griseum of the nucleus preopticus (fig. 25, *tr.po.i.*), which probably represents the portion of that tract which is missing in the other series and is provisionally so labelled in the figure. This tract seems to be comparable with the tractus olfacto-habenularis from the preoptic nucleus to the habenula.

17. Tractus habenulo-peduncularis

This tract, the fasciculus retroflexus of Meynert, pursues a typical course from the habenula to the interpeduncular nucleus. The tract is unmyelinated, but myelinated fibers of diverse sorts are added to it from the thalamus and other parts. For the first part of its course the fibers run nearly horizontally under the extensive pars intercalaris diencephali, then they turn ventrally in a course parallel with the fibers of the posterior commissure but farther rostrally (figs. 3 to 9, 28, 29, 30, 39, 41, 44, 46, 47, 49, 58, 59, 60, 63, 64, 68, *f.retr.*). They distribute chiefly without decussation, in the interpeduncular nucleus, both before and behind the fovea isthmi and far caudad under the motor tegmentum (fig. 61).

18. *Tractus thalamo-peduncularis*

This name is given to an extensive and very complex connection between the pars intercalaris of the diencephalon and the dorsal and ventral parts of the thalamus, on one hand, and the cerebral peduncle in the vicinity of the tuberculum posterius, on the other hand. This system of fibers is quite distinct from that described above under the name tractus thalamo-hypothalamicus et peduncularis cruciatus and decussating in the postoptic commissure. The fibers here under consideration are partly myelinated, but chiefly unmyelinated. They are directed caudad and ventrad and run in diffuse formation, some in the deepest level of the stratum album, some very superficially, and some at intermediate levels. Many of the superficial and intermediate fibers form compact fascicles of a few fibers each which retain their individuality for long distances. They are for the most part uncrossed, but some of the more ventral fibers of the complex decussate in the rostral part of the commissure of the tuberculum posterius. These crossed fibers are distinguished from those of the tractus thalamo-peduncularis cruciatus already described (p. 259) by the fact that the latter tract decussates in the postoptic commissure.

The fibers from the pars ventralis thalami may be termed *tractus thalamo-peduncularis ventralis* (fig. 68, *tr th.p.v.*), and they form three groups, deep, superficial, and intermediate. The deep fibers (tractus thalamo-peduncularis ventralis profundus) form a thin sheet of both myelinated and unmyelinated fibers at the boundary between the gray and white layers. They extend backward and ventralward into the tuberculum posterius, some decussating in the commissure of the tuberculum posterius to enter the opposite motor tegmentum laterally of the fasciculus longitudinalis medialis (figs. 30, 41, 43, 44, 58, 59, *tr.th.p.v.p.*). The superficial fibers (tractus thalamo-peduncularis ventralis superficialis) pass directly lateralward from the pars ventralis thalami in slender wisps, some myelinated and some unmyelinated. They usually accompany the blood vessels to the surface of the brain and here spread out. All

probably ultimately effect connections with other parts of the motor tegmentum. Some of these fibers can be followed to the region laterally of the nucleus of the III nerve (figs. 2, 3, 18, 25, 26, 27, 44, 45, 48, 49, *tr.th.p.v.s.*). Fibers of this system also descend into the cerebral peduncle at intermediate levels in company with the lateral forebrain tract (tractus thalamo-peduncularis ventralis intermedius, figs. 25, 44, 48, *tr.th.p.v.i.*).

The fibers from the pars dorsalis thalami and pars inter-calaris are termed *tractus thalamo-peduncularis dorsalis* (fig. 68, *tr.th.p.d.*). These are chiefly unmyelinated and, as in the preceding case, some are deep, some are superficial, and some occupy intermediate levels.

From the pars dorsalis thalami fibers which are chiefly un-myelinated, with a few myelinated fibers among them, collect and join the tractus habenulo-peduncularis in the deeper levels of the stratum album. They can be followed to the region of the tuberculum posterius. Other deep fibers take courses farther ventrally and rostrally. All of these fibers belong to the tractus thalamo-peduncularis dorsalis profundus (figs. 42, 43, 57, 58, *tr.th.p.d.p.*).

There is also a rather compact fascicle of unmyelinated fibers which passes ventro-caudad from the pars dorsalis thalami in a superficial position (tractus thalamo-peduncularis dorsalis superficialis). Most of these fibers evidently connect with the neurons of the nucleus of the tuberculum posterius and others with neurons of the pars ventralis thalami; some may also reach the pars dorsalis hypothalami. So far as observed they are all uncrossed. Some slender strands of unmyelinated fibers belonging to this tract come also from the nucleus of the pars optica thalami.

We may also consider here as belonging to this system a very definite tract of fibers which arises in the pars intercalaris of the diencephalon and passes ventrad and slightly caudad along the extreme outer surface of the brain in the superficial groove which separates the mesencephalon from the dien-cephalon. These fibers are chiefly unmyelinated with a few mye-linated fibers among them; they reach the ventro-lateral border of

the cerebral peduncle laterally of the fovea isthmi and appear to terminate in the area of neuropil here termed the area lateralis tegmenti (see pp. 242 and 295) which is also related with the so-called basal optic tract and other fiber systems. The most dorsal of these fibers spring from the extreme dorsal surface of the pars intercalaris where there is a collection of neurons with dense bushy dendrites. These axons pass downward across the pars dorsalis thalami and may receive additions of both myelinated and unmyelinated fibers from this part (figs. 38, 52, 53, *tr.th.p.d s.*). At the level of the pars ventralis thalami they are joined by slender strands of unmyelinated fibers from the gray layer of the pars ventralis thalami.

In both Necturus and Amblystoma a strand of unmyelinated fibers which seems to be a part of this system is seen to arise from the habenula in company with the tractus habenulo-penduncularis. It turns sharply lateralward across the dorsal aspect of the pars intercalaris to the dorso-lateral surface (figs. 39, 41, *tr.th.p.d.s.*), then ventralward and caudalward. Its further course is parallel with that of the tractus habenulo-pe-duncularis, but more superficial close to the outer surface of the brain. There are extensive connections between the thalamus and the habenula, but their details have not been worked out.

The dorsal thalamo-peduncular tracts above described are probably for the most part composed of descending fibers from the sensory centers of the thalamus and epithalamus to the motor centers of the pars ventralis thalami and tegmentum, though other types of fibers may be mingled with them. Some of these fibers may be collaterals from the mamillo-peduncular fibers, comparable with the mamillo-thalamic tract of mammals. Some of our Golgi preparations suggest this, but no definite region can be identified as homologous with the mammalian nucleus anterior thalami.

Accompanying the slender axons running from the pars dorsalis thalami to the cerebral peduncle coarser contorted fibers are seen in Golgi preparations. These are dendrites from the nucleus of the tuberculum posterius which extend far forward and dorsalward to break up in the neuropil of the pars dorsalis

thalami and even still farther dorsally in the pars intercalaris diencephali. Similar dendrites from neurons of the pars ventralis thalami extend dorsally among the fibers of this system. This arrangement suggests relations analogous with those of the 'corpus glomerosum,' or nucleus rotundus of Fritsch, with the nucleus anterior thalami, as described by V. Franz ('12, pp. 426–431) in fishes.

19. Tractus dorso-ventralis thalami

Short, slender axons, to which this name is applied, pass directly from the pars dorsalis to the pars ventralis of the thalamus. Many of them at their origin (figs. 58, 68, *tr.d.v.th.*) are mingled with fibers of the tractus thalamo-peduncularis dorsalis profundus. They really are of the same type as the latter tract and hardly merit a separate name.

20. Tractus mamillo-peduncularis

The hypothalamus has not been exhaustively studied. At the rostral end of the commissure of the tuberculum posterius there is a decussation of hypothalamic fibers, the decussatio hypothalamica posterior; and there are fibers passing backward from the pars dorsalis hypothalami into the motor tegmentum which probably constitute the tractus mamillo-peduncularis and are so designated on the figures (figs. 8, 9, 29, 30, *tr.mam.ped.*). None of these connections have been fully demonstrated. In figures 46, 47 and 49 there is shown a collection of peculiar fine varicose terminals laterally of the tuberculum posterius and fovea isthmi which probably belong to this system and are provisionally so designated. They make their appearance in the preparations close to the stratum griseum dorsally of the cell bodies of the more medial part of the pars dorsalis hypothalami, and they pass directly caudad mingled with dendrites of the neurons of the tuberculum posterius and adjacent parts. Some of them cross in the ventral commissure both above and below the fovea isthmi, and some of them reach the chief terminal nucleus of the tractus habenulo-peduncularis below the fovea.

21. The basal forebrain bundle

The basal forebrain bundle of Amphibia was described by Osborn ('88, p. 78) as containing both ascending and descending fibers relating the cerebral hemisphere with lower regions as far back as the spinal cord. This was confirmed by Van Gehuchten ('97, p. 53) on the basis of Golgi preparations of Salamandra. It seems probable that the complicated connections of the fibers of this bundle as described by Van Gehuchten represent, not the course of a single tract, but the confusion of several distinct fiber systems no one of which extends through the entire length of the brain. Recent authors have separated the basal forebrain bundle into two parts, a lateral forebrain bundle related to the lateral wall of the cerebral hemisphere, and a medial forebrain bundle related to the medial wall. Each of these bundles is a complex containing both ascending and descending fibers, whose analysis has not yet been completely effected.

1) *The lateral forebrain bundle* (fasciculus lateralis telencephali). This is the most compact and obvious fasciculus in the brain of Necturus. Its course through the ventro-lateral wall of the telencephalon has been figured by Kingsbury ('95, figs. 28 to 31), McKibben ('11, figs. 9 to 18), and Röthig ('11, figs. 5 to 15). In this contribution it is illustrated in figures 1 to 11, 15 to 20, 25, 28, 43, 46, 49, 55, 57, 68, (*f.lat.t.*). It contains both myelinated and unmyelinated fibers of several sorts, some of which will next be enumerated.

Tractus strio-thalamicus et tegmentalis. These fibers descend from the ventro-lateral area of the cerebral hemisphere (primordial corpus striatum) and are strongly myelinated, at least in part. At the transverse level of the postoptic commissure they begin to scatter (figs. 3 to 6, 19, 20, 55, 57, *f.lat.t.*) and many of them end in the adjacent pars ventralis thalami (*tractus strio-thalamicus*, fig. 57, *tr.st.th.*). The remainder turn dorsalward and enter the cerebral peduncle, where they join the bundles of motor fibers in the tegmentum (*tractus strio-tegmentalis*, figs. 55, 57, *tr.st.t.*). Their lower termination

has not been determined. In larval Amblystoma I have followed some of these fibers as far as the upper end of the medulla oblongata ('14 a, figs. 4 to 8, *tr.lat.t.*).

Tractus thalamo-frontalis. These fibers ascend from the thalamus to the lateral wall of the cerebral hemisphere. They are arranged in two imperfectly separated groups, anterior and posterior. The tractus thalamo-frontalis posterior is ·a diffuse collection of a few myelinated fibers passing from the thalamus into the lateral forebrain bundle (fig. 66 *tr.th.f.p.*). They are connected chiefly with the caudal part of the pars dorsalis thalami, viz., the area of distribution of the thalamic fibers of the spinal and acoustico-lateral lemnisci (pp. 245, 246.)

The tractus thalamo-frontalis anterior is a more extensive and more compact system of unmyelinated fibers which arise in the middle and most massive portion of the pars dorsalis thalami. Golgi sections show them as exceedingly fine axons arising from the bases of the dendrites of the neurons of this region (figs. 1 to 5, 17 to 20, 40 to 46, 48, 55, 57, 66, *tr.th.f.a.*). These fibers are arranged in slender compact fascicles which pass ventralward through the intermediate depths of the stratum album to reach the lateral forebrain bundle. At the dorsal border of this bundle they enter it and turn forward, forming a compact fascicle of unmyelinated fibers embedded among the myelinated fibers in the dorsal part of its cross section (figs. 1, 2). The origin of this tract from the thalamus was seen and figured by Kingsbury ('95, p. 195).

These thalamo-frontal fibers of urodeles are probably the precursors of the thalamo-cortical sensory radiations of mammals and were, accordingly, designated tractus thalamo-corticalis in my account of Ambylstoma ('10, p. 434). In view of the fact that there is no true cortex in the urodele hemispheres, it seems better to use the more conservative name thalamo-frontal tract in these animals. I have no satisfactory evidence that in Necturus any fibers from the pars optica thalami enter this thalamo-frontal tract, i.e., there seems to be no true optic radiation in Necturus.

Tractus pallii. Still another component of the lateral fore-brain bundle is a tract which I have recognized in the frog ('10, p. 444 and fig. 41) and compared with Johnston's account of the tractus pallii of fishes. This tract is well developed in all of the urodeles which I have studied and its fibers are always unmyelinated. It probably contains both ascending and descending fibers, for I have seen numerous free arborizations at both ends of the tract. It can be followed from the postero-lateral wall of the cerebral hemisphere into the ventral part of the cross section of the lateral forebrain tract (figs. 1 to 5, *tr.pal.*) and then back to the level of the postoptic commissure. Near the caudal end of this commissural complex the tractus pallii fibers turn abruptly ventralward (fig. 5) and part of its fibers decussate in the commissure. Both the crossed and the un-crossed fibers are related to a fairly distinct nucleus in the hypo-thalamus near the mid-plane and enclosing the most caudal fibers of the postoptic commissure (fig. 6, *nuc.tr.pal.*).

This tract may be the forerunner of the olfactory projection tract of mammals. As described by Ramón y Cajal ('11, p. 721), these fibers connect the pyriform lobe and amygdala of the hemisphere with the motor tegmentum. This tract has been seen by Johnston in the turtle ('15, figs. 16, 49, 52 to 54) and by Miss Crosby in the alligator ('17, p. 373). The former author ('15, p. 464, fig. 54) comments upon the fact that in turtles, as in mammals, this tract reaches the tegmental region of the midbrain, a connection which the tract in question in Necturus does not make.

Mingled with the axons of the lateral forebrain bundle are long, slender dendrites; some of these come from the caudal end of the ventro-lateral part of the cerebral hemisphere (striatal area) and reach as far caudad as the chiasma ridge, others come from the pars ventralis thalami and nucleus of the tuberculum posterius and extend as far forward as the chiasma ridge.

2) The medial forebrain bundle (fasciculus medialis telen-cephali). This is a broad connection between the medial wall of the cerebral hemisphere and the hypothalamus. The fibers are unmyelinated with a few myelinated fibers among them

and they lie ventro-medially of the more compact lateral fore-brain bundle (figs. 1 to 5, 46, 55, 57, 58, *f.med.t.*). Johnston ('15, p. 464, fig. 54) mentions that in turtles almost all of the fibers of this tract enter the tegmentum of the midbrain instead of connecting with the hypothalamus as commonly described. In Necturus most of these fibers clearly connect with the hypothalamus; but some of them can be seen in parasagittal sections to reach the ventral part of the tegmentum of the midbrain (fig. 55). Some such fibers are also seen in a horizontal Golgi section (fig. 46.) These probably represent a tractus olfacto-tegmentalis. Long dendrites of neurons of the hypothalamus are mingled with these fibers, some of them extending forward beyond the level of the chiasma ridge.

The stratum album of the cerebral peduncle and motor tegmentum farther caudad receives the numerous tracts from the tectum mesencephali, from the diencephalon, and from the telencephalon to which reference has already been made. In addition to these there are numerous descending tracts which arise within this region. These intrinsic tegmental systems fall into two groups, which are here called the dorsal and ventral tegmental fascicles. All of the tracts of the cerebral peduncle are enumerated on pages 292–297.

22. The dorsal tegmental fascicles

These comprise five clearly defined bundles of large size and some smaller ones, whose fibers are largely, though not exclusively, unmyelinated (figs. 10 to 14, 24, 33, 35, 68, *f.d.t.*). They lie ventrally of the lemniscus systems and in the deeper layers of the stratum album. When followed forward in transverse Weigert sections these fascicles are seen to lose their individuality at about the level of the tuberculum posterius (fig. 10). When followed caudad they break up in the eminentia subcerebellaris tegmenti below the isthmus (fig. 14). Though our preparations do not exclude the possibility that individual fibers of these fascicles may extend much farther, the fascicles as such clearly run between the nucleus of the tuberculum posterius and the eminentia subcerebellaris tegmenti.

The fascicles of this group begin rostrally in that dorsal region of the motor tegmentum where the lateral forebrain bundle spreads out and rapidly breaks up, and they end in the region below the isthmus where the great descending bulbar systems of fibers are beginning to form. Sagittal Cajal sections show that the fascicles of the lateral forebrain bundle which enter the rostral end of the tegmentum where these dorsal tegmental fascicles arise are not continuous with them, but a synapse is here interposed. It therefore appears probable that these dorsal tegmental fascicles serve, in part at least, to transmit nervous impulses from the basal forebrain bundle to the centers of motor distribution in the medulla oblongata.

23. *The ventral tegmental fascicles*

These bundles, like the dorsal fascicles, arise within the pedunculus cerebri, but lie farther ventrally and are composed of myelinated fibers. Many of their fibers, moreover, extend farther caudad into the medulla oblongata, though large numbers end in the eminentia subcerebellaris tegmenti (figs. 9 to 14, 31, 32, 33, 35, 46, 47, 55, 68, *f.v.t.*).

24. *Tractus tegmento-bulbaris*

The eminentia subcerebellaris tegmenti receives the terminals of the dorsal and ventral tegmental fasciculi, as already described, including some endings from the fasciculus longitudinalis medialis. Myelinated fibers are directed downward and lateralward from this eminence into the reticular formation of the medulla oblongata, the tractus tegmento-bulbaris (fig. 68, *tr.teg.b.*).

25. *Fasciculus longitudinalis medialis*

This is a highly specialized member of the system of ventral tegmental fascicles (figs. 7 to 14, 24, 29, 31, 32, 33, 46, 47, 49, 68, *f.l.m.*). Fibers of this system arise from neurons of the nucleus of the tuberculum posterius and especially from a collection of very large neurons scattered through the dorsal part

of this nucleus and extending somewhat dorsally of the ventricular eminence formed by it (figs. 8, 23, 59, 60, *nuc.com.post.*). These neurons form the nucleus of the posterior commissure, as already described (p. 252), and are grouped among the terminals of this commissure. They might with equal propriety be called the nucleus of the fasciculus longitudinalis medialis, for they appear to be differentiated to receive nervous impulses from the tectum by way of the commissura posterior and to transmit these impulses into the fasciculus longitudinalis medialis. In higher vertebrates separate nuclei for these two tracts are described in this region, but in Necturus this differentiation seems not to have taken place.

Some fibers of the fasciculus longitudinalis medialis decussate in the commissure of the tuberculum posterius. The fasciculus rapidly increases in size as it is followed caudad and below the level of the fovea isthmi (figs. 11 to 14) it is composed of several fascicles of heavily myelinated fibers. There is a particularly strong decussation of the fibers of the fasciculus both rostrad and caudad of the fovea isthmi (figs. 46, 47). As the medulla oblongata is approached, this fasciculus, like the other members of the motor tegmentum, diminishes in size, largely by discharge of its fibers among the neurons of the eminentia subcerebellaris tegmenti. The larger number of its fibers, however, accompany the remnant of the fibers of the ventral tegmental fascicles into the medulla oblongata.

26. Tractus tegmento-interpeduncularis

This name is given provisionally to a well defined fascicle of fibers of uncertain significance. They are unmyelinated and extend forward and ventralward from the eminentia subcerebellaris tegmenti at the boundary between the gray and white layers, running parallel with the brachium conjunctivum and a little rostrally of it. The tracts of the two sides converge at the fovea isthmi, immediately caudad of which they turn abruptly ventralward close to the median plane and spread out in the dense neuropil of the interpeduncular nucleus, which occupies

the ventro-medial part of the brain in this region. Their direction of conduction is uncertain, but their course and arrangement suggest that they arise from cells of the subcerebellar eminence. They seem to end in the nucleus of Edinger-Westphal of the III nerve (fig. 68, *tr.teg.i.*).

V. THE COMMISSURES OF THE MIDBRAIN AND THALAMUS

Under this head the commissures and decussations of these regions will be enumerated, and so far as their component tracts have been described in the preceding pages they will be summarized here. These commissures fall into a dorsal and a ventral series. The dorsal series extends continuously forward from the cerebellar commissures in the decusssatio veli to the commissura habenularum, with a short interruption in the recessus posterior mesencephali and a more extensive interruption at the pineal recess. The ventral series of decussations extends continuously forward from the ventral commissure of the medulla oblongata to the optic chiasma except for a very short interruption at the fovea isthmi and a more extensive break at the wide infundibulum.

Commissura habenularum (superior commissure)

This contains chiefly decussations of various components of the stria medullaris, which are not considered in this paper. As we have seen, a decussation of fibers of the parietal nerve in this commissure, which has been described in some other vertebrates, does not occur in Necturus.

Commissura tecti diencephali

This is Gaupp's name for the fibers crossing in the diencephalic roof between the epiphysis and the commissura posterior, i.e., those contained in the pars intercalaris diencephali. Some of these are probably true commissural fibers between the adjacent regions. There is also included the decussation of the tractus tecto-habenularis cruciatus (see p. 261).

Commissura posterior

At the rostral border of the roof of the midbrain this commissure is a conspicuous massive crossing of heavily myelinated fibers, whose composition is only imperfectly revealed in our preparations. Many of these fibers appear to be truly commissural between the optic parts of the tectum mesencephali; but most of them probably form a decussation between the tectum of one side and the region of the tuberculum posterius of the opposite side. Here are the very large neurons of the nucleus of the posterior commissure and fasciculus longitudinalis medialis, which seem to form the chief avenue of discharge for this decussation (p. 271). It is probable that this commissure has been differentiated principally to connect the tectum opticum with the fasciculus longitudinalis medialis. Some of its fibers, however, appear to go farther and connect directly with dendrites of the nucleus of the III nerve.

Commissura tecti mesencephali

Extending backward from the posterior commissure and quite uniformly developed throughout almost the entire length of the midbrain roof is the commissura tecti of the midbrain (Sylvian commissure of C. L. Herrick), of which the posterior commissure is merely a more highly differentiated portion. What proportion of these fibers are truly commissural has not been determined; but most of them appear to effect a decussation between the tectum (chiefly its non-optic portion, or colliculus inferior) and the motor tegmentum of the opposite side.

Decussatio veli

The commissura tecti mesencephali is nearly but not quite continuous with the decussatio veli immediately behind the isthmus, the roof of the recessus posterior mesencephali containing no commissural fibers (fig. 62; cf. also Herrick, '14, p. 28, fig. 25). The composition of the decussatio veli in Necturus has been given in my paper on the cerebellum ('14, p. 9),

where the following components were described: (1) decussation of the roots of the IV nerve, (2) decussation of fibers of the mesencephalic root of the V nerve, (3) probably fibers of the tractus tecto-cerebellaris, (4) unmyelinated fibers of the commissura cerebelli lateralis, (5) fine myelinated fibers of the commissura cerebelli, (6) coarser myelinated fibers of the commissura cerebelli derived from the tractus spino-cerebellaris. In fishes the decussatio veli contains a large commissure between the secondary visceral nuclei (Rindenknoten of Mayser). I have not found this in Necturus, though the secondary visceral nucleus is here very small and such a tract might easily be overlooked.

The ventral decussations in the floor of the midbrain form a very complex system which is the direct forward continuation of the ventral commissure of the medulla oblongata and isthmus regions, with a slight interruption at the fovea isthmi in the region of the III nerves. The portion of this system lying below the fovea will here be termed the ventral tegmental commissure; the portion lying in front of the fovea will be termed the commissure of the tuberculum posterius.

Ventral tegmental commissure

Whether this region contains any true commissural fibers in Necturus is not clear. The components here enumerated are all decussations. These include the following crossed tracts: (1) brachium conjunctivum (p. 251), (2) tractus tecto-bulbaris cruciatus (p. 252), (3) tractus tecto-peduncularis superficialis (p. 253), (4) tractus tecto-peduncularis profundus (p. 254), (5) tractus tegmento-interpeduncularis (p. 272).

Commissura tuberculi posterioris

In the region of the tuberculum posterius, that is between the fovea isthmi and the infundibulum, there is a dense accumulation of commissural and decussating fibers of very diverse sorts, derived from the midbrain, the epithalamus, the thalamus, and the hypothalamus. Some of the tracts which cross in

this region wholly or in part are as follows: Tractus tecto-peduncularis superficialis (p. 253), (2) tractus tecto-peduncularis profundus (p. 254), (3) the fasciculus longitudinalis medialis (p. 271), (4) tractus habenulo-peduncularis (p. 262), (5) tractus thalamo-peduncularis ventralis (p. 263), (6) nervus terminalis (p. 236), (7) various hypothalamic connections which have not been fully analyzed (decussatio hypothalamica posterior). This group includes some crossed fibers of the tractus mamillo-peduncularis. Some Golgi preparations indicate the presence of true commissural fibers here, connecting the dorsal parts of the hypothalamus, or of the pars ventralis thalami farther forward, of the two sides.

Optic chiasma and commissura postoptica

The optic chiasma is described on page 241. The other fibers which cross in the chiasma ridge in the aggregate constitute the postoptic commissure, which is much more extensive in Necturus than the optic chiasma (fig. 67). There are doubtless some true commissural fibers in this complex, but the most important components appear to be decussations. Of these I have especially distinguished three important systems: (1) tractus tecto-thalamicus et hypothalamicus cruciatus (p. 255), (2) tractus thalamo-hypothalamicus et peduncularis cruciatus (p. 259), (3) decussation of the tractus pallii (p. 269). My observations upon these decussations may be summarized as follows.

This aggregation of fibers is exceedingly complex and it has not been fully analyzed in any vertebrate. It is very extensive in all Amphibia and in Necturus it is more easily isolated from the associated optic tracts than in most other species because the optic tracts are here wholly unmyelinated, while the postoptic commissure complex is partly myelinated. Its analysis is, however, even here very difficult and our studies are still incomplete.

The postoptic commissure system as a whole evidently puts the entire tectum mesencephali and pars dorsalis thalami into functional relation with the pars ventralis thalami, hypothalamus, and cerebral peduncle of the opposite side. There may

also be true commissural fibers present connecting any or all
of these regions, as well as other elements. Its individual
components are not as sharply isolated as in teleostean and
higher brains, and a determination of the homologies of its
various components in different vertebrates cannot be definitely
made at this time. It should be frankly stated, moreover,
that the conclusions summarized in the immediately following
paragraphs must be regarded as tentative rather than as fully
demonstrated. An enormous amount of urodele material has
been faithfully studied, but the complete unravelling of this
fiber complex has not been sucessfully accomplished.

1. *Tractus tecto-thalamicus et hypothalamicus cruciatus.* This
complex may be described in general as a connection between
the tectum mesencephali of one side and the ventral part of
the thalamus, the hypothalamus, and to a less extent the cere-
bral peduncle of the opposite side. Many of these fibers, more-
over, or collaterals from them, connect with the homolateral
pars ventralis thalami.

This system of fibers is divisible into two parts depending
upon the part of the tectum with which its fibers are connected.
The *pars anterior* is related with the optic tectum (colliculus
superior). The number of these fibers is relatively small and
they are unmyelinated save for a few myelinated fibers of very
large size. After decussation in the most rostral part of the
chiasma ridge in close association with the optic tracts, they
distribute to the hypothalamus, pars ventralis thalami, and
nucleus of the tuberculum posterius. There is evidence that
this part is functionally complex, part of its fibers carrying
chiefly optic impulses derived from the optic tracts, and part
carrying chiefly non-optic impulses derived from the terminals
of the lemniscus systems in the colliculus superior (see p. 255).
The *pars posterior* is related with the non-optic part of the
tectum (colliculus inferior) and especially with the nucleus
posterior tecti. This contains a large number of myelinated
fibers and many which are unmyelinated; its distribution is
similar to that of the pars anterior. This part will probably
be found to be a complex containing at least two components

related respectively with the colliculus inferior in general and the nucleus posterior tecti.

This system as a whole I compare provisionally with the mammalian commissure of Gudden; but if this comparison holds, it is evident that the homology is only partial. The commissure of Gudden (commissura arcuata transversa of Hannover, decussatio supraoptica ventralis of Edinger, '11, p. 346) seems to be a very complex system which has been only incompletely analyzed. Sterzi ('15, p. 660) recognizes five components: (1) a commissure between the medial geniculate bodies, (2) a commissure between the inferior colliculi, (3) a decussation between the medial geniculate body and the opposite globus pallidus, (4) a decussation between the medial geniculate body and the opposite temporal cortex, (5) fibers connected with the nucleus subthalamicus. The only regions here enumerated which can be recognized in Necturus are the inferior colliculus and the subthalamus (pars ventralis thalami). The mammalian connections of Gudden's commissure with the two parts last mentioned may, however, be represented in our tractus tecto-thalamicus et hypothalamicus cruciatus.

In fishes, where the postoptic commissure complex is very highly differentiated, the corresponding connection is in many cases very clearly seen. This is the commissura transversa of Haller. Victor Franz ('12, p. 433) describes this tract in Gadus as comprising a true commissural connection between the torus semicircularis of the two sides (the teleostean colliculus inferior), a decussation between the torus and the opposite hypothalamus, and associated with the last an uncrossed tract between the torus and the hypothalamus. This is essentially similar to my findings in Necturus. I have confirmed Franz' description of this commissure by personal observations on Gadus and some other teleosts. The tractus tecto-lobaris of Acipenser, as described by Johnston ('01, p. 71), contains similar elements, although the uncrossed fibers are much more numerous and the number of fibers decussating in the postoptic commissure is very small. Here most of the crossed fibers for the hypothalamus decussate in the ventral commissure of the midbrain instead of in the postoptic commissure. Many other variations of these relations are found in different species of fishes, but in all cases there seems to be a connection between the equivalent of the colliculus inferior and the hypothalamus associated with the postoptic commissure. It is possible that the commissura minor of C. L. Herrick ('92. p. 37—the commissura Herricki of Edinger, Goldstein and Kappers) is the teleostean representative of the myelinated fibers of the pars anterior of this complex which in Necturus are related to the optic part of the tectum; for Kappers ('06, p. 28) has traced this commissure in Gadus to the superficial layer of the most rostral end of the tectum opticum. The teleostean commissura horizontalis of Fritsch, which reaches the rostral border of the tectum opticum, may also correspond in part with the amphibian pars anterior.

2. *Tractus thalamo-hypothalamicus et peduncularis cruciatus.*
These fibers are both myelinated and unmyelinated. They
arise in the pars dorsalis thalami and after decussation distribute
to the hypothalamus more medially than the tract last described
and to the cerebral peduncle. Unmyelinated fibers reach the
region of the tuberculum posterius and one fascicle of myelinated
fibers has been followed as far caudad in Amblystoma larvae
as the level of the superficial origin of the V cranial nerve (trac-
tus thalamo-peduncularis cruciatus).

The mammalian equivalent of this system cannot be clearly de-
termined. The commissure of Meynert clearly contains a decus-
sation related on one side with the body of Luys in the subthalamus
(pars ventralis thalami). On the other side its connections are ob-
scure; some say with the thalamus or nucleus lentiformis, but there
are no precise accounts of the striatal connections. These fibers may
well pass on to connect with the thalamus or some other part farther
caudad. The commissure of Ganser (commissura hypothalamica
anterior of Sterzi, '15, p. 653; decussation of Forel of Darkschewitsch
and Pribytkow, '91) has been shown experimentally to be a crossed
connection between the subthalamus or dorsal part of the hypothala-
mus near the third ventricle and some point on the opposite side far-
ther laterally and dorsally, and this may also contain thalamo-hypo-
thalamic fibers. Edinger ('11, p. 347) figures his decussatio supraoptica
dorsalis in the kangaroo exactly in the position of the medial limb of
Ganser's commissure between the columna fornicis and the ependyma
of the third ventricle, and he adds that its fibers have been followed
back into the midbrain, "where they occupy the most medial part
of the medial fillet." These fibers might well represent the descend-
ing limb of a crossed thalamo-peduncular connection.

3. *Tractus pallii.* Most of the fibers of this tract appear
to enter the cerebral hemisphere without decussation; but the
nucleus of this tract lies embedded within the caudal part of
the postoptic commissure very close to the median plane and
some of the fibers of the tract clearly decussate here.

VI. REVIEW AND DISCUSSION OF THE FUNCTIONAL ANALYSIS OF THE MIDBRAIN AND THALAMUS

In this section we shall review the principal functionally de-
fined subdivisions of the midbrain and thalamus as enumerated
on pages 220 to 232, and summarize briefly the fiber connec-

tions of each.   Some additional histological details will be added, though no attempt has been made to give a complete account of all of the types of neurons represented.   The epithalamus and hypothalamus are not included in this analysis, and the telencephalic connections will be mentioned in only a few cases.

The classification of primary cerebral centers and tracts into afferent and efferent types is easily effected; but this becomes increasingly difficult (and unimportant) when applied to correlation centers and tracts of higher orders.   Nevertheless most correlation centers of the dorsal parts of the midbrain and thalamus are evidently more intimately connected with the lower afferent systems than with the lower efferent systems. Conversely, the ventral parts of the midbrain and thalamus are in more direct relation with the lower motor centers.

In the midbrain the roof (tectum) is the receptive center for sensory impulses coming in by way of the mesencephalic V root and from the retina by way of the optic tracts, and for afferent fibers of other systems by way of the spino-tectal and bulbo-tectal lemniscus systems.   The floor of the midbrain (cerebral peduncle) and isthmus region give rise to efferent fibers of the III and IV nerves and to the great descending motor systems, and receive important tracts from the cerebellum, from the tectum, from all parts of the diencephalon, and from the cerebral hemispheres.   The significance of its connections with the nervus terminalis and nervus parietalis is still obscure.

The epithalamus and hypothalamus are correlation centers dominated by descending olfactory tracts.   The pars dorsalis thalami is a receptive center for various somatic sensory tracts and it discharges downward into the pars ventralis thalami, cerebral peduncle, and hypothalamus; it also sends many fibers (thalamo-frontal tracts) forward into the lateral wall of the cerebral hemisphere.   The pars ventralis thalami receives fibers chiefly from the tectum mesencephali, from the pars dorsalis thalami and from the lateral wall of the cerebral hemisphere by way of the lateral forebrain bundle.   At its anterior end it also receives fibers from the primordium hippocampi through the tractus cortico-thalamicus and eminentia thalami (p. 290).

The tectum mesencephali

In ordinary histological preparations it is evident that the neurons are crowded in a thicker and more dense layer in the stratum griseum of the tectum than in that of the cerebral peduncle (figs. 9 to 14). The boundary between these regions in the lateral wall is obscurely indicated by this difference in structure, especially as seen in Golgi preparations (figs. 9 to 14, 32, 33).

The stratum griseum of the tectum is obscurely three layered. The deepest layer is the stratum ependymale, though clearly the bodies of some of the neurons are found here also. An ependymal element from the caudal part of the tectum is seen in figure 34. These cells are usually clearly distinguished in Golgi preparations from the neurons by their delicately plumose appearance.

The stratum ependymale at the rostral end of the tectum is greatly thickened to form a peculiar massive structure below the posterior commissure which has been commented on by many authors and which is named by Dendy ('10) and Dendy and Nicholls ('10) the subcommissural organ (figs. 5, 6, 7, *o.sc.*). The origin of Reissner's fiber from this organ is seen in figure 62 (*f.R.*), as found in a sagittal Weigert series. At the caudal end of the tectum the roof of the recessus posterior mesencephali immediately in front of the velum medullare anterius shows a similar, though less pronounced, thickening of the ependyma, associated with which is a dorsal medial collection of neurons of the mesencephalic V nucleus.

Most of the neurons of the tectum are found in the two outer layers of the stratum griseum, which are incompletely separated by a thin irregular band of neuropil (figs. 9 to 14). I have not been able to demonstrate any constant difference between the neurons of these layers, though further study may reveal it. In this band of neuropil are numerous very fine unmyelinated fibers extending tangentially between the tectum and the cerebral peduncle. The character of these fibers has not been determined; probably they are mostly dendrites. At the outer

surface of the stratum griseum is a similar layer of tangential fibers, chiefly unmyelinated, which carry nervous impulses from the tectum to the underlying cerebral peduncle and motor tegmentum (tractus tecto-peduncularis profundus, see p. 254).

The histological organization of the tectum exhibits a very primitive pattern with very imperfect functional localization. My preparations reveal no important structural differences between the optic and the somesthetic parts of the tectum, except that the walls of the latter are somewhat thicker and the neuropil of the stratum album is more dense (figs. 9 to 14). The individual neurons of the tectum, so far as shown by our preparations, are of several types, of which the one most commonly impregnated in our Golgi sections has a single thick dendrite which passes through the stratum griseum and branches as soon as the stratum album is reached (figs. 21, 22, 23, 31, 32, 33, 35). These branches are thick, contorted and very widely spread throughout the stratum album, frequently ending in dense irregular tufts approaching the form of glomeruli (figs. 22, 23). They differ in this respect from the large and equally widely spread dendrites of the neurons of the pedunculus cerebri, which are smoother and more diffusely branched at their termini (fig. 23). The axon usually springs from one of the larger dendrites near its base in the deeper levels of the stratum album (fig. 31, ax.) These axons are impregnated for a very short distance and probably acquire myelin sheaths at once. The dendrites of a single neuron of this type may spread throughout the entire dorso-ventral extent of the tectum and thus effect functional connections with diverse afferent systems. Figures 21, 22, 23, 32 illustrate neurons, some of whose dendrites arborize in the terminal area of the optic tract, while others reach only the terminals of the lemniscus systems. An element of this type may, therefore, receive either optic or tactile stimuli or both of these simultaneously, this being the simplest possible type of a correlation center. Other neurons lying farther ventrally in the tectum may send one large dendrite into the terminal area of the lemniscus (colliculus inferior) and another into the tegmentum (figs. 31, 32), thus being able to respond to excita-

tions coming in either by the lemniscus from the bulbar and spinal centers or by the pedunculus cerebri from the higher regions of the thalamus and cerebral hemispheres.

As we have seen, the dorso-medial part of the tectum only is reached by the optic tracts and this part may, therefore, be termed tectum opticum and considered homologous with the mammalian colliculus superior. Most of the optic fibers end by free arborizations in the rostral end of this region (figs. 36, 37); whether any of them reach as far as the caudal end of the tectum in Necturus is not clear—apparently not. Within the tectum opticum there is an area of neuropil in the stratum album where the most numerous optic synapses are found. This area is rather sharply separate from the larger and denser area of neuropil lying farther ventrally which receives the lemniscus terminals only and which, accordingly, corresponds with the colliculus inferior of mammals (figs. 9 to 14, 36, 37).

While the terminals of the optic tract seem to be confined to the dorsal part of the tectum and chiefly to its rostral end, the terminals of the acoustico-lateral and spinal lemniscus tracts, on the other hand, reach all parts of the tectum and are not limited to the ventral and caudal parts (the colliculus inferior). Some fibers of both of these tracts are clearly seen to spread throughout the optic region also, at a deeper level than the optic termini (fig. 24).

No definite regions of the tectum can be assigned as the terminal nuclei of the acoustico-lateral and spinal lemnisci, though probably the tectal distribution of these tracts is not exactly coextensive. The tractus bulbo-tectalis (trigeminal lemniscus?), on the other hand, is definitely distributed to the nucleus posterior tecti (figs. 52, 53) and the ascending secondary visceral tract is even more sharply limited to the superior secondary visceral nucleus (figs. 51, 52).

The nucleus posterior tecti has just been mentioned as a special area at the caudal end of the tectum which contains in both Necturus and Amblystoma a specially dense group of neurons with thick contorted dendrites. The mesencephalic ventricle is here dilated to form the recessus posterior mes-

encephali, a relation which is much more obvious in young
larvae of urodeles. The nucleus posterior tecti is the differen-
tiated wall of this ventricular recess. It contains dorsally a
special group of neurons of the mesencephalic V nucleus, and
more laterally the terminal nucleus of the tractus bulbo-tectalis.
From the latter area an important component of the tractus
tecto-thalamicus et hypothalamicus cruciatus arises (p. 257),
and also the tractus tecto-peduncularis posterior (p. 254).

The superior secondary visceral nucleus has been described
on page 248, where it is shown that it receives fibers from the
ascending secondary visceral tract derived from the nucleus
of the fasciculus solitarius. This small nucleus appears to
correspond with the 'Rindenknoten' of Mayser ('82) in teleosts;
but the commissural fibers which connect these nuclei through
the decussatio veli in teleosts are not apparent in Necturus.
The tertiary visceral tract leaving this nucleus, which I have
demonstrated in teleosts ('05), is but feebly developed in Nec-
turus and most of the fibers of the secondary tract appear to
pass forward beyond the level of the secondary nucleus in com-
pany with the less numerous tertiary fibers. Their rostral
terminus has not been clearly demonstrated.

It is quite probable that the differentiation of the tectum
opticum from the remainder of the tectum mesencephali is
incidental to the partial degeneration of the optic apparatus in
Necturus, rather than an expression of primitive relations; for
Johnston ('05 a, p. 237) has commented upon the fact that such
a differentiation is not evident in the generalized fishes. It
seems more probable that the tectum mesencephali was primi-
tively a correlation center for impulses received from the skin,
the muscles, the ear, the lateral line organs, and the eye, and
that these special systems obtained separate localized centers
later in the evolutionary history, the optic system developing
the colliculus superior and the acoustic system the colliculus
inferior, the muscle sense of the head retaining its primitive
relations with the mesencephalic V nucleus, and the cutaneous
and proprioceptive systems in general for the most part being
transferred to the correlation centers of the thalamus. This

view receives further support from the fact that lemniscus fibers are distributed throughout the optic tectum (p. 246) and from the arrangement of the tectal neurons described above.

Bellonci ('88, p. 31) established a true principle in general when he affirmed that the differentiation of the roof of the midbrain into superior and inferior colliculi arose from the fact that this region receives its chief afferent fibers from two sources—the thalamus and the medulla oblongata. The optic fibers coming in from the thalamus led to the differentiation of the colliculus superior away from the colliculus inferior. (The principle which he enunciated stands in spite of the fact that in the frog he incorrectly identified the nucleus magnus of Reissner ('64), or ganglion isthmi of Edinger, with the corpus posterior, or inferior colliculus) But the separation of these functionally distinct regions from the primitive tectum mesencephali has advanced only a very short way in Necturus.

From the preceding account it is evident that the tectum of Necturus lacks the well defined lamination which is so characteristic a feature of most other vertebrates. Since all of the usual functional systems except the optic are well represented here, it follows that the customary type of lamination is correlated with the optic apparatus.

The fibers of the stratum album have the following arrangement. At the rostral end of the midbrain the fibers of the commissura posterior pass downward close to the central gray for their entire length (figs. 7, 8, *com.post.*). Farther caudad the commissura tecti occupies the same position in the middorsal plane, but farther ventrally these fibers are displaced laterally (figs. 9 to 14). Medially of the latter fibers are those of the mesencephalic V root (figs. 11 to 14, *r.V.mes*) and farther ventrally at the boundary between the gray and white layers is the tractus tecto-peduncularis profundus (figs. 9 to 14, *tr.t.ped.p.*). The spino-tectal and spino-thalamic tracts lie ventrally of the mesencephalic V root and immediately laterally of the tractus tecto-peduncularis profundus (figs. 9 to 14). Farther laterally is the acoustico-lateral lemniscus (*lm.*) and the associated neuropil of the colliculus inferior, and farther

forward in this level the tractus tecto-thalamicus rectus (figs. 5 to 8). Scattered through this level ventrally run also the fibers of the tractus tecto-bulbaris (figs. 11 to 14, *tr.t.b.*). At the extreme caudal end of the tectum the most superficial fibers are those of the tractus bulbo-tectalis, together with the associated neuropil of the nucleus posterior tecti and the secondary visceral tract and nucleus (figs. 14, 50, 51, 52). The fibers of the tractus tecto-thalamicus et hypothalamicus cruciatus run slightly external to those of the acoustico-lateral lemniscus and the uncrossed tractus tecto-thalamicus, but they are not strictly superficial. In the rostral part of the tectum the fibers of the optic tract form a thin layer close to the pial surface, immediately internal to which is the neuropil of the optic tectum.

These relations may give some clues to the sequence of evolution of the tectal systems, for it is a general rule (with, however, many exceptions) that more recently developed fiber systems are added superficially to the more primitive systems.

In summary, the tectal fiber tracts are as follows. The afferent systems include the following tracts: radix mesencephalica trigemini, tractus opticus, tractus spino-tectalis, tractus bulbo-tectalis, lemniscus acoustico-lateralis, tractus visceralis ascendens. The efferent systems include: tractus tecto-bulbaris, tractus tecto-peduncularis, tractus tecto-habenularis, tractus tecto-thalamicus rectus, tractus tecto-thalamicus et hypothalamicus cruciatus. The posterior commissure and commissura tecti diencephali also transmit nervous impulses from the tectum to the pedunculus cerebri.

The pars dorsalis thalami

This is the great receptive center of the diencephalon for somatic afferent impulses, which enter it by the lemniscus and tecto-thalamic systems and by the optic tracts (which might well be called the optic lemniscus). The lemniscus systems which ascend from the spinal cord and medulla oblongata in part traverse the midbrain to connect directly with the pars dorsalis thalami; but the larger part of these fibers are inter-

rupted in the tectum mesencephali and neurons of a higher order
carry on the nervous impulses to the thalamus. This latter
arrangement appears to be the primitive one, and as we ascend
the phylogenetic series an increasingly larger proportion of the
lemniscus fibers effect direct connection with the thalamus.

The pars dorsalis thalami exhibits little evidence of separate
localization of specific nuclei for the optic, acoustic, somesthetic,
and other functional systems which enter it, though there are
some indications of the early stages of such functional localiza-
tion. Its posterior portion receives the scanty thalamic ter-
minals of the spinal and acoustico-lateral lemnisci; its middle
and most massive portion receives the greater part of the strong
uncrossed tecto-thalamic tract and possibly hypothalamic fibers
representing the tractus mamillo-thalamicus (p. 265); its
anterior portion contains the sharply circumscribed neuropil
to which we apply the name pars optica thalami. This neuropil
receives collaterals from fibers of the marginal optic tract, and
probably also of the axial optic tract, as has been more fully
described on page 244. It may also receive some terminals from
the anterior part of the tractus tecto-thalamicus et hypothal-
amicus cruciatus after decussation in the postoptic commissure
(p. 256). Dendrites of neurons from all adjacent parts of the
thalamus may enter this neuropil, but there is one group of
neurons whose dendrites seem to be related exclusively with
this area. These neurons form a slight ventricular eminence
in the rostral end of the pars dorsalis thalami immediately
caudad of the eminentia thalami, and are termed the nucleus
of the pars optica thalami (figs. 42 to 45, 63, 64, 65, 67, 68, *nuc.p.o.*
th.). The axons from this nucleus are directed caudad through
the pars ventralis thalami, thus forming the most ventral fibers
of the tractus thalamo-peduncularis dorsalis (figs. 42 to 45, 48).

The neurons of the more massive middle portion of the pars
dorsalis thalami have longer dendrites than those of the nucleus
of the pars optica. Some of these dendrites reach the pars
optica, as just mentioned; but most of them spread out among
the terminals of the tractus tecto-thalamicus rectus and other
fibers of the overlying stratum album. Their axons in part

enter the tractus thalamo-peduncularis dorsalis for the cerebral peduncle (p. 264) and the tractus dorso-ventralis thalami for the pars ventralis thalami (p. 266); but the larger part enter the tractus thalamo-frontalis anterior. These are mostly very fine unmyelinated fibers which are gathered into several very compact fascicles which descend abruptly ventralward, at the same time curving slightly caudalward, to enter the lateral forebrain bundle and then turn forward to end in the lateral wall of the cerebral hemisphere (p. 268). A few scattered myelinated fibers are found among these fascicles, and from the caudal end of the pars dorsalis thalami there are more of these myelinated fibers (tractus thalamo-frontalis posterior).

The caudal part of the pars dorsalis thalami lying ventrally of the pars intercalaris diencephali receives chiefly the thalamic terminals of the spinal and acoustico-lateral lemnisci. Its efferent fibers are in part directed backward and downward to the cerebral peduncle in the tractus thalamo-peduncularis dorsalis, and in smaller part downward and then forward to enter the tractus thalamo-frontalis posterior, as just mentioned.

The pars dorsalis thalami is the great somatic sensory receptive center of the diencephalon. Its fiber connections show that in Necturus it is concerned chiefly with intrinsic thalamic reflexes of a very primitive sort, the efferent pathways for these responses being short axons discharging into the pars ventralis thalami and cerebral peduncle. In addition to these connections, there is a small but clearly defined thalamo-frontal path discharging into the lateral wall of the cerebral hemisphere and probably concerned with correlations of a higher order. These tracts are the precursors of the sensory thalamic radiations of mammals. The differentiation of specific functional areas in the pars dorsalis has begun, but has advanced only a very short distance in the direction of the mammalian conditions. It is probable that its functional character resembles most closely that of the medial nucleus of higher forms. The lateral and ventral nuclei are, however, represented, though not spatially separate. The pars optica seems to be concerned wholly with the intrinsic thalamic reflexes; it cannot, therefore, be compared

directly with the lateral geniculate body and pulvinar of mammals, which are chiefly cortical dependencies. The medial geniculate body is represented in a quite undifferentiated form in the caudal end of the pars dorsalis.

The pars ventralis thalami

This region is dominated by the great somatic efferent systems leading downward into the cerebral peduncle (tractus thalamo-peduncularis ventralis, page 263). It receives nervous impulses (1) from the tectum mesencephali by fibers of the tractus tecto-thalamicus et hypothalamicus cruciatus both before and after their decussation in the postoptic commissure (p. 257); (2) from the pars dorsalis thalami directly (tractus dorso-ventralis thalami, page 266) and also by way of the tractus thalamo-hypothalamicus et peduncularis cruciatus from the same side and from the opposite side (p. 259); (3) from the lateral walls of the cerebral hemispheres by way of the lateral forebrain bundle (tractus strio-thalamicus, page 267).

The neurons of this region have long, thick, widely branched dendrites which are contorted but not varicose or thorny, the axon arising from the base of the dendrite or one of its chief branches (figs. 27, 28, 42 to 45). These dendrites spread out among the fibers of the tracts mentioned in the preceding paragraph and into the surrounding regions, notably into the pars dorsalis thalami and the nucleus of the tuberculum posterius. The tecto-thalamic and lateral forebrain connections appear to be the most important sources of nervous impulses for this part of the brain. Figure 27 illustrates the way in which dendrites of these neurons turn ventrally to accompany the fibers of the tractus tecto-thalamicus et hypothalamicus cruciatus as they approach their decussation. They are similarly related to the fibers of the tractus thalamo-hypothalamicus et peduncularis cruciatus. Some of these dendrites appear to cross the medial plane in the commissure.

In general, the pars ventralis thalami of Necturus appears to be a center devoted to the collection of nervous impulses

from various somatic sensory centers (tectum, thalamus and cerebral hemisphere) and their distribution by way of relatively short axons into the cerebral peduncle.

The eminentia thalami and tractus cortico-thalamicus. Although having no direct mesencephalic connections and, therefore, not properly falling within the scope of this paper, some details of the structure of the eminentia thalami are incidentally shown in the illustrations and these will be briefly referred to here.

This eminence, as has already been mentioned (p. 230), is a strong projection of the ventricular surface immediatly behind the interventricular foramen and above and behind the hippocampal commissure, its caudal border being in imediate contact with the pars ventralis thalami, from which it is separated by a vertical sulcus (figs. 41, 42, 43, 48, 63, 64, 68, *em.th.*). Its relations are essentially as I have described them in Amblystoma ('10, pp. 419, 426 and figs. 17, 18). I have provisionally regarded it as a diencephalic structure, though further embryological research may show that it should be placed in the telencephalon medium. It is in contact anteriorly with the fibers of the commissura hippocampi, of the tractus cortico-habenularis medialis and of the stria medullaris. From the first of these tracts, and probably from the others also, collateral fibers are freely discharged into the eminentia thalami.

Golgi sections show that the neurons of this eminence are very small and densely crowded. Their thick, contorted dendrites spread out in every direction (fig. 48). Some are directed forward into the cerebral hemisphere among the fibers of the commissura hippocampi and tractus cortico-habenularis medialis (see Herrick, '10, p. 428) just as these fibers enter the commissure ridge. Others are directed lateralward and backward among the fibers of the stria medullaris. Horizontal sections by the Golgi method taken through the commissura hippocampi just dorsally of its decussation show very delicate unmyelinated fibers leaving this tract, which are probably collaterals of its fibers. This tract is called the tractus cortico-thalamicus (fig. 43, *tr.c.th.*). Its fibers are very short and immediately break

up in freely branched arborizations among the dendrites of the neurons of the eminentia thalami (only one of which is impregnated in this preparation; cf. fig. 48, *em.th.*).

Slender axons are seen to pass between the stria medullaris and the eminentia thalami; but the preparations do not show whether these fibers arise from the stria and end in the eminence or arise in the eminence and enter the stria. This connection between the stria medullaris and the eminentia thalami is much more clearly shown in our Golgi preparations of larval Amblystoma. There is also an obscure fibrous connection seen in some preparations made by the method of vom Rath between the eminentia thalami and the underlying preoptic nucleus, the nature of which is not revealed.

The axons of the neurons of the eminentia thalami seem to be very short. In numerous Golgi preparations I have seen them passing directly caudad to end apparently in the rostral end of the pars ventralis thalami, though some of them may continue still farther caudad in the tractus thalamo-peduncularis ventralis. Some of these axons are shown in figure 48. In some of our Golgi sections of larval Amblystoma taken in the horizontal plane these fibers are well impregnated. Here they are clearly seen to break up in an open neuropil within the pars ventralis thalami a very short distance behind the eminentia thalami. So short are these axons and so widely spread are their terminal arborizations that these neurons might almost be considered as belonging to Golgi's type II if it were not for the fact that, in spite of their short course, their axons evidently transmit nervous impulses from one functionally differentiated region of the brain to another.

The eminentia thalami is thus seen to receive nervous impulses from the cerebral hemisphere by way of the fibers of the commissura hippocampi (apparently as collaterals of these fibers) and probably also in a similar way from the stria medullaris. It discharges nervous impulses into the adjacent pars ventralis thalami, of which I consider it to be a specially differentiated portion.

Our analysis of the eminentia thalami is still incomplete. So far as shown by our preparations it appears to serve as an intermediary between the primordium hippocampi (the olfacto-visceral pallial area) and the somatic parts of the thalamus. In reptiles and mammals the nucleus anterior thalami stands in somewhat similar intermediate relation between the mammillary body and the somatic parts of the thalamus by way of the mammillo-thalamic tract of Vicq d'Azyr. And the mammillary body, in turn, receives part of its afferent nerve supply from the hippocampus. It may be that in the course of vertebrate evolution when the direct cortico-thalamic pathway found in Amphibia was interrupted its place was taken functionally by the longer and more complex connection by way of the column of the fornix and the mammillo-thalamic tract.

The pedunculus cerebri and motor tegmentum

The cell bodies of the neurons of this region are arranged in the stratum griseum in somewhat more open formation than are those of the overlying tectum and the stratum griseum is here somewhat thinner. Their dendrites are long and they branch widely throughout the stratum album, but in general they are less contorted than those of the tectum (figs. 23, 24, 31, 32, 33). Dendritic branches are given off at the boundary between the gray and white layers which run tangentially to the gray layer and form dense arborizations among the tangential axons of this region (fig. 32).

Among the motor tracts of the tegmentum are occasional tract neurons whose cell bodies lie in the stratum album and whose dendrites spread out in the neuropil between the tegmental fascicles (figs. 24, 35). The dendrites of some of the most ventral cells of the tegmentum are directed downward to arborize in the neuropil of the interpeduncular nucleus (fig. 61).

The stratum album of the cerebral peduncle receives the following tracts: the brachium conjunctivum from the cerebellum; the tractus tegmento-interpeduncularis from the eminentia subcerebellaris tegmenti; fibers from the tectum by way

of the commissura tecti mesencephali and the commissura posterior, the tractus tecto-peduncularis, and probably some fibers from the tractus tecto-thalamicus et hypothalamicus cruciatus; fibers from the habenula by the tractus habenulo-peduncularis; a few fibers of the parietal nerve (these may be efferent in function); fibers from the pars intercalaris, the pars dorsalis, and the pars ventralis of the diencephalon by the tractus thalamo-peduncularis; fibers from the pars dorsalis thalami by the tractus thalamo-hypothalamicus et peduncularis cruciatus; fibers of the nervus terminalis (these may be efferent in function); fibers from the lateral walls of the cerebral hemispheres by the lateral forebrain bundle; fibers from the medial walls of the cerebral hemispheres by the medial forebrain bundle; fibers from the hypothalamus which have not yet been fully analyzed.

The known efferent connections from this region (including the tegmentum both above and below the fovea isthmi) are as follows: the peripheral neurons of the III and IV nerves; possibly fibers of the nervus parietalis and nervus terminalis; the fasciculus longitudinalis medialis; the tractus pedunculo-bulbaris. The dorsal and ventral tegmental facicles (p. 270) arise and terminate within the region here under consideration. They conduct descending impulses into the eminentia subcerebellaris tegmenti, and after a synapse here into the medulla oblongata.

I have been able to demonstrate very few fibers of passage in Necturus, which traverse the cerebral peduncle without effecting functional connections either within it or within the eminentia subcerebellaris tegmenti. Some fibers of the lateral forebrain bundle and of the tractus tecto-thalamicus et peduncularis cruciatus may extend beyond the limits of these structures into the medulla oblongata, but most fibers of even these systems are interrupted by a synapse in the motor tegmentum under the tectum. On the other hand, the fibers of both the direct and crossed tractus tecto-bulbaris pass uninterruptedly through into the medulla oblongata.

The fiber tracts of the motor tegmentum which have been described in the preceding paragraphs are arranged in the

following order, from within outward. At the boundary between the gray and white layers are the brachium conjunctivum, tractus tecto-peduncularis profundus, and tractus tegmento-interpeduncularis. These are largely unmyelinated. Next to these are the dorsal tegmental fascicles, and farther ventrally the ventral tegmental fascicles, the fasciculus longitudinalis medialis, the tractus thalamo-peduncularis cruciatus from the postoptic commissure, and the tractus strio-peduncularis from the lateral forebrain bundle. External to these are the tecto-bulbar tracts and the superficial tecto-peduncular fibers. Below the isthmus region at the level of the auricular lobes of the oblongata the acoustico-lateral lemniscus and other lemniscus systems come to lie superficially of all these tegmental tracts.

Correlated with the fact that most of the nervous impulses which descend through the midbrain of Necturus are interrupted by at least one synapse under the tectum, we find three specially differentiated regions, one above and one below the fovea isthmi and one (the interpeduncular nucleus) extending throughout the length of this region close to the mid-ventral plane. These will next be reviewed.

The nucleus of the tuberculum posterius. This ventricular eminence lying dorsally and rostrally of the tuberculum posterius (figs. 63, 64, 68, *nuc.tub.p.*) is an important coordination station for assembling various types of descending motor fibers. Its neurons are large, with long widely branched dendrites (figs. 7, 8, 9, 23, 24, 30, 59, *nuc.tub.p.*). In the dorsal part of this eminence and extending somewhat farther dorsally than its border, are the very large neurons of the nucleus of the posterior commissure and fasciculus longitudinalis medialis (see pp. 252, 271 and figs. 8, 23, 59, 60, *nuc.com.post.*), which seem to be more specialized members of the same functional complex, though the ventricular eminence itself is not produced by these neurons. There is a very slight ventricular eminence running transversely just behind the nucleus of the tuberculum posterius which is formed by these large cells and by the commissure itself (figs. 63, 64 and compare figs. 7 and 8).

Some of the dendrites of the neurons of the nucleus of the tuberculum posterius are very long, reaching dorsalward in company with the axons of the tractus thalamo-peduncularis to the pars dorsalis thalami and even to the pars intercalaris; others reach forward and ventralward as far as the nuclus of the tractus pallii immediately behind the postoptic commmissure. These dendrites receive nervous impulses by way of extensive tracts from the hypothalamus, from the medial and lateral forebrain bundles, from the thalamo-penclular tracts, from the posterior commissure, and from the midbrain and thalamus by way of the postoptic commissure. The efferent discharges from this region go in part to the nuclei of the III and IV nerves and in larger part into the dorsal and ventral tegmental fascicles and the fasciculus longitudinalis medialis.

Laterally of the tuberculum posterius is an elongated superficial area of dense neuropil, the area lateralis tegmenti (figs. 8, 9, 10, 31, 32, 54, 55, *a.l.t.*), which is reached by dendrites with thick bushy terminals from neurons of the nucleus of the tuberculum posterius. In this neuropil are the much branched termini of the thick unmyelinated axons of the supposed basal optic bundle (p. 242). It is also reached by fibers of the medial forebrain bundle, by fine freely branched axons from the motor tegmentum farther caudad (especially well shown in some of our preparations of larval Amblystoma), by axons from the hypothalamus and from the pars intercalaris diencephali. There are indications in vom Rath and Cajal preparations that the fibers ascending into it from the motor tegmentum are derived from the ascending visceral tract (visceral lemniscus) and superior secondary visceral nucleus, but of this there is no clear demonstration. If these latter indications should be confirmed, it would identify this neuropil as in part a visceral (olfacto-gustatory) correlation center of a higher order. In the frog I have seen in the corresponding region quite superficially and immediately rostrally of the superficial origin of the III nerve a small nucleus, whose cells are arranged as a hollow sphere surrounding a core of dense neuropil, which may be a more

specialized form of the same structure; but of the fiber connections of these neurons nothing is known.

The eminentia subcerebellaris tegmenti. This is a very large ventricular eminence in the motor tegmentum under the nucleus posterior tecti and cerebellum and extending as far forward as the fovea isthmi (figs. 14, 63, 64, 68, *em.s.t.*). The dendrites of its neurons are widely spread among the fibers of the motor tegmentum, and especially are directed dorso-laterally into the area of termination of the dorsal fasciculi of the motor tegmentum. These dendrites form dense packets between the longitudinal fascicles of the motor tegmentum. They receive direct and crossed fibers from the tectum and from regions farther forward by the tegmental fascicles.

The axons of some of these neurons (chiefly unmyelinated) are directed forward and ventrally parallel with the brachium conjunctivum. These fibers reach the mid-ventral plane within the interpeduncular nucleus immediately caudad of the fovea isthmi and appear to end in relation with the ventro-medial nucleus of the III nerve (the supposed representative of the nucleus of Edinger-Westphal, see p. 235). They are provisionally named the tractus tegmento-interpeduncularis (p. 272).

There is a group of very large neurons at the lateral border of the stratum griseum of the eminentia subcerebellaris whose myelinated axons are directed laterad and caudad without decussation into the ventral fasciculi of the motor tegmentum of the oblongata, thus constituting a tractus tegmento-bulbaris (p. 271). Other fibers of this system doubtless decussate in the ventral commissure. These fibers put the motor tegmentum under the tectum mesencephali into functional relation with the reticular formation of the oblongata. Golgi sections show that the dendrites of these large neurons spread widely through the motor tegmentum, especially caudo-laterally into the eminentia ventralis cerebelli ('14, p. 4), where they come into relation with descending fibers of the dorsal tegmental fascicles and ascending fibers of the tractus spino-bulbaris ('14 a, p. 375). Large bipolar neurons with widely branched dendrites are occasionally impregnated in the stratum album laterally of the

eminentia subcerebellaris tegmenti. One of these is shown in
figure 35 and two of them are figured in my former paper ('14,
p. 25, fig. 18). Their axons have not been seen.

The nucleus interpeduncularis. This is an area of very dense
neuropil lying ventrally of the ventral commissure from the tuber-
culum posterius far backward into the medulla oblongata. It is
not well developed rostrally of the fovea isthmi, but it becomes
large immediately caudad of that region. The neurons bor-
dering the extreme ventral part of the mesencephalic ventricle
send their dendrites downward through the fibers of the ventral
commissure. Here they branch freely, forming a compact
entanglement of bushy and thorny dendritic branches, through
which slender unmyelinated longitudinal axons descend into
the medulla oblongata (figs. 61, 68, *nuc.ip.*). These axons in
part are derived from the tractus habenulo-peduncularis and
in part they arise from the neurons of the interpeduncular nu-
cleus itself.

VII. CONCLUSION

This paper is concerned with the descriptive anatomy of
the midbrain and thalamus and their connections in Necturus.
The topography and subdivisions of the mesencephalon and
diencephalon are presented in Section II, and the internal
structure and functional connections of each of these regions
are given in Section VI. Some of these relations are shown in
figures 62 to 68. These facts need not be again summarized
here. The specific morphological questions suggested by these
data can best be discussed at a later time after the functional
analysis of the amphibian diencephalon and telencephalon
has been more fully elucidated. Attention may be called here,
however, to some of the more general problems related to these
researches.

Some of these questions have been suggested by the pre-
viously published studies of Coghill and the present writer on
the nervous system of Amblystoma. Coghill ('02) finds that
the functional components of the cranial nerves at about the
time of metamorphosis present the typical vertebrate pattern,

and I find the same relations in half grown larvae. Coghill
('16) has studied the development of each of these functional
systems in the youngest larvae. Here, as in the development of
the spinal nerves (Coghill, '14 and Herrick and Coghill, '15), the
elaboration of the pattern of the peripheral nerve components
is accompanied by striking changes in the orgnization of the
central nervous system.

From these researches it is suggested that functional differ-
entiation in the phylogeny, as in the ontogeny, began at the
periphery; and here the elaboration of functionally specific end-
organs and conduction paths advances much more rapidly on the
sensory side than on the motor side of the reflex circuits.[1] At
an early stage when all of the sense organs and afferent nerve
components are differentiated substantially as in the adult the
motor mechanisms of the spinal cord and nerves may show very
little evidence of capacity for diversified response, simple swim-
ming movements toward or away from the source of the stimulus
being almost the only possible reactions. In other words, the
elaborately diversified receptor mechanisms converge into a
very simply organized final common path. Even in the half
grown larva the motor apparatus of the spinal cord seems to be
organized to serve chiefly total reactions of the simplest loco-
motor type.

In the medulla oblongata of the half grown larva we can
follow some of the steps in the gradual emergence of functionally
specific primary sensory centers and their correlation tracts
from a relatively equipotential receptive apparatus which origin-
ally discharged directly and without functional analysis of
different sense qualities into the motor final common path (Her-
rick, '14 a). In these larvae various lemniscus systems which
discharge specific kinds of sensory nervous impulses upward

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[1] This does not mean that the sensory paths become functional earlier than
the motor paths. In fact, in the development of the spinal cord of Amblystoma
larvae the converse is true, as Coghill ('13) has shown. But it does imply that
in the progress of differentiation the sense organs are structurally adapted to
respond in a selective way to a great variety of external stimuli at a stage when
the motor apparatus is so simply organized that there is possible but little
variety of modes of response to these excitations.

into the midbrain and thalamus are recongizable, though they are not functionally completely segregated. Essentially the same relations are found in adult Necturus.

Parallel with the differentiation of the several ascending afferent systems of tracts from their respective specific primary lower centers, the mechanism of integration is elaborated in the higher correlation centers. The complexity of these higher centers need not be great so long as the effector apparatus is relatively unspecialized, i.e., so long as the range of variety of possible responses to stimulation is relatively small.

This seems to be the condition realized in the brain of Necturus. Here the tectum mesencephali, the entire diencephalon, and probably to a less extent the cerebral hemispheres serve this integrative function and in connection therewith organize the afferent impulses in such a way as to ensure their discharge into the appropriate motor centers. But the nervous connections described in this contribution indicate that the functional specificity of these regions is even less sharply differentiated than in the case of the primary centers in the medulla oblongata. Mechanisms are provided for summation of stimuli of diverse sorts and doubtless for various inhibitions from conflict of sensory impulses and of these with mnemonic vestiges of previous experience; but there is very imperfect provision of the apparatus for functionally separated reflex connections with clearly defined localization in space adapted for diversified specific responses to particular kind of excitation. The movements, though of a very precise character of high adaptive value, are still largely on the plane of total reactions to a general situation, rather than diversified movements each of which is in response to some particular factor in the stimulus complex (on the behavior of Necturus, see Whitman, '99, p. 295, Eycleshymer, '06, Reese, '06, Sayle, '16; on Amblystoma, see Haecker, '12).

The nervous centers and conduction pathways described in this paper are concerned, for the most part, with somatic sensorimotor reactons, as contrasted with those of the visceral and olfactory systems. Further investigation of the diencephalon and telencephalon of urodeles is necessary before the correlation

centers of the latter systems can be fully described and their relations to the somatic systems determined. For such studies this paper is preliminary.

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ABBREVIATIONS

a.l.t., area lateralis tegmenti
aq., aqueduct of Sylvius
ax., axon
br.conj., brachium conjunctivum
cb., cerebellum
ch., chiasma optica
col.inf., colliculus inferior
col.sup., colliculus superior
com.ant., commissura anterior
com.cb., commissura cerebelli (myelinated component)
com.cb.l., commissura cerebelli (lateral unmyelinated component)
com. hab., commissura habenularum (commissura superior)
com.hip., commissura hippocampi
com.po., commissura postoptica
com.post., commissura posterior
com.t.d., commissura tecti diencephali
com.t.m., commissura tecti mesencephali
com.tub.p., commissura tuberculi posterioris
com.v., commissura ventralis
d., dendrite
em.s.t., eminentia subcerebellaris tegmenti
em.th., eminentia thalami
ep., epiphysis
epen., ependyma
F., foramen interventriculare
f.d.t., fasciculus dorsalis tegmenti
f.i., fovea isthmi
f.lat.t., fasciculus lateralis telencephali (lateral forebrain bundle)
f.l.m., fasciculus longitudinalis medialis
f.med.t., fasciculus medialis telencephali (medial forebrain bundle)
f.R., fiber of Reissner
f.retr., tractus habenulo-peduncularis (fasc. retroflexus)
f.v.t., fasciculus ventralis tegmenti
hab., habenula
hyp.g., hypophysis, pars glandularis
hyth., hypothalamus
inf., infundibulum
lm., acoustico-lateral lemniscus

m.n., multipolar neuron of stratum album
n.II, nervus opticus
n. III, nervus oculomotorius
n.IV, nervus trochlearis
n.IV.x., root of trochlear nerve proximally of its decussation
n.par., nervus parietalis
n.term., nervus terminalis
nuc.com.post., nucleus of commissura posterior
nuc.III, nucleus of oculomotor nerve
nuc.ip., nucleus interpeduncularis
nuc.mes.V, nucleus mesencephalicus trigemini
nuc.po., nucleus preopticus
nuc.po.m., nucleus preopticus, pars magnocellularis
nuc.p.o.th., nucleus of pars optica thalami
nuc.p.t., nucleus posterior tecti
nuc.tr.pal., nucleus of tractus pallii
nuc.tub.p., nucleus tuberculi posterioris
nuc.vis.s., secondary visceral nucleus
o.sc., organon subcommissurale (subcommissural organ of Dendy)
optc., optocoele
par., paraphysis
p.d.hyth., pars dorsalis hypothalami
p.d.th., pars dorsalis thalami
ped., pedunculus cerebri
p.i.th., pars intercalaris diencephali
pl.c.d., plexus chorioideus diencephali
p.o.th., pars optica thalami
prim.hip., primordium hippocampi
p.v.hyth., pars ventralis hypothalami
p.v.th., pars ventralis thalami
r.l., recessus lateralis rhombencephali
r.pin., recessus pinealis
r.p.m., recessus posterior mesencephali
r.po., recessus preopticus
r.V.mes., radix mesencephalica trigemini
s.a., stratum album
sac.d., saccus dorsalis
sac.v., saccus vasculosus
s.d., sulcus dorsalis thalami
s.ep., stratum ependymale

s.g., stratum griseum

s.is., sulcus isthmi

s.m., sulcus medius thalami

s.sh., sulcus subhabenularis

str.med., stria medullaris

s.v., sulcus ventralis thalami

tect., tectum mesencephali

tr.b.t., tractus bulbo-tectalis

tr.c.hab.l., tractus cortico-habenularis lateralis

tr.c.th., tractus cortico-thalamicus

tr.d.v.th., tractus dorso-ventralis thalami

tr.hab.th., tractus habenulo-thalamicus

tr.mam.ped., tractus mamillo-peduncularis

tr.ol.hab.ant., tractus olfacto-habenularis anterior

tr.ol.hab.lat., tractus olfacto-habenularis lateralis

tr.ol.hab.med., tractus olfacto-habenularis medialis

tr.op., tractus opticus, marginal bundle

tr.op.ax., axial bundle of the optic tract distally of its decussation

tr.op.ax.x., axial bundle of the optic tract proximally of its decussation

tr.op.b., tractus opticus, basal bundle of Wlassak

tr.pal., tractus pallii

tr.po.i., tractus preoptico-intercalaris

tr.sp.t., tractus spino-tectalis

tr.sp.th., tractus spino-thalamicus

tr.st.t., tractus strio-tegmentalis

tr.st.th., tractus strio-thalamicus

tr.t.b., tractus tecto-bulbaris

tr.teg.b., tractus tegmento-bulbaris

tr.teg.i., tractus tegmento-interpeduncularis

tr.t.hab., tractus tecto-habenularis

tr.t.hab.c., tractus tecto-habenularis cruciatus

tr.th.f., tractus thalamo-frontalis

tr.th.f.a., tractus thalamo-frontalis anterior

tr.th.f.p., tractus thalamo-frontalis posterior

tr.th.h.p.c., tractus thalamo-hypothalamicus et peduncularis cruciatus

tr.th.h.p.c.x., tractus thalamo-hypothalamicus et peduncularis cruciatus, after decussation

tr.th.p.d., tractus thalamo-peduncularis dorsalis

tr.th.p.d.p., tractus thalamo-peduncularis dorsalis profundus

tr.th.p.d.s., tractus thalamo-peduncularis dorsalis superficialis

tr.th.p.i., tractus thalamo-peduncularis intermedius

tr.th.p.v., tractus thalamo-peduncularis ventralis

tr.th.p.v.i., tractus thalamo-peduncularis ventralis intermedius

tr.th.p.v.p., tractus thalamo-peduncularis ventralis profundus

tr.th.p.v.s., tractus thalamo-peduncularis ventralis superficialis

tr.t.ped., tractus tecto-peduncularis

tr.t.ped.i., tractus tecto-peduncularis intermedius

tr.t.ped.p., tractus tecto-peduncularis profundus

tr.t.ped.post., tractus tecto-peduncularis posterior

tr.t.ped.s., tractus tecto-peduncularis superficialis

tr.t.th.c.p., free endings in tectum of *tr.t.th.h.c.p.*

tr.t.th.h.c.a., tractus tecto-thalamicus et hypothalamicus cruciatus, pars anterior

tr.t.th.h.c.a.x., tractus tecto-thalamicus et hypothalamicus cruciatus anterior, ventral limb after decussation

tr.t.th.h.c.p., tractus tecto-thalamicus et hypothalamicus cruciatus, pars posterior

tr.t.th.r., tractus tecto-thalamicus rectus

tr.v.a., tractus visceralis ascendens (secondary gustatory tract)

tr.v.t., tertiary visceral tract

t.th., taenia thalami

tub.p., tuberculum posterius

v.l., ventriculus lateralis

v.m.a., velum medullare anterius

v.3, third ventricle

DESCRIPTION OF FIGURES

All of the figures are drawn from preparations of the brain of Necturus maculatus (*Raf.*). The figures of microscopic preparations are all drawn from single sections unless the contrary is explicitly stated. The drawings made from Golgi preparations aim to reproduce as faithfully as possible with pen and ink the actual appearance of the sections. They are schematized only by the omission of irrelevant detail, and in a few cases (which are specially noted) some features are introduced from adjacent sections.

Figs. 1 to 14 A series of transverse sections through the thalamus and midbrain of Necturus, stained by the method of Weigert. The sections were cut serially 15 μ in thickness. The myelinated fibers are printed in blue and the reference lines related to myelinated tracts are also printed in blue. $\times$ 25. The serial numbers of the sections drawn are included in the descriptions (in these and all other descriptions of figures the series numbers given refer to preparations of the brain of Necturus in the private collection of Professor P. S. McKibben), from which the exact intervals between these sections can be readily computed. Compare also figures 62 to 68, which are based on reconstructions from sagittal Weigert sections. For the external appearance of this part of the brain, see McKibben, '13, figures 1 and 2; see also the figures of Kingsbury, '95. Series of sections through the cerebral hemispheres and thalamus of Necturus are illustrated in the papers by McKibben, '11, and Röthig, '11a. Transverse sections through the upper part of the medulla oblongata are illustrated in figures 4 to 16 of my paper, '14, on the cerebellum of Necturus, and transverse sections through the entire medulla oblongata of larval Amblystoma, which is very similar to Necturus, are figured in my paper, '14 a.

Fig. 1 At the level of the optic chiasma (civ, 692).

Fig. 2 At the level of the habenular commissure (civ, 703).

Fig. 3 Through the thickest part of the chiasma ridge and postoptic commissure (civ, 723).

Fig. 4 Through the middle of the pars intercalaris of the diencephalon (civ, 733).

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Fig. 5 Through the commissura tecti of the diencephalon and the caudal part of the chiasma ridge immediately in front of the partial decussation of the tractus pallii (civ, 745).

Fig. 6 Through the extreme caudal end of the thalamus (civ, 757).

Fig. 7 Through the rostral end of the mesencephalon at the level of the posterior commissure (civ, 772).

Fig. 8 Through the nucleus of the commissura posterior (civ, 790).

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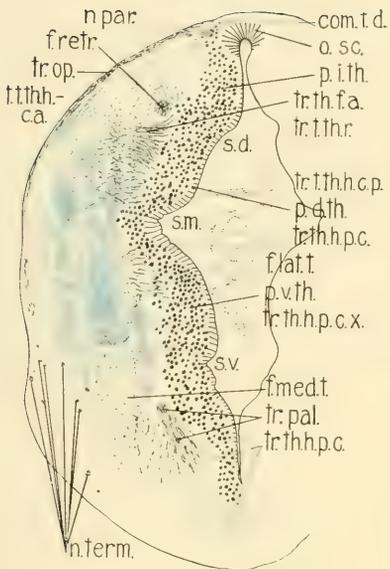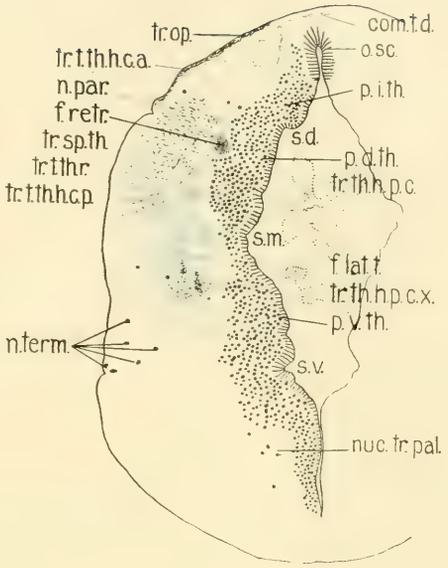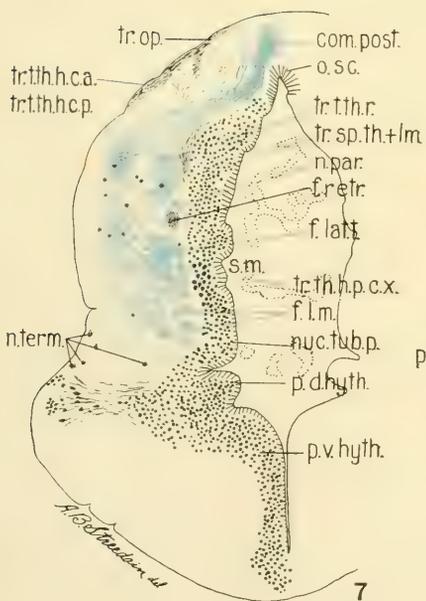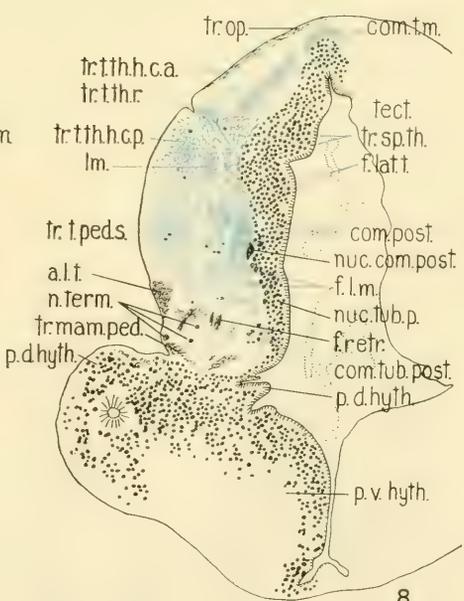
Fig. 9   Through the tuberculum posterius (civ, 802).

Fig. 10   Immediately below the attachment of the hypothalamus to the cerebral peduncle (civ, 819).

Fig. 11   At the level of the root of the III nerve and fovea isthmi (civ, 833).

Fig. 12   Through the middle of the tectum mesencephali (civ, 860).

Fig. 13   Through the lower third of the tectum.   The optic part of the tectum (colliculus superior) apparently does not extend farther caudad than this level (civ, 875).

Fig. 14   Through the nucleus posterior tecti.   The auricular lobe of the medulla oblongata appears in the section and the cerebellum lies a short distance farther caudad.   The fibers of the brachium conjunctivum (*br. conj.*) in this and the preceding sections are mingled with those of the tractus tecto-peduncularis profundus (*tr.t.ped.p.*) (civ, 890).
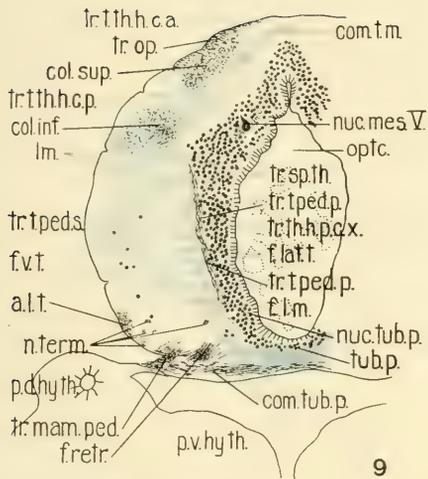
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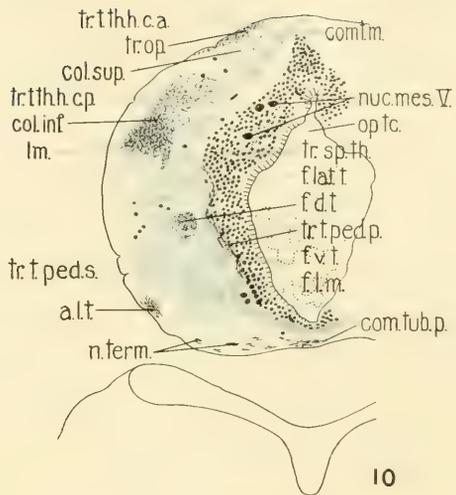
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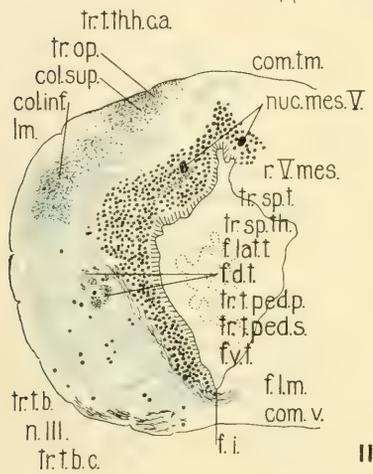
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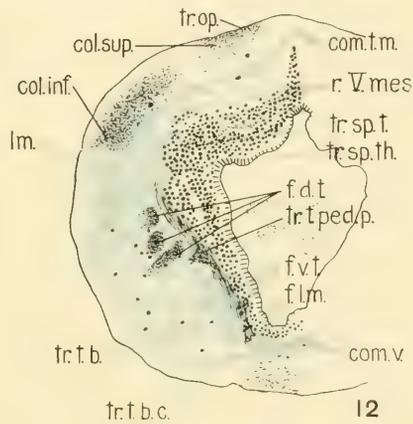
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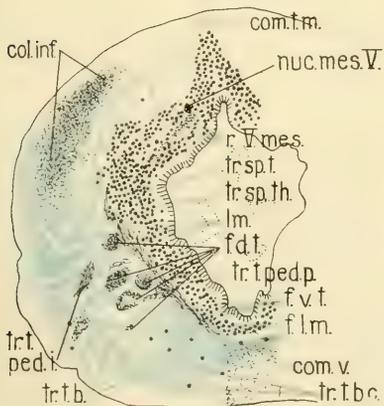
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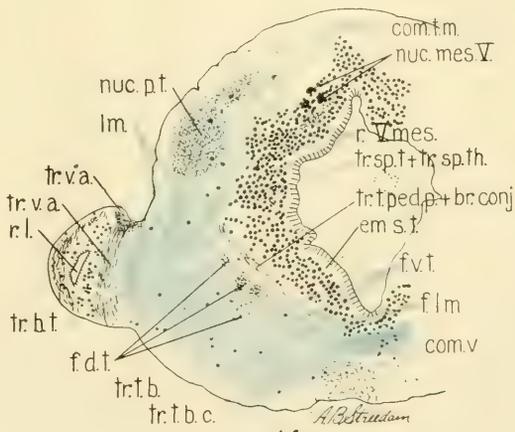
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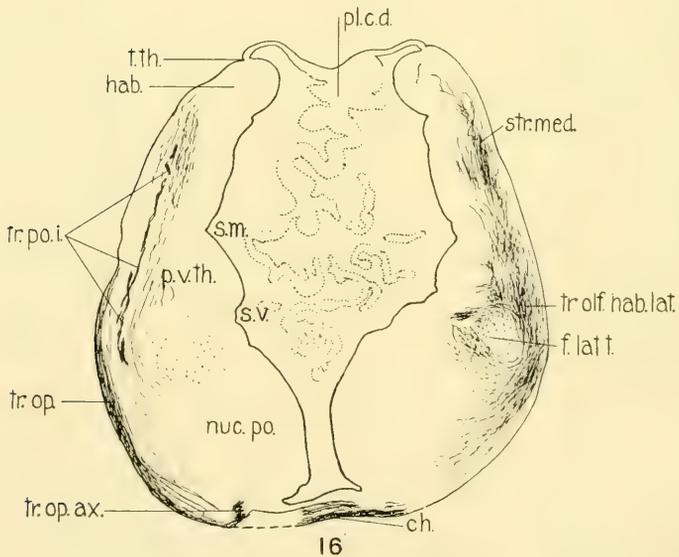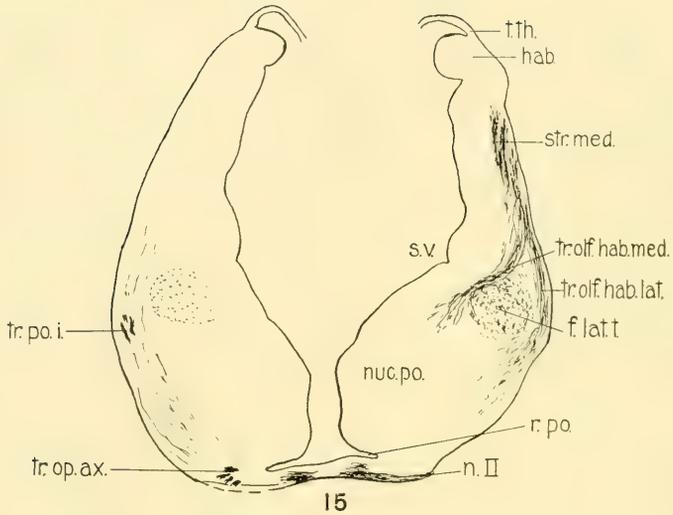
Figs. 15 to 24 Drawings from a series of transverse sections (each about 50 μ thick) through the thalamus and midbrain by the Golgi method.

Fig. 15 Through the preoptic recess (*r.po.*) immediately in front of the optic chiasma. . The origins of the lateral and medial olfacto-habenular tracts from the nucleus preopticus (*nuc.po.*) are shown on the right side, and on the left side the tractus preoptico-intercalaris (*tr.po.i.*) near its connection with the preoptic nucleus; cf. figure 25. × 22 (cvic, 145).

Fig. 16 Two sections farther caudad through the rostral end of the optic chiasma. × 22 (cvic, 147).

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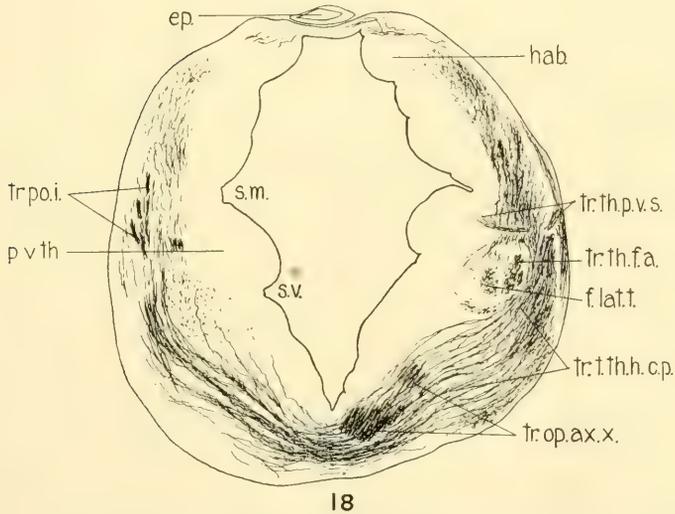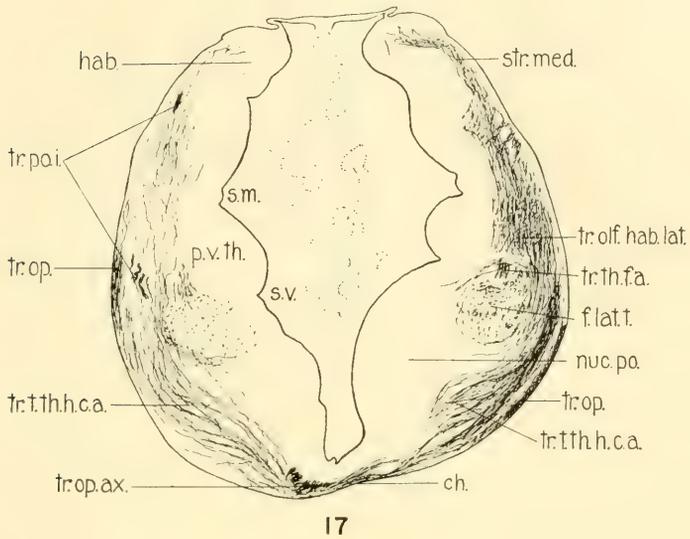
Fig. 17 Three sections farther caudad through the optic chiasma. × 22 (cvic, 150).

Fig. 18 Three sections farther caudad through the rostral end of the postoptic commissure. × 22 (cvic, 153).
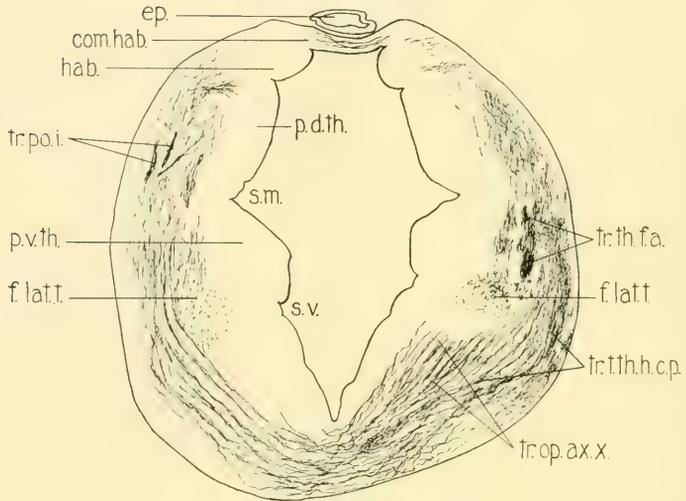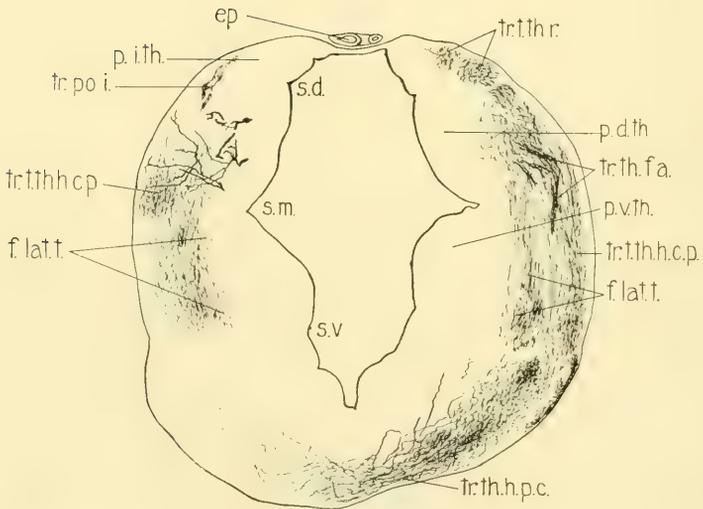
Fig. 19 Two sections farther caudad through the habenular commissure (*com.hab.*) and postoptic commissure. $\times$ 22 (cvic, 155).

Fig. 20 Five sections farther caudad through the pars intercalaris diencephali (*p.i.th.*), illustrating the connection of the tractus preoptico-intercalaris (*tr.po.i.*) with this part. The origin of the tractus thalamo-frontalis anterior (*tr.th.f.a*) from the pars dorsalis thalami (*p.d.th.*) is seen on the right. $\times$ 22 (cvic, 160).
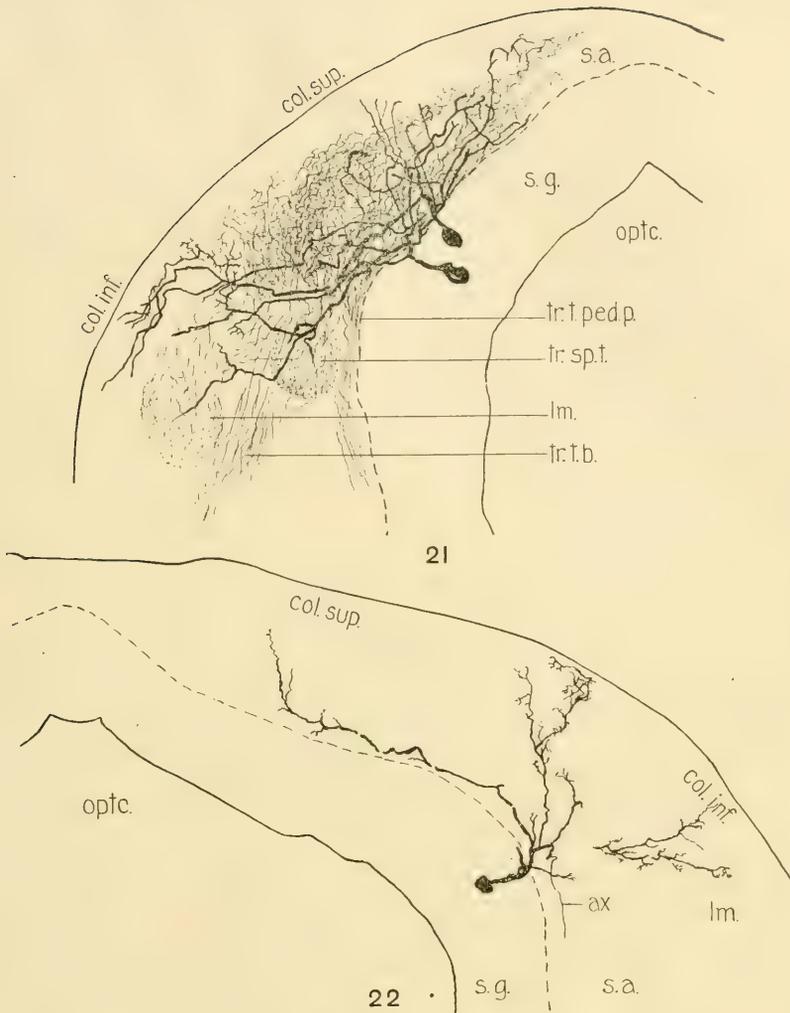
Fig. 21   Detail of the optic part of the tectum (left side) near its rostral end. The optic tract is not impregnated, but the area of termination of this tract, as revealed by other preparations, is marked *col.sup.*   Two neurons of the optic tectum are shown, the lower one being drawn in from the next adjacent section in front.   Some dendrites of each of these neurons arborize in the neuropil reached by the optic terminals, while others pass farther ventrally into the colliculus inferior (*col.inf.*).   Terminals from both the acoustico-lateral lemniscus (*lm.*) and the spinal lemniscus (*tr.sp.t.*) are seen to reach the neuropil of the optic part of the tectum, though their chief area of distribution is farther ventral and caudal.   × 74 (cvic, 176).

Fig. 22   A single neuron from the right side of the tectum one section caudad of figure 24.   Some of the dendrites were cut off and are drawn in from the adjacent sections by superposing camera outlines made on tracing paper.   The position of the acoustico-lateral lemniscus is indicated at *lm*, though the tract itself is not drawn.   × 74 (cvic, 182).
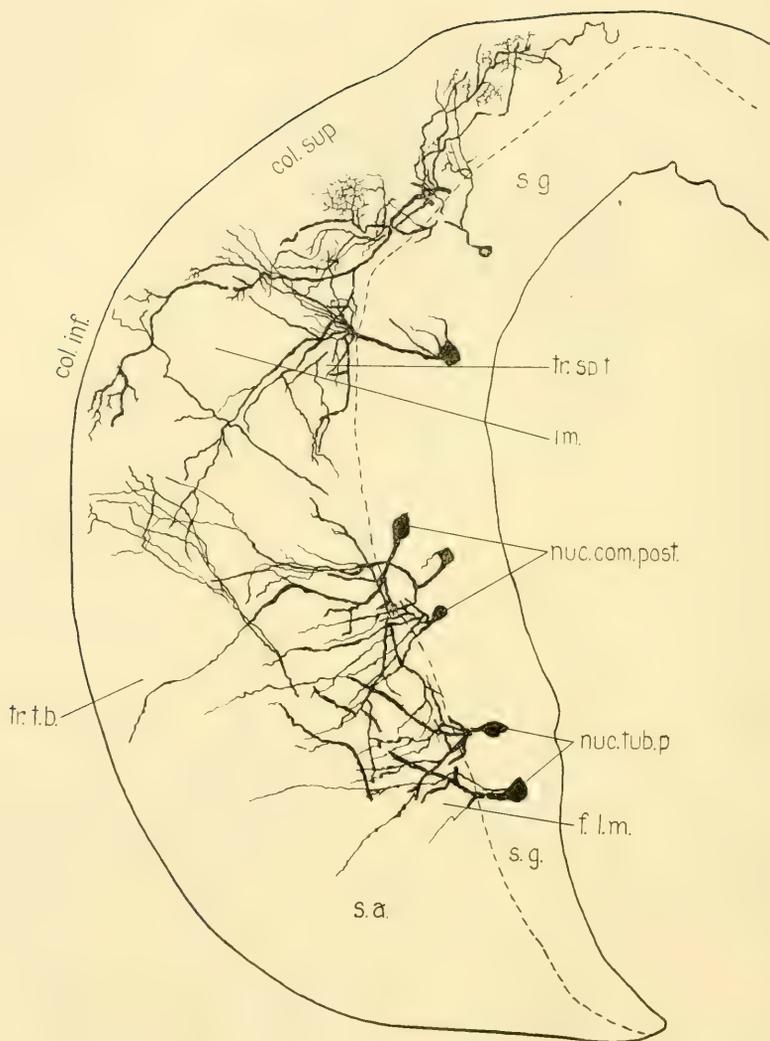
Fig. 23 Section immediately in front of the tuberculum posterius. Some details of the neurons are added from the two adjacent sections rostrad and caudad. Dendrites of neurons of the tectum spread out in the area of distribution of the optic tract (*col.sup.*) and also farther ventrally in the colliculus inferior (*col.inf.*). In the motor tegmentum are three neurons of the nucleus of the posterior commissure (*nuc.com.post.*) and two of the nucleus of the tuberculum posterius (*nuc.tub.p.*). The dendrites of the tectal neurons tend to develop tufted glomerulus-like terminals, in contrast with the more open and smoother terminals of those of the motor tegmentum. The locations of some of the fiber tracts are indicated on the figure, though to avoid confusion they are not drawn (cf. fig. 24, three sections farther caudad, where these tracts are drawn). In this section terminals of the acoustico-lateral lemniscus and spino-tectal tract are freely spread out in the colliculus superior substantially as shown in figure 24. × 74 (evic, 178).

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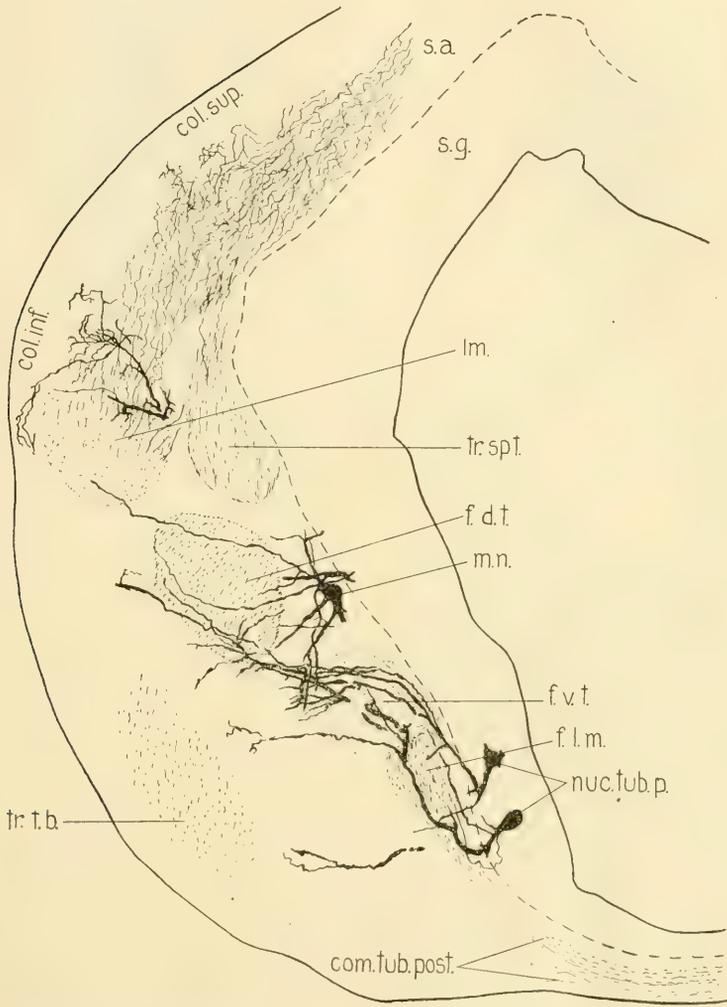
Fig. 24   Through the tuberculum posterius.   Two neurons of the nucleus of the tuberculum posterius (*nuc.tub.p.*) are impregnated and, farther dorsally, a neuron of the stratum album of the motor tegmentum (*m.n.*).   The locations of some of the fiber tracts are indicated and also free terminals from the acoustico-lateral and spinal lemnisci in the neuropil of the tectum opticum (*col.sup.*). $\times$ 74 (cvic, 181).
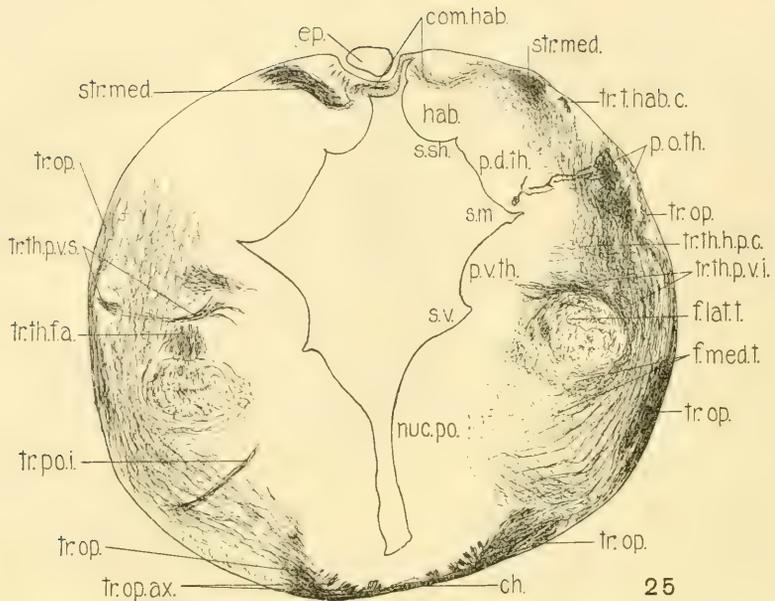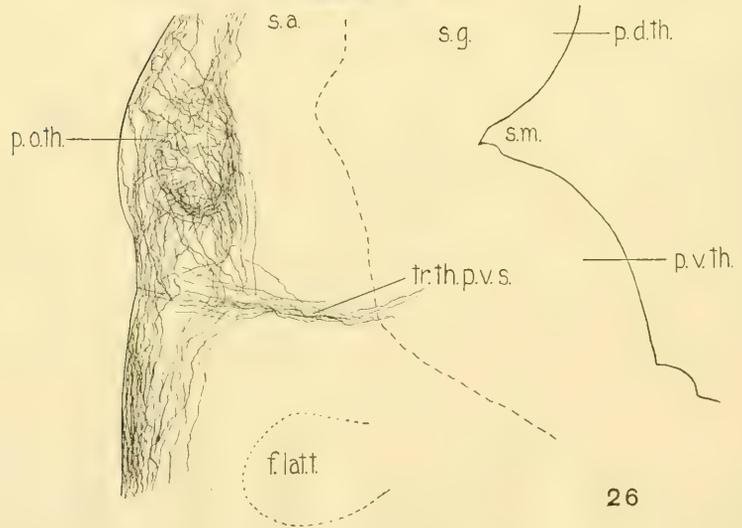
Figs. 25 and 27 to 35   Drawings of selected sections from a transverse series
by the Golgi method.   The left side of these sections is a little farther caudad
than the right.

Fig. 25   Through the habenular commissure (*com.hab.*) and optic chiasma
(*ch.*).   On the right side a single neuron of the pars dorsalis thalami (*p.d.th.*)
sends its dendrites lateralward into the neuropil of the pars optic thalami (*p.o.
th.*).   × 60 (cvc, 2–6–11).

Fig. 26   A detail of the pars optica thalami of the left side from a different
specimen from the proceding figure; transverse section, Golgi method.   None

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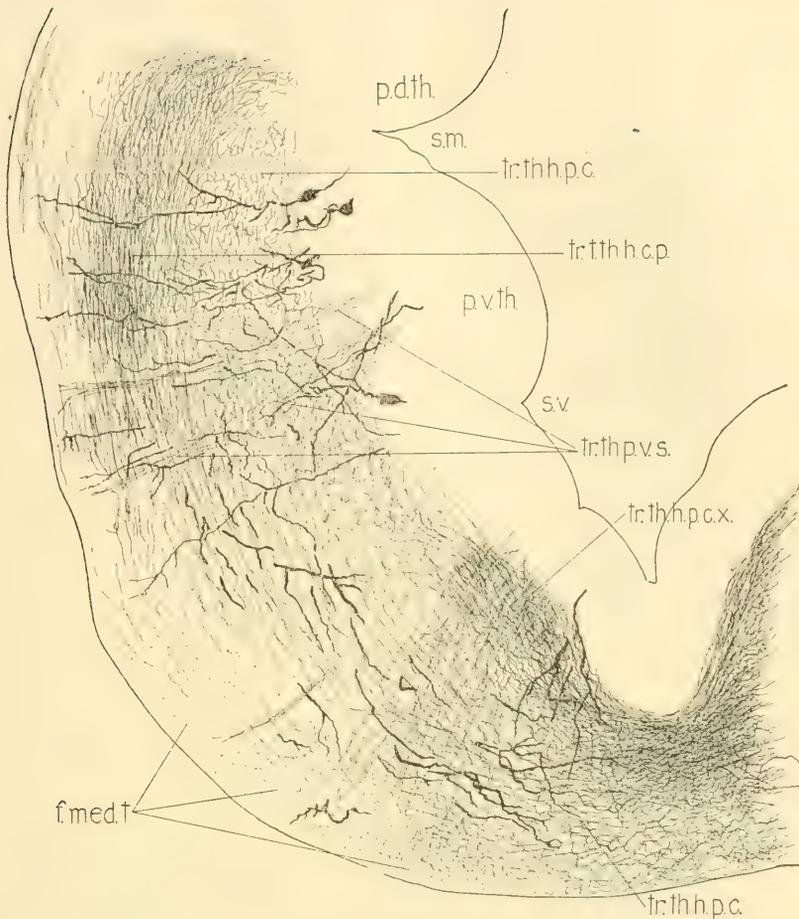
of the dendrites entering this superficial neuropil are impregnated. The vertical superficial fibers which discharge collaterals into this neuropil probably belong to the marginal optic tract (cf. fig. 54), though it is possible that they belong to the axial optic tract or to the tractus tecto-thalamicus et hypothalamicus cruciatus anterior after their decussation (cf. fig. 56). × 74 (cciii, 3–1–6).

Fig. 27 Detail of a section through the ventral part of the thalamus at the level of the middle of the chiasma ridge (cf. fig. 3, which is taken from about the same place). Drawn from the same series as figure 25. The fibers of the lateral forebrain bundle are not impregnated, but a part of those of the medial bundle are shown (*f.med.t.*). At this level the chiasma ridge is filled with a dense neuropil composed chiefly of contorted axons of the tractus thalamo-hypothalamicus et peduncularis cruciatus mingled with dendrites of the neurons lying farther dorsally. The section is too thin to reveal the sources of all the dendrites, but the indications are that they come from both the hypothalamus and the pars ventralis thalami. Those from the latter source are related more especially with the tractus tecto-thalamicus et hypothalamicus cruciatus. × 60 (cvc, 3–1–8).
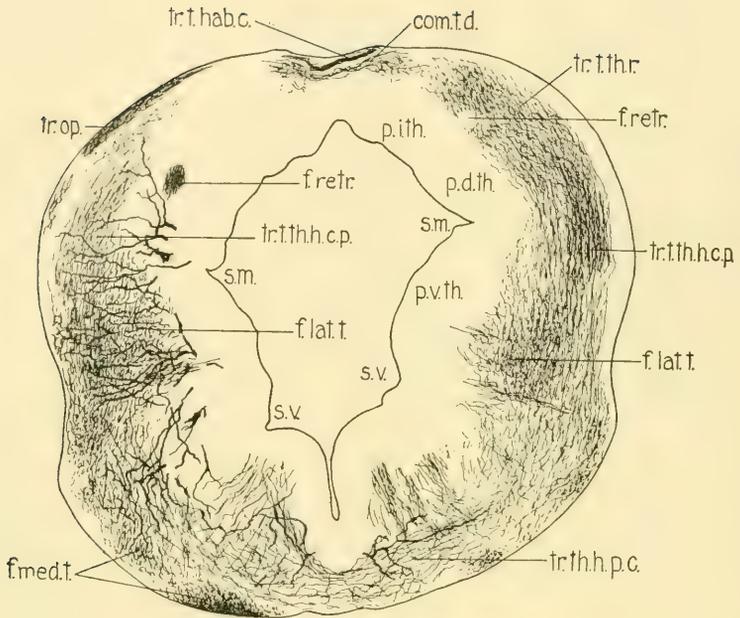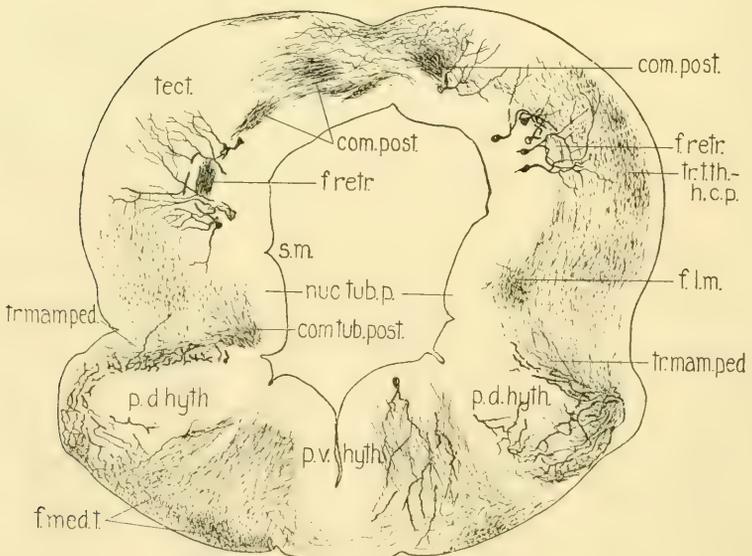
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Fig. 28 Through the commissura tecti diencephali (*com.t.d.*) and caudal part of the thalamus (cf. fig. 5). Dendrites from the pars ventralis thalami are seen to spread widely among the fibers of the postoptic commissures and the lateral and medial forebrain bundles. × 30 (cvc, 3–2–3).
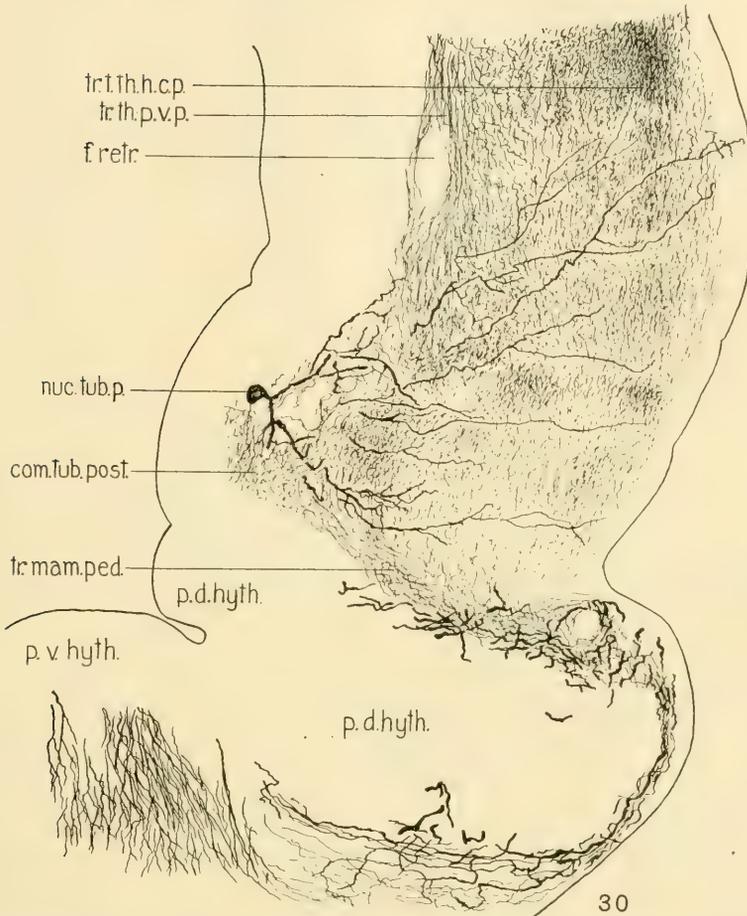
Fig. 29 Through the commissura posterior (*com.post.*) and the anterior end of the pars dorsalis hypothalami (*p.d.hyth.*); cf. figure 7.   The cell bodies of the neurons of the pars dorsalis hypothalami are not impregnated, but their dendrites and axons are shown, the latter forming the tractus mamillo-peduncularis, part of which appears to enter the commissure of the tuberculum posterius, or commissura hypothalami posterior (*com.tub.post.*).   × 30 (cvc, 3-2-6).

Fig. 30 Detail of the nucleus of the tuberculum posterius (*nuc.tub.post.*) and pars dorsalis hypothalami (*p.d.hyth.*) of the right side, two sections caudad of figure 29.   The plane of section lies between those of figures 7 and 8.   Dendrites and axons from the pars dorsalis hypothalami are impregnated, and also a single neuron of the nucleus of the tuberculum posterius.   Some dendrites related to the latter nucleus are drawn in from the adjacent sections rostrad and caudad.   × 74 (cvc, 3-2-8).
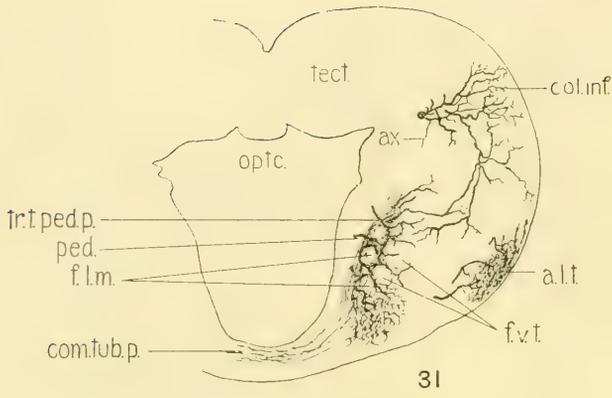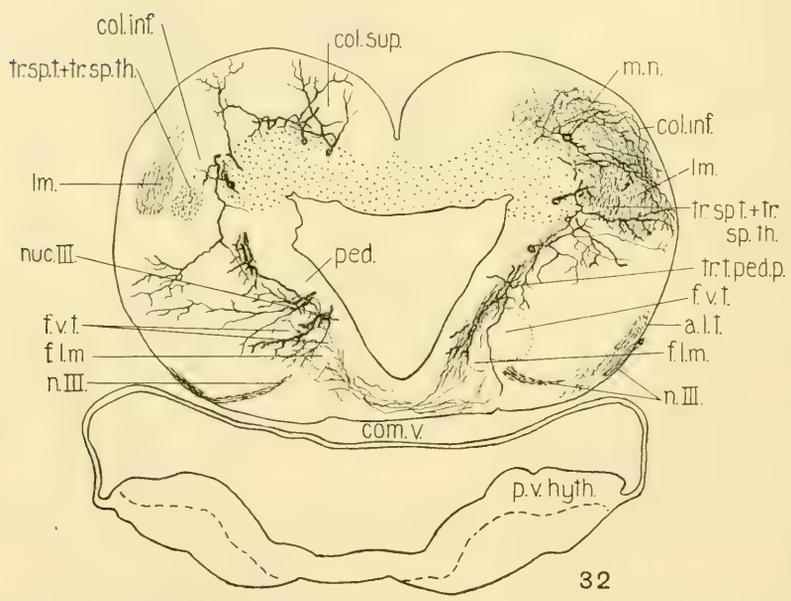
Fig. 31 Through the tuberculum posterius in the same plane as figure 10. Comparison of figures 31 to 35 with figures 10 to 14 shows that in the former series the tectum mesencephali has collapsed into the optocoele producing some distortion of the tectum. A single neuron of the colliculus inferior (*col.inf.*) is impregnated, some of its dendrites spreading throughout the field of the acoustico-lateral lemniscus and others extending farther ventrally into the cerebral peduncle. The axon is directed ventrad, probably to enter the tractus tecto-bulbaris or tecto-peduncularis profundus. Dendrites of several neurons of the cerebral peduncle are impregnated (*ped.*). × 30 (cvc, 3–4–1).

Fig. 32 At the level of the superficial origin of the III nerve; cf. figure 11. In this and the next following figure the stratum griseum of the tectum mesencephali is coarsely stippled; that of the pedunculus cerebri is unshaded. On the left side two neurons of the optic part of the tectum (*col.sup.*) are impregnated, one of which sends dendrites also into the colliculus inferior (*col. inf.*).

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col.inf.
m.n.
m.n.
col.sup.
r. V. mes.
tr. sp.T.
lm.
tr. sp. th.
lm.
f.d.t.
f.v.t.
tr.t.ped.p.
f.d.t.
f.v.t.
f.l.m.
com. v.
f.l.m.

33

s. a.
s. g.
optc.

34
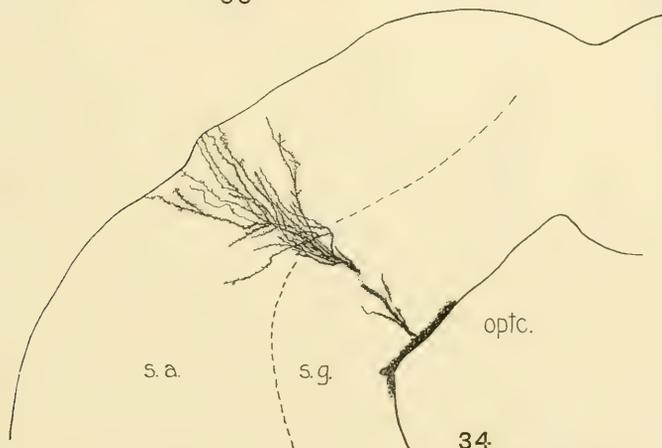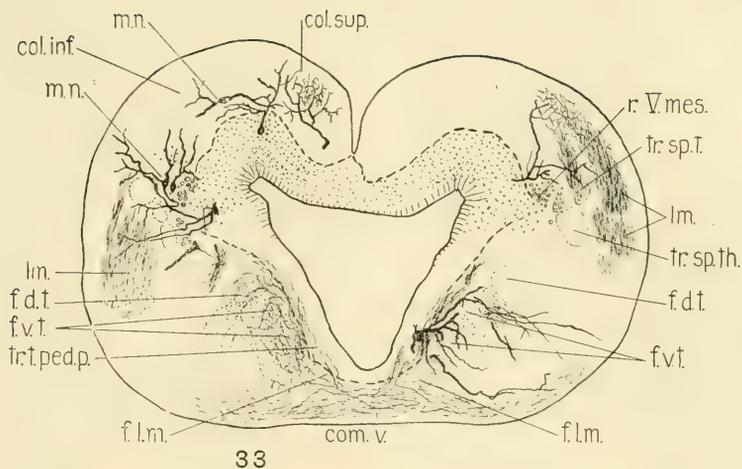
Farther ventrally there is an imperfect impregnation of two neurons of the colliculus inferior, one of which sends dendrites also into the pedunculus cerebri (*ped.*). In the latter region a single neuron of the III nerve is impregnated (*nuc.III*). On the right side a multipolar neuron (*m.n.*) of the stratum album of the tectum is impregnated. × 30 (cvc, 3–4–7).

Fig. 33 Through the middle of the tectum mesencephali; cf. fig. 12. The dendrite of a neuron of the tectum opticum is impregnated (*col.sup.*) and also several neurons of the colliculus inferior (*col.inf.*). On the left side are seen several multipolar neurons of the stratum album (*m.n.*). On the right side is a typical neuron of the motor tegmentum within the pedunculus cerebri. × 30 (cvc, 3–5–4).

Fig. 34 An ependymal element from the caudal part of the tectum mesencephali. From a transverse Golgi section. Throughout these Golgi preparations the ependyma can be readily distinguished from the neurons by their delicately plumose appearance. × 74 (cvic, 194).
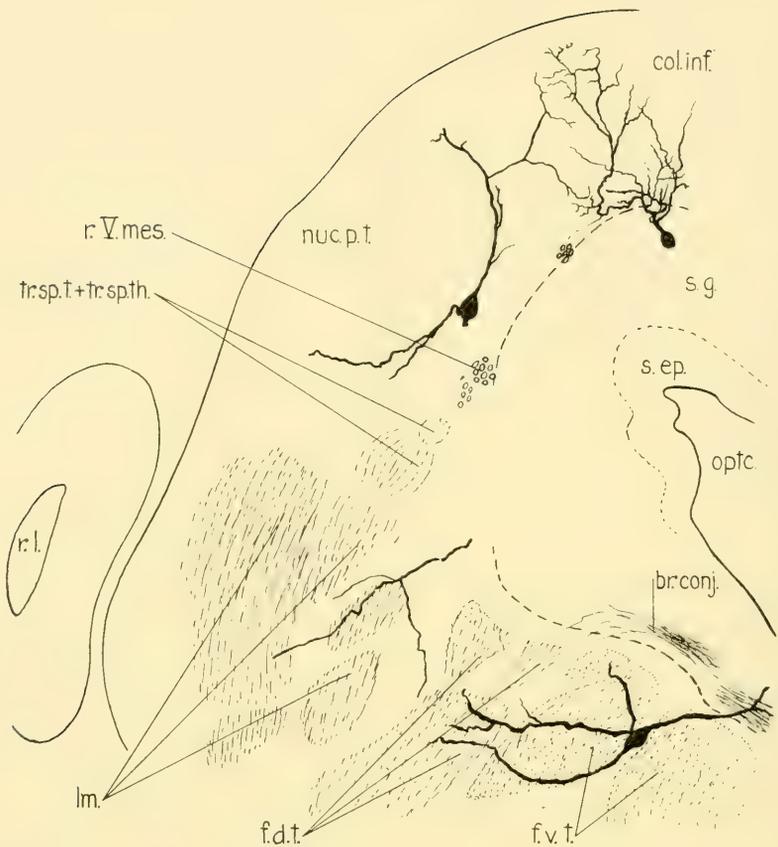
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Fig. 35 Through the caudal part of the tectum mesencephali and the nucleus posterior tecti (*nuc.p.t.*); cf. figure 14. In the latter nucleus is a tengential neuron of the stratum album, and in the motor tegmentum is a similar neuron whose cell body lies among the bundles of the ventral tegmental fascicles (*f.v.t.*). × 74 (cvc, 3–5–7).

Figs. 36 to 47 Drawings of selected sections from a horizontal series through the thalamus and midbrain. Golgi method. The right side is slightly farther ventral than the left.

Fig. 36 Through the dorsal part of the tectum mesencephali, including the nucleus posterior tecti. The relations of the neuropil of the optic part of the tectum (*col. sup.*) and the colliculus inferior (*col.inf.*) are shown. The most lateral fibers of the tractus tecto-thalamicus et hypothalamicus cruciatus posterior (*tr.t.th.h.c.p.*) are seen to be related to the nucleus posterior tecti (*nuc.p.t.*). Sections immediately ventrally show very long dendrites of the nucleus posterior tecti reaching far forward on the lateral surface and arborizing among the fibers of this tract. × 30 (cvc, 3–5–7).

Fig. 37 Three sections ventrally of the preceding. The habenulae lie in the plane of section farther forward, but are not drawn. On the right side is a dense neuropil in the regions marked *col.sup.* and *col.inf.*, the details of which are not drawn in. On the left side two widely branched free endings of fibers of the tractus tecto-thalamicus et hypothalamicus cruciatus posterior (*tr.t.th.c.p.*) are

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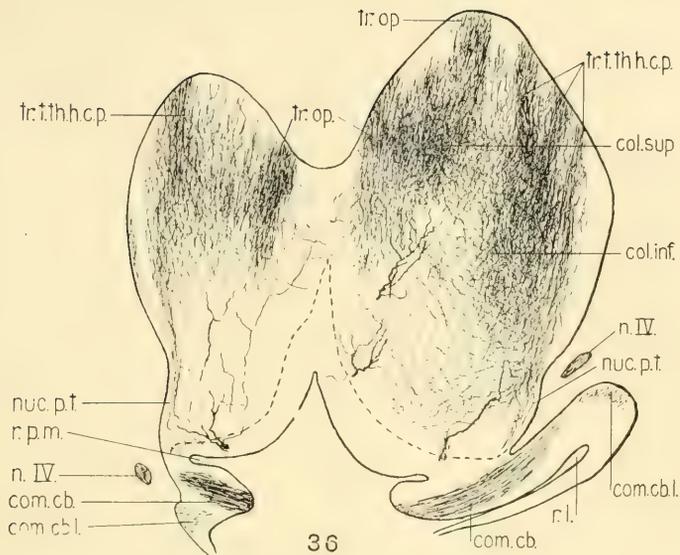
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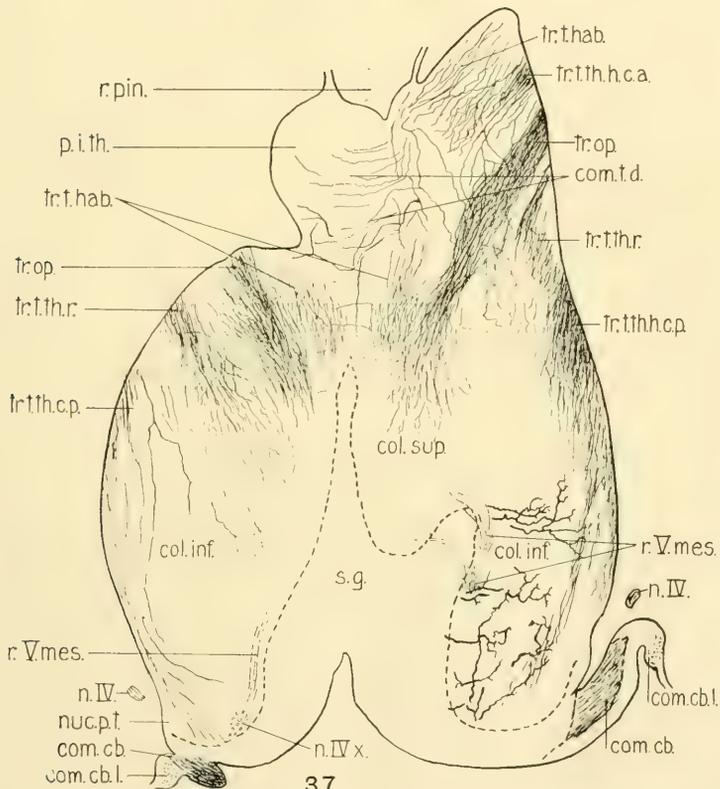
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seen; these are probably commissural fibers from the opposite tectum. The trac-
tus tecto-thalamicus et hypothalamicus cruciatus anterior (*tr.t.th.h.c.a*) joins the
optic tract farther ventrally. It exhibits some free endings in the pars intercala-
ris diencephali. Medially of it are a few scattered fibers (*tr.t.hab.* on the right
side) which branch freely and send some collaterals across the meson in the com-
missura tecti diencephali (*com.t.d.*). × 30 (cviic, 4).
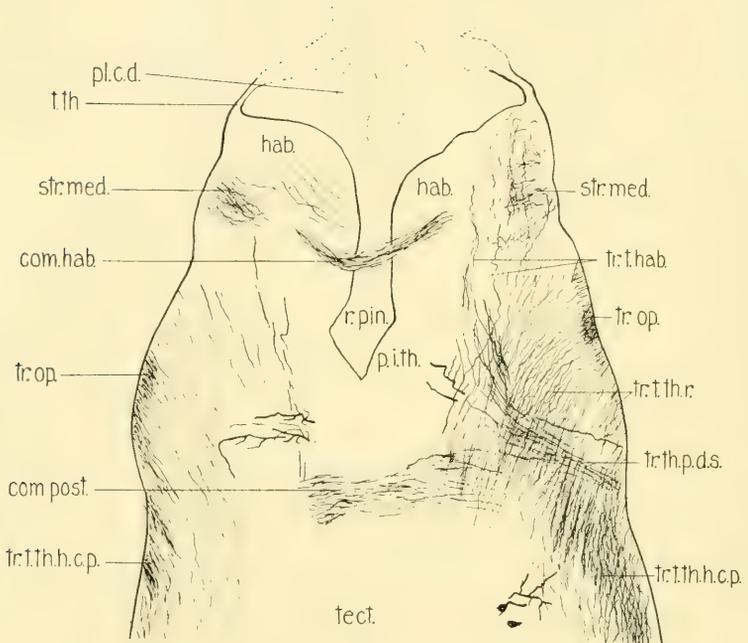
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Fig. 38   Three sections farther ventrally, including the habenula, pars in-
tercalaris diencephali, commissura posterior, and the rostral end of the tectum
mesencephali.   The tractus tecto-habenularis (tr.t.hab.) shows free endings
within the habenula.   Fibers of the tractus thalamo-peduncularis dorsalis
superficialis (tr.th.p.d.s.) are seen arising from the pars intercalaris diencephali,
and some of these fibers seem to arise farther forward in the habenula.   × 30
(cviic, 7).

Fig. 39   Three sections farther ventrally through the subcommissural organ
(o.sc.) and the ventral part of the habenula (hab.) and pars intercalaris dien-
cephali (p.i.th.).   The slender neuron in the rostral part of the pars intercalaris
on the right side is drawn in from the adjacent section dorsally.   Its axon is
directed toward the tractus thalamo-peduncularis dorsalis superficialis.   Many
similar neurons are impregnated, one imperfectly on the left side of this section.
Their dendrites spread among the fibers of the tractus tecto-thalamicus rectus
and tractus spino-thalamicus.   On the left side is seen the connection of a
slender wisp of fibers of the tractus thalamo-peduncularis dorsalis superficialis
(tr.th.p.d.s.) with the caudo-ventral part of the habenula.   See figure 41 for
the further course of these fibers.   On the left side is a superficial area of neu-
ropil laterally of the superior commissure, which is composed of peculiar very
fine and freely branched varicose fibers.   They are apparently terminal arbori-
zations of axons of unknown origin.   There are some indications that these are
short axons arising from the cells of the stratum griseum of the pars intercalaris
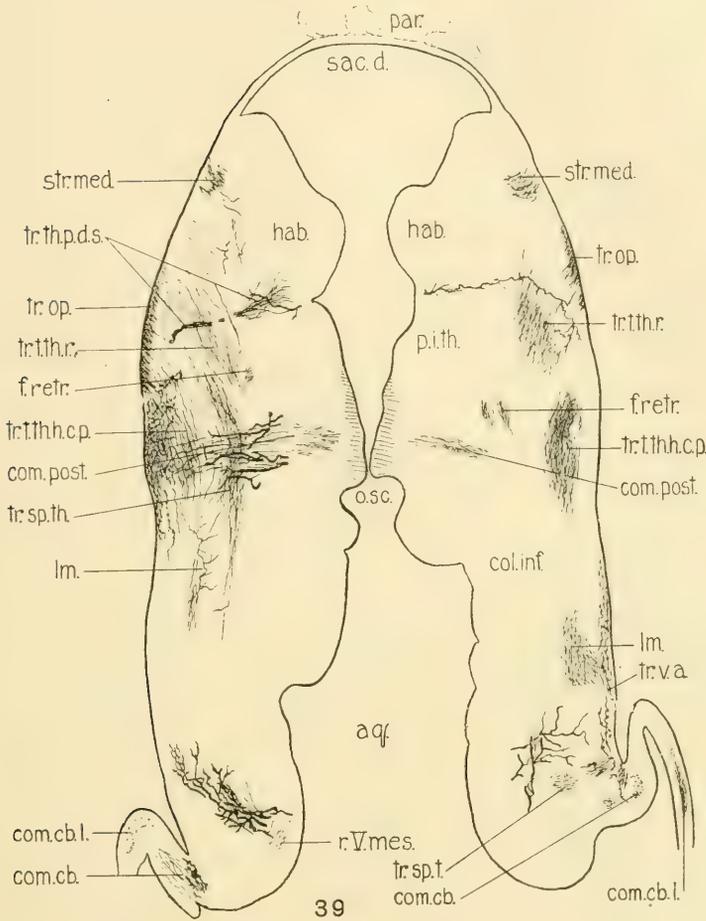
diencephali. This neuropil is reached by dendrites of neurons whose cell bodies lie adjacent to the subcommissural organ, some of which are imperfectly impregnated in this preparation. In several cases axons are seen arising from the bases of these dendrites and directed laterally without decussation among the fibers of the commissura posterior. These neurons may be related at their bases with the subcommissural organ, thus providing a correlation mechanism between stimuli arising at the attachment of Reissner's fiber (fig. 62, *f.R.*; cf. Nicholls, '12 and '17) and the fibers of the peripheral neuropil. But further details are necessary before this suggestion can be adopted. On the right side in the area marked *col.inf.* is a dense neuropil (not drawn in) reached by terminals of the acoustico-lateral lemniscus (*lm.*). A few free terminals of this lemniscus are shown on the left side; cf. figure 56. × 23 (cviic, 10).
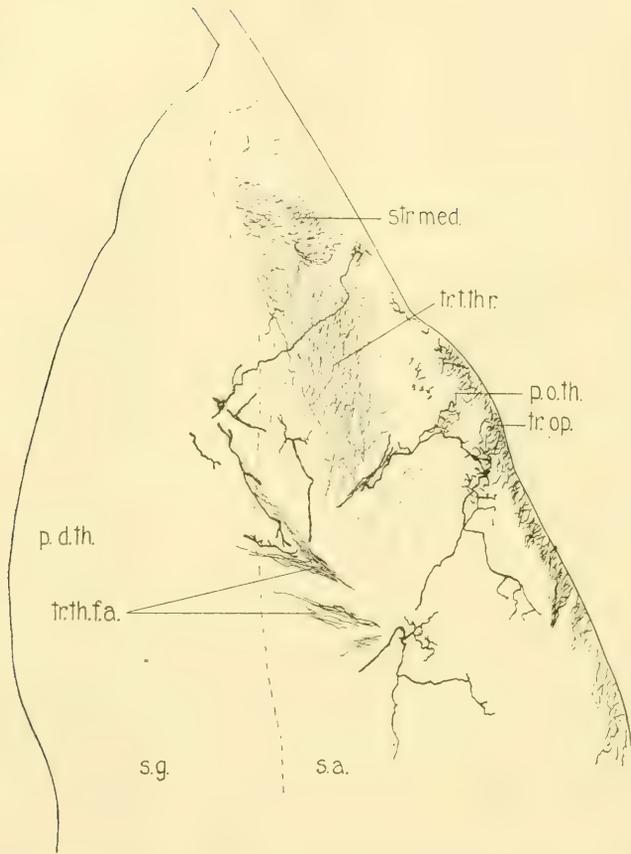
Fig. 40 Detail from the extreme dorsal part of the pars dorsalis thalami of the right side (one section ventrally of fig. 39). The origin of the tractus thalamo-frontalis anterior (*tr.th.f.a.*) is shown and the endings of a few dendrites of neurons of the pars dorsalis thalami. Those in the more caudal part of the region engage chiefly the tractus spino-thalamicus; those in the rostral part chiefly the tractus tecto-thalamicus rectus (*tr.t.th.r.*); and some reach the extreme dorsal part of the pars optica thalami (*p.o.th.*), which is not at this level well differentiated. $\times$ 74 (cviic, 11).
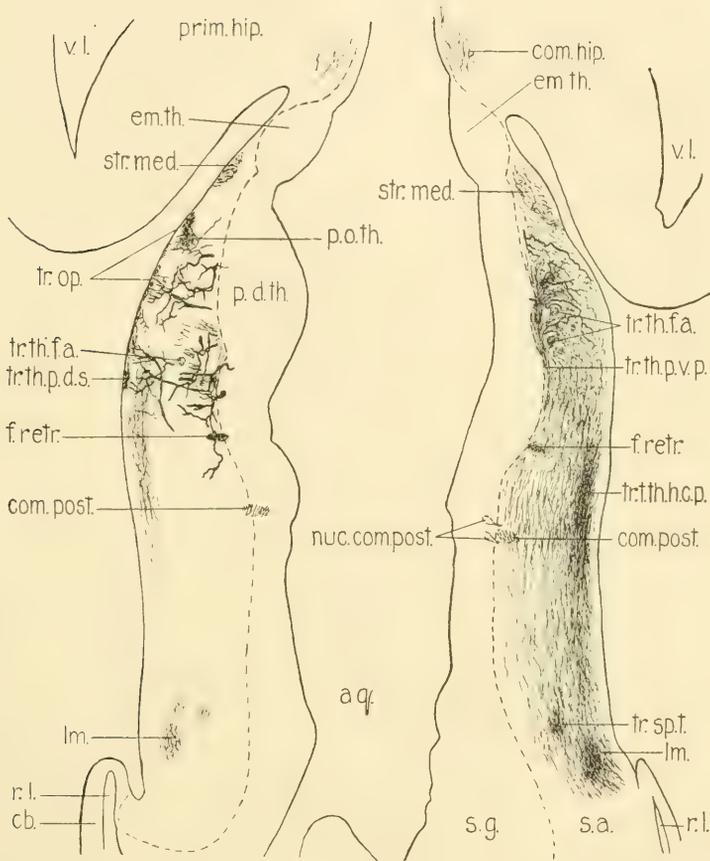
Fig. 41 Through the diencephalon, including on the left the ventral border of the pars dorsalis thalami, and on the right passing through the sulcus medius thalami. On the left side collaterals are leaving the marginal optic tract (*tr.op.*) to enter the pars optica thalami (*p.o.th.*).   × 23 (cviic, 16).
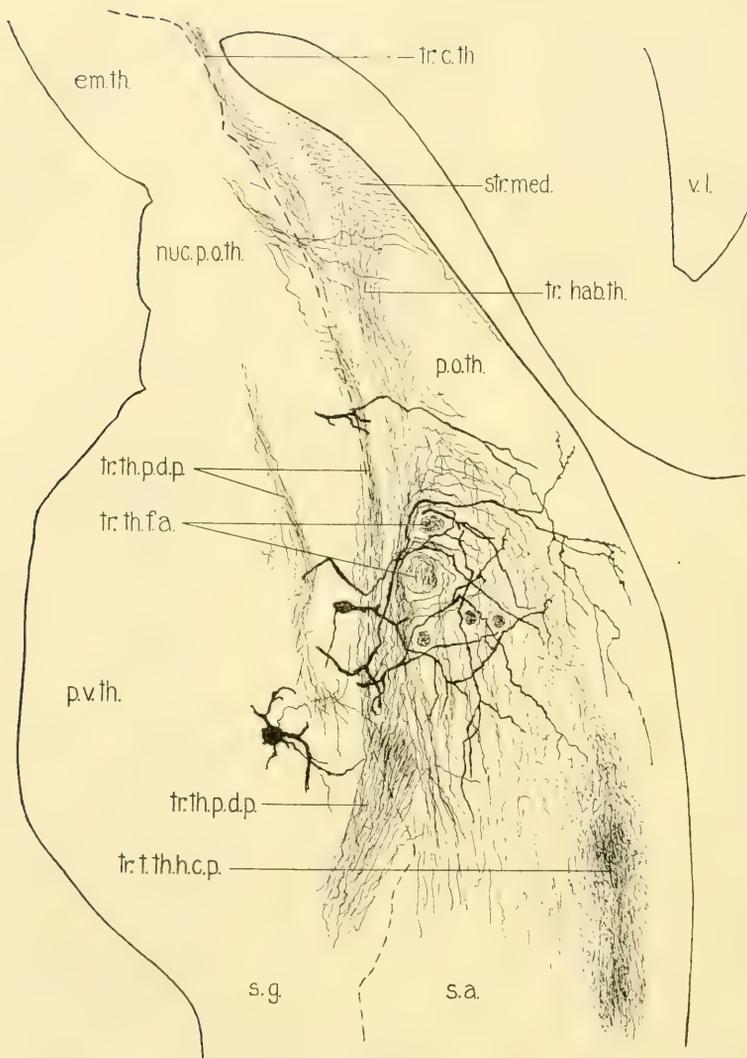
Fig. 42 Through the dorsal part of the pars ventralis thalami of the right side. At the rostral end of the figure is the eminentia thalami (*em.th.*). The fibers of the tractus cortico-thalamicus (*tr.c.th.*) laterally of it are in part sketched in from sections farther ventrally. No neurons of the nucleus of the pars optica thalami (*nuc.p.o.th.*) are impregnated (*cf.* fig. 48), but the more rostral fibers of the tractus thalamo-peduncularis dorsalis profundus (*tr.th.p.d.p.*) shown appear to be axons from this nucleus. Mingled with them are probably axons of the tractus thalamo-peduncularis ventralis profundus from the pars ventralis thalami and eminentia thalami. The significance of the tract marked *tr.hab.th.* is obscure. Comparison with other preparations suggests a connection between the habenula and the pars ventralis thalami termed provisionally tractus habenulo-thalamicus. × 74 (cviic, 16).
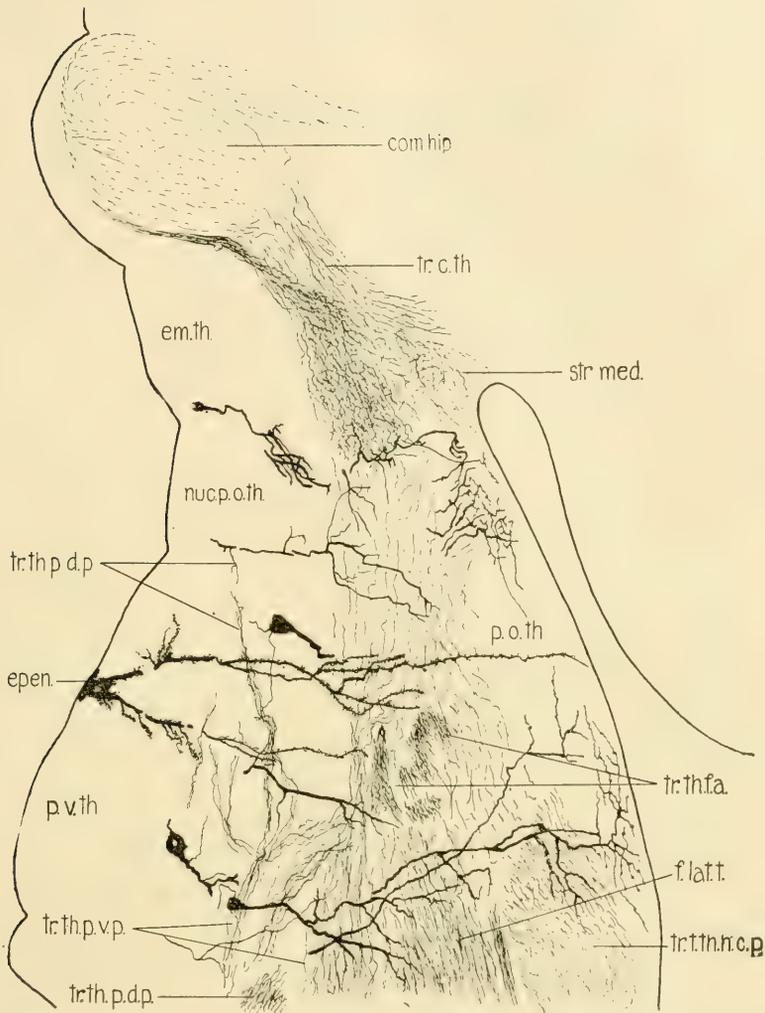
Fig. 43 Through the ventral part of the right pars ventralis thalami and immediately dorsally of the crossing of the hippocampal commissure (*com.hip.*). From the fibers of the latter, which are unimpregnated, delicate fibers (probably collaterals) are stained; these pass backward to arborize among the dendrites of the neurons of the eminentia thalami (*em.th.*, cf. fig. 48). A single neuron from the caudal end of the eminentia thalami is partially impregnated. This neuron is transitional to those of the nucleus of the pars optica thalami (*nuc.p. o.th.*). A few fibers of the tractus thalamo-peduncularis dorsalis profundus (*tr.th.p.d.p.*) from the nucleus of the pars optica thalami are shown, to which are added farther caudad similar fibers of the tractus thalamo-peduncularis ventralis profundus from the pars ventralis thalami. A single ependyma element (*epen.*) of the pars ventralis thalami is impregnated. × 74 (cviic, 21).

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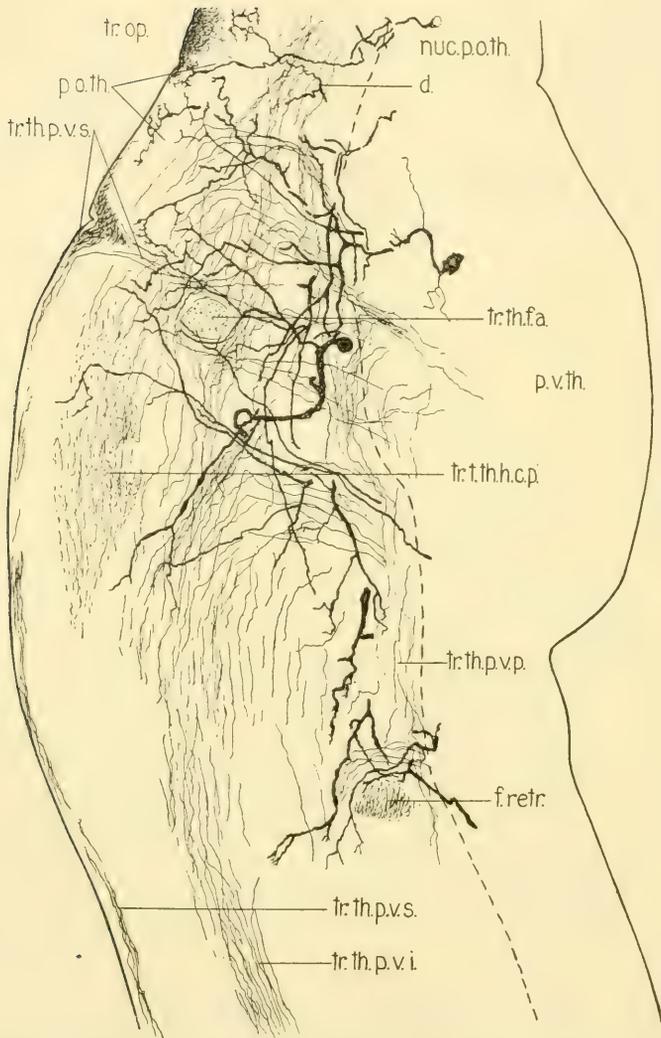
Fig. 44 Through the pars ventralis thalami of the left side, from the same section as figure 43. Probably all of the axons here drawn except those otherwise designated belong to the tractus thalamo-peduncularis ventralis system. The dendrite marked *d* is probably a branch of the more rostral of the two neurons shown in figure 45. Some neurons of the nucleus of the pars optica thalami (*nuc.p.o.th.*) are imperfectly impregnated. × 74 (cviic, 21).
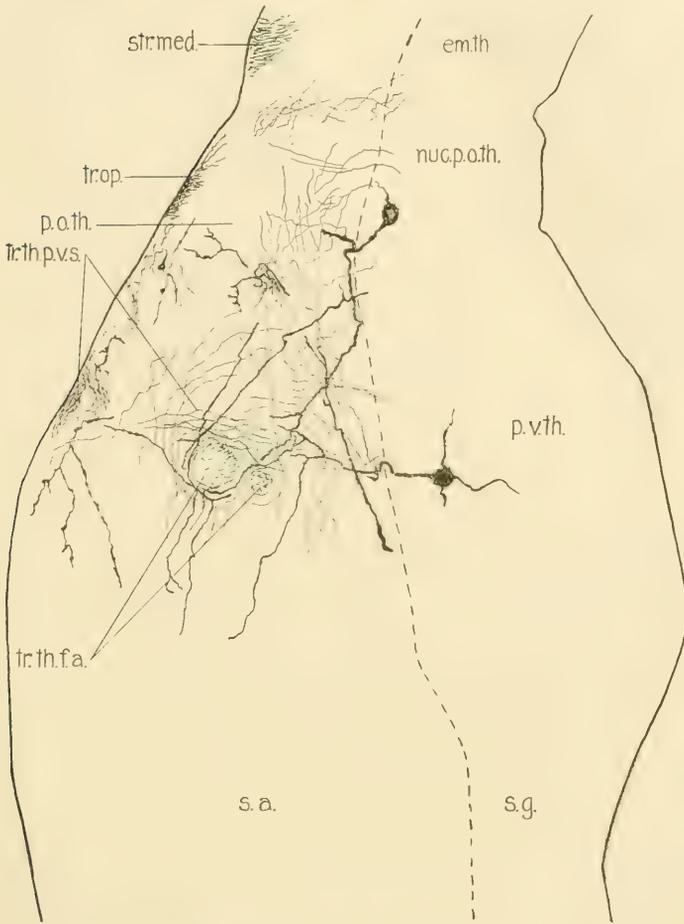
Fig. 45 The pars ventralis thalami of the left side from the next section ventrally of the last. The more rostral of the two neurons whose cell bodies are impregnated is apparently transitional between the pars ventralis and the nucleus of the pars optica. One large dendrite is directed backward into the stratum album of the pars ventralis, and another which passes out of the plane of the section is directed toward the pars optica. This dendrite, or an entirely similar one, is shown in figure 44 at *d*. The axons here drawn probably all belong to the tractus thalamo-peduncularis system, a few at the rostral end coming from the eminentia thalami, then a few from the nucleus of the pars optica, the remainder coming from the pars ventralis. × 74 (cviic, 22).
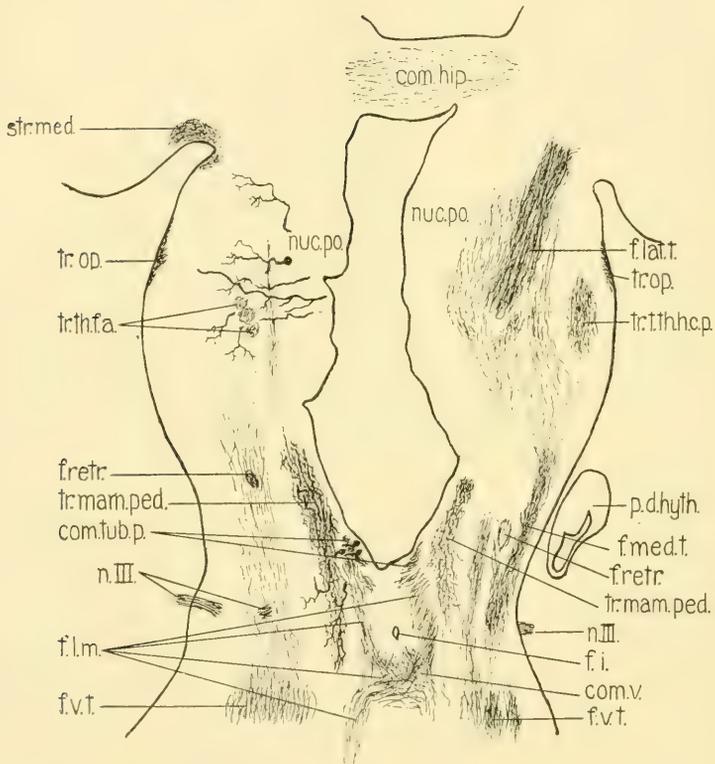
Fig. 46 Through the commissura hippocampi, the dorsal part of the nucleus preopticus, the tuberculum posterius, and the fovea isthmi. Some of the imperfectly impregnated neurons of the preoptic nucleus are sketched in from the adjacent section dorsally. The component of the medial forebrain bundle which enters the cerebral peduncle (*f.med.t.*) is probably the tractus olfacto-tegmentalis, though the source of its fibers is unknown. The slender varicose fibers provisionally marked *tr.mam.ped.* are of doubtful significance. Mingled with them are coarse dendrites of the cells of the tuberculum posterius. The appearances suggest that they come from the pars dorsalis hypothalami, though their connection with these neurons has not been observed. They end by free arborizations laterally of the tuberculum posterius and fovea isthmi (cf. fig. 47). × 23 (cviic, 25).
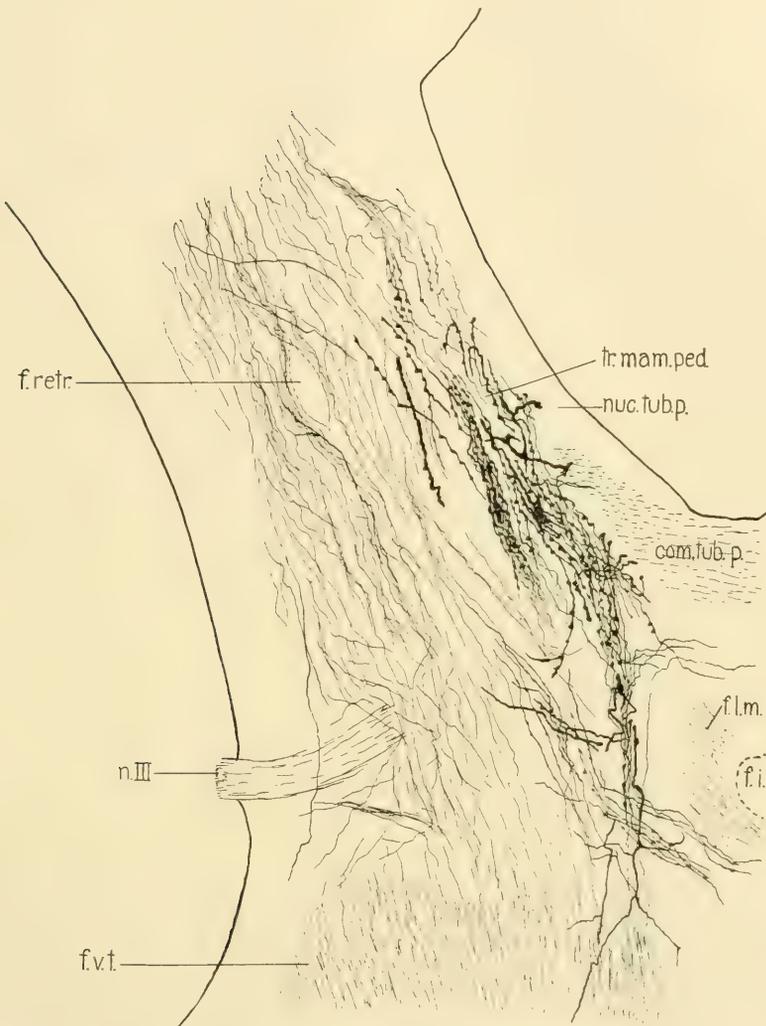
Fig. 47   Detail of the region laterally of the tuberculum posterius and fovea isthmi of the left side, from the next section ventrally of figure 46, to illustrate the mode of ending of the fibers of the supposed tractus mamillo-peduncularis (*tr.mam.ped.*).   Some of these fibers decussate in the ventral commissure both before and behind the fovea isthmi (*f.i.*).   × 74 (cviic, 26).
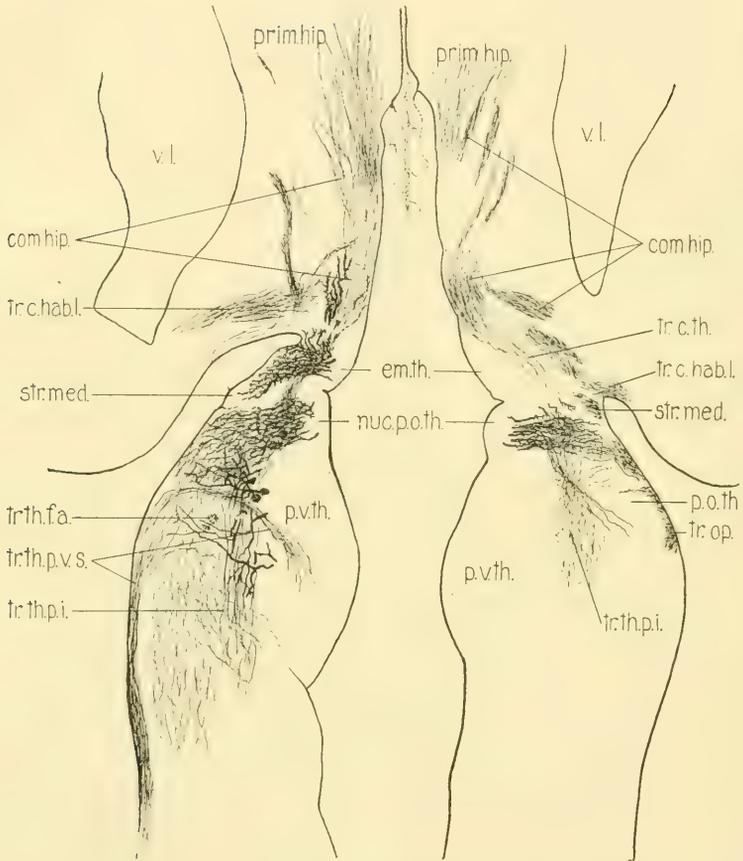
Fig. 48 A horizontal section through the eminentia thalami, nucleus of pars optica thalami, and pars ventralis thalami. The right side is slightly farther ventral. The fibers of the commissura hippocampi (*com.hip.*) are converging from the various parts of the primodium hippocampi to descend to their crossing. Dendrites from the eminentia thalami (*em.th.*) are directed forward among these fibers and also laterally to engage the tractus cortico-thalamicus (*tr.c.th.*) and stria medullaris (*str.med.*). Dendrites of the nucleus of the pars optica thalami (*nuc.p.o.th.*) are directed laterally into the ventral part of the pars optica thalami (*p.o.th.*). Axons from both of these classes of neurons are directed backward into the tractus thalamo-peduncularis (*tr.th.p.i.*). The tract so designated in this and the next following figure contains, accordingly, fibers of both the dorsal and ventral thalamo-peduncular tracts. × 30 (civc, 1–2–9).
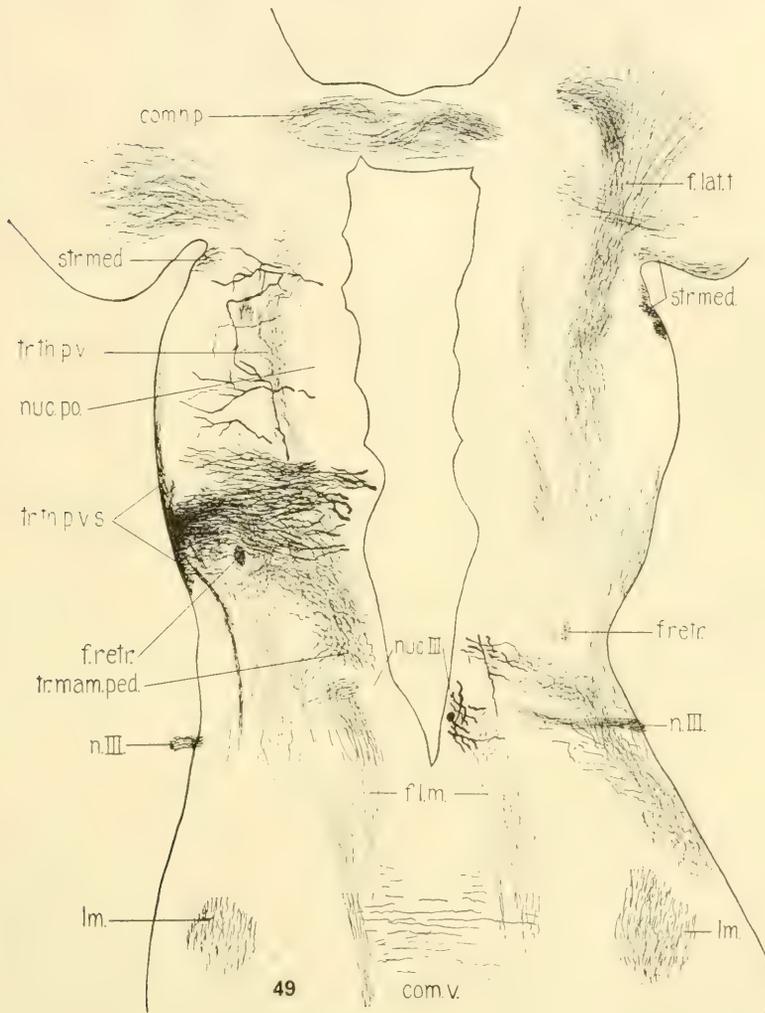
Fig. 49 Horizontal section from the same specimen as the last a little farther ventrally through the commissura hippocampi (*com.hip.*) and dorsal margin of the nucleus preopticus (*nuc.po.*). Regarding the varicose fibers marked *tr. mam.ped.*, see the description of figure 46. × 30 (civc, 1–2–4).

Figs. 50 to 60 A series of parasagittal sections through the thalamus and midbrain. They are taken from several specimens prepared by the Golgi method. In some cases there is a slight deviation from the true parasagittal plane, which is noted. The notations of the series numbers of the sections included in the descriptions will enable the reader to determine which sections come from the same specimen. In some of the series sections are illustrated

Fig. 50 ... (labels: tr.v.t., tr.b.t., tr.b.t., nuc.vis.s., s.a., s.g., tr.op.b., com.cb, cb, com.cb.l., r Ⅴ mes., tr.op., tr.t.th.h.c.p., tr.th.p.d.s., tect., tr.t.ped.post., nuc.p.t., tr.v.a., tr.b.t., nuc.vis.s., com.cb., cb., com.cb.l., tr.t.ped.post., tr.b.t., tr.v.a., nuc.vis.s., com.cb., cb., com.cb.l., s.a., s.g.)

50   51   52
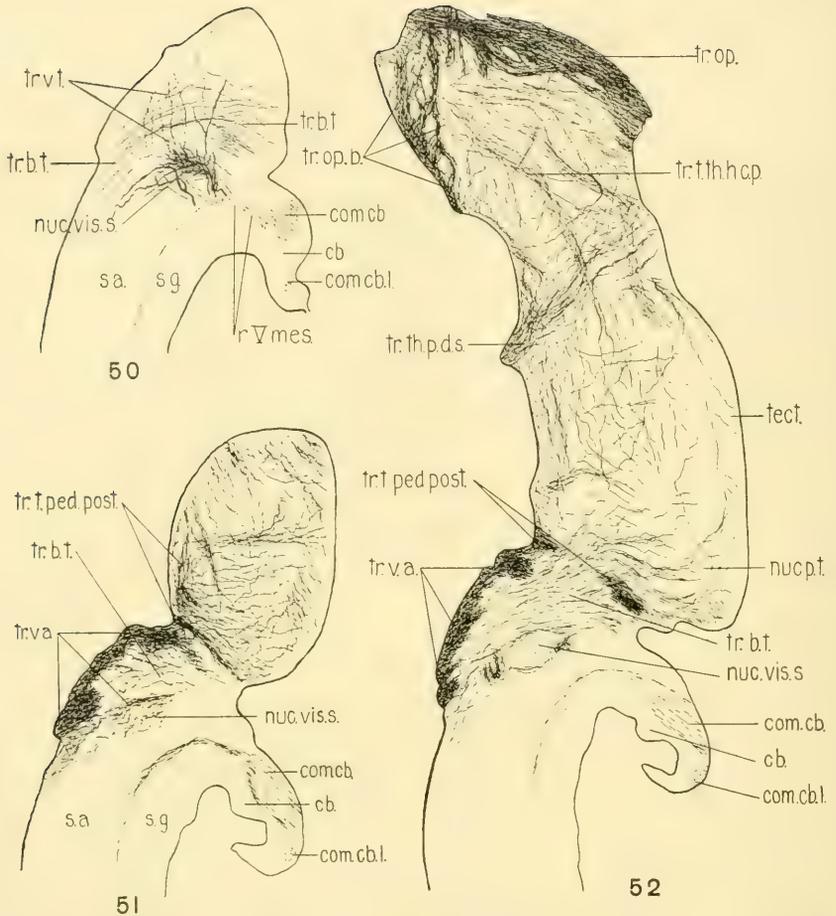
from both the right and the left side of the brain. The rostral end in all cases is above and the ventral side to the left.

Fig. 50 Section taken close to the lateral border in the region of the isthmus. Ventrally and rostrally of the cerebellum are the dendrites of a few neurons of the superior secondary visceral nucleus (*nuc.vis.s.*), which form a dense neuropil. The varicose axons of these neurons are directed forward and ventralward as the tertiary visceral tract (*tr.v.t.*). These axons cross at right angles the smoother axons of the tractus bulbo-tectalis (*tr.b.t.*). The ascending secondary visceral tract is not impregnated; cf. figure 51. × 30 (cic, 1-1-9).

Fig. 51 Section slightly farther medial than the last from a different specimen. In this and the following figures 52 to 55 the plane of section is slightly oblique to the sagittal plane, the dorsal side being farther medial than the ventral. The ascending secondary visceral tract (*tr.v.a.*) ascends along the lateral margin of the oblongata, engages the dendrites of the secondary visceral nucleus (*nuc.vis.s.*), which are not here impregnated (cf. fig. 50), and passes forward along the ventro-lateral margin of the motor tegmentum. × 30 (U, 24).

Fig. 52 The adjacent section medialward from the same series as figure 51. The relations of the ascending secondary visceral tract (*tr.v.a.*) are but little

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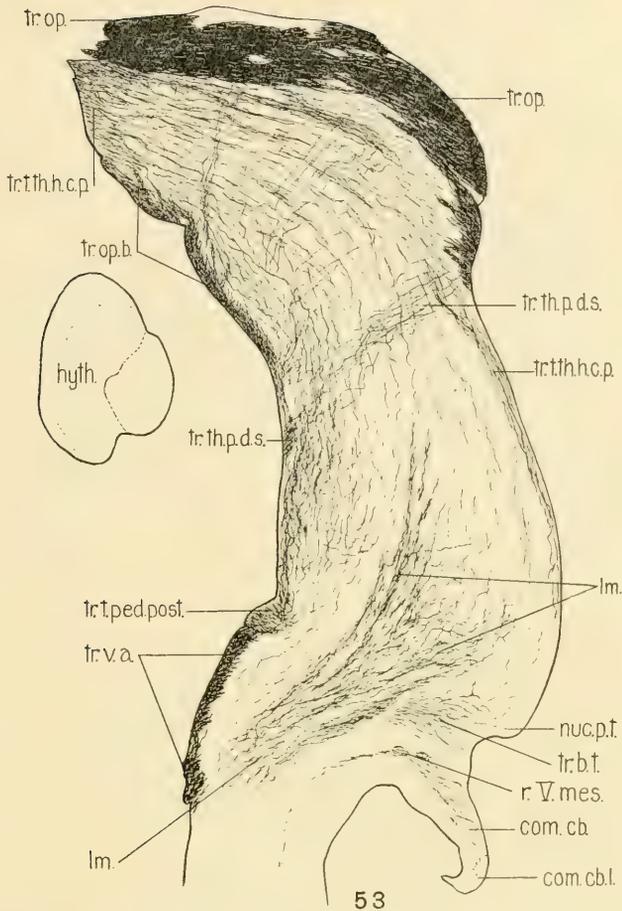
changed. The tractus bulbo-tectalis (*tr.b.t.*) is spreading out and ending by free arborizations in the nucleus posterior tecti (*nuc.p.t.*). Descending from the nucleus posterior toward the cerebral peduncle is a compact fascicle belonging to the superficial tecto-peduncular system, the tractus tecto-peduncularis posterior (*tr.t.ped.post.*). At the boundary between thalamus and midbrain are fibers of the tractus thalamo-peduncularis dorsalis superficialis (*tr. th.p.d.s.*) which descend from the pars intercalaris diencephali to the cerebral peduncle in the vicinity of the area lateralis tegmenti (cf. fig. 53). The marginal optic tract (*tr.op.*) is seen crossing the thalamus, and farther ventrally the so-called basal optic bundle (*tr.op.b.*) traverses the extreme lateral border of the hypothalamus. × 30 (U, 25).

Fig. 53 From the same series two sections farther medialward. The marginal optic tract (*tr.op.*) is heavily impregnated; mingled with these fibers may be some of the tractus tecto-thalamicus et hypothalamicus cruciatus anterior. Terminals of the tractus bulbo-tectalis (*tr.b.t.*) are still seen in the nucleus posterior tecti (*nuc.p.t.*). The acoustico-lateral lemniscus (*lm.*) reaches the remainder of the tectum. × 30 (U, 27).

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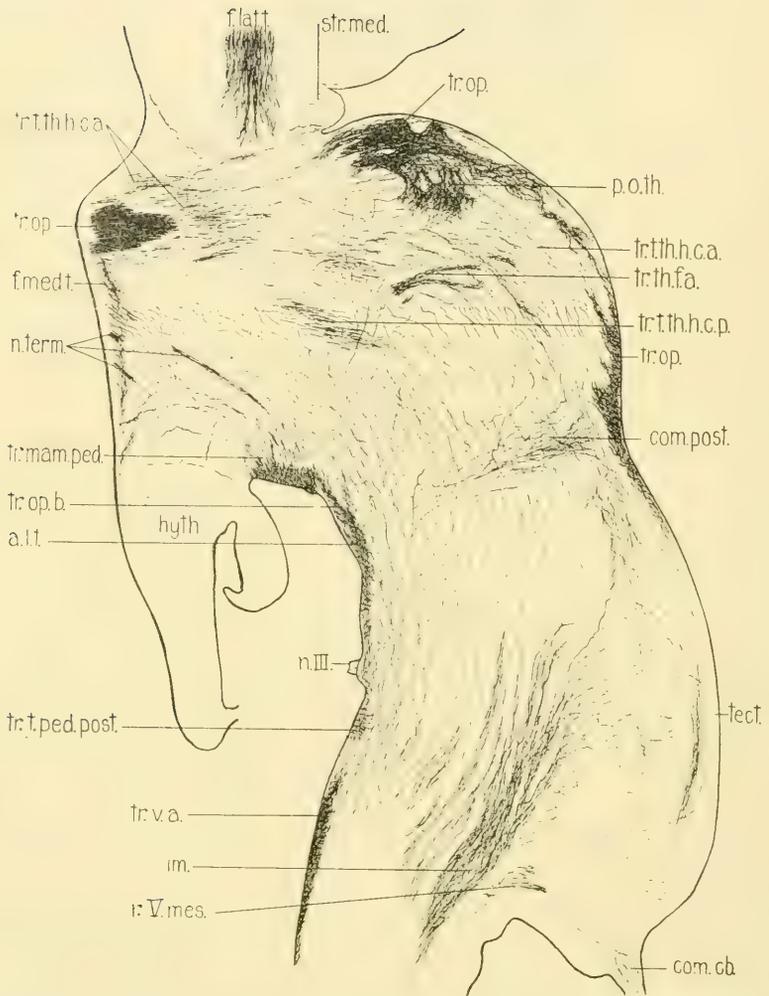
Fig. 54 From the same series three sections farther medialward. The caudal part of the hypothalamus is defective. From the heavily impregnated marginal optic tract (*tr.op.*) dense fascicles of collaterals pass off into the pars optica thalami (*p.o.th.*). The fibers of the unmyelinated component of the tractus tecto-thalamicus et hypothalamicus cruciatus anterior (*tr.t.th.h.c.a.*) are finer and less deeply impregnated. They do not appear to participate in the neuropil of the pars optica thalami. The fibers of the so-called basal optic bundle (*tr.op.b.*) appear to end in the area lateralis tegmenti (*a.l.t.*) in front of the III nerve. These fibers are mingled with others of uncertain origin, probably from the hypothalamus (*tr.mam.ped.*), and probably also with fibers of the ascending visceral tract (*tr.v.a.*) and tractus tecto-peduncularis posterior (*tr.t.ped.post.*) farther medially. The tractus tecto-thalamicus et hypothalamicus cruciatus posterior (*tr.t.th.h.c.p.*) is for the most part unimpregnated; cf. fig. 56. × 30 (U, 30).

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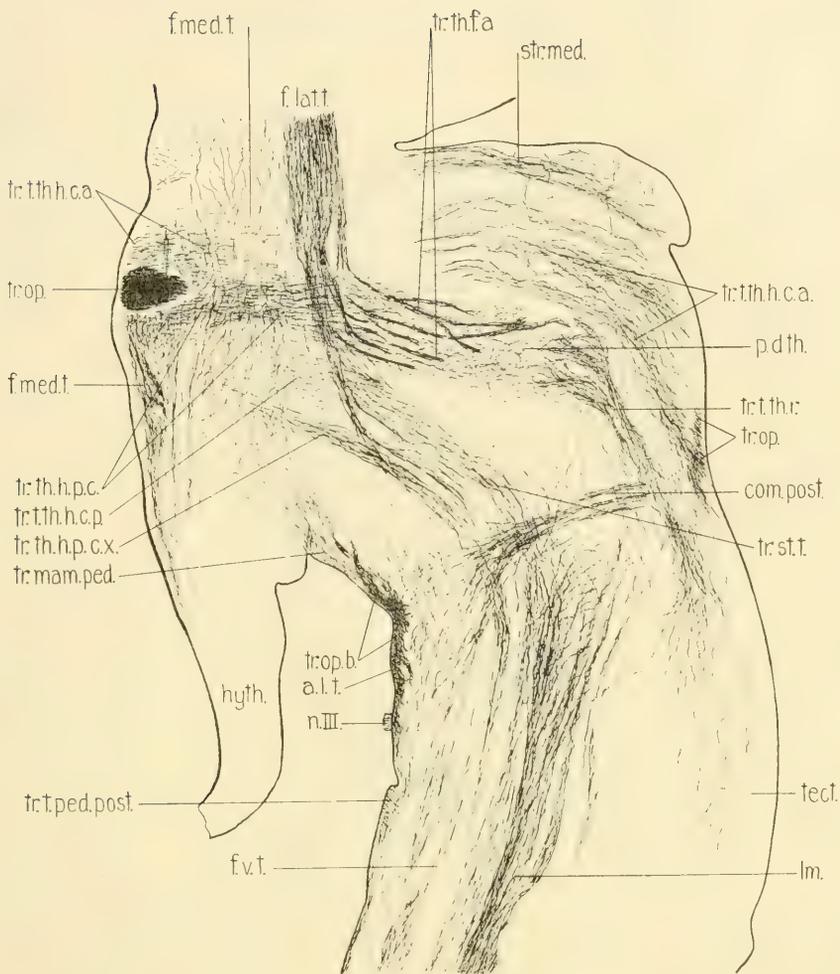
Fig. 55 From the same series two sections farther medialward. Two of the components of the lateral forebrain bundle (*f.lat.t.*) are clearly shown. Axons of the tractus thalamo-frontalis anterior (*tr.th.f.a.*) leave the pars dorsalis thalami (*p.d.th.*) in slender wisps of closely crowded fibers to turn forward in the lateral forebrain bundle. Other fibers of this bundle (*tr.st.t.*) enter the ventral tegmental fascicles (*f.v.t.*). The more slender and scattered fibers of the tractus thalamo-hypothalamicus et peduncularis cruciatus (*tr.th.h.p.c.*) are seen to arise from the pars dorsalis thalami mingled with those of the tractus thalamo-frontalis. Some of the fibers of the former tract, after their decussation, are shown (*tr.th.h.p.c.x.*) to enter the ventral tegmental fascicles. The tractus tecto-thalamicus rectus (*tr.t.th.r.*) is seen entering the pars dorsalis thalami. No attempt has been made to represent the dense neuropil in this region, composed of the three types of axons figured and dendrites from the underlying stratum griseum. The fibers of the medial forebrain bundle (*f.med.t.*) are very incompletely impregnated. Some enter the hypothalamus and some the ventral part of the motor tegmentum of the cerebral peduncle. × 30 (U, 32).

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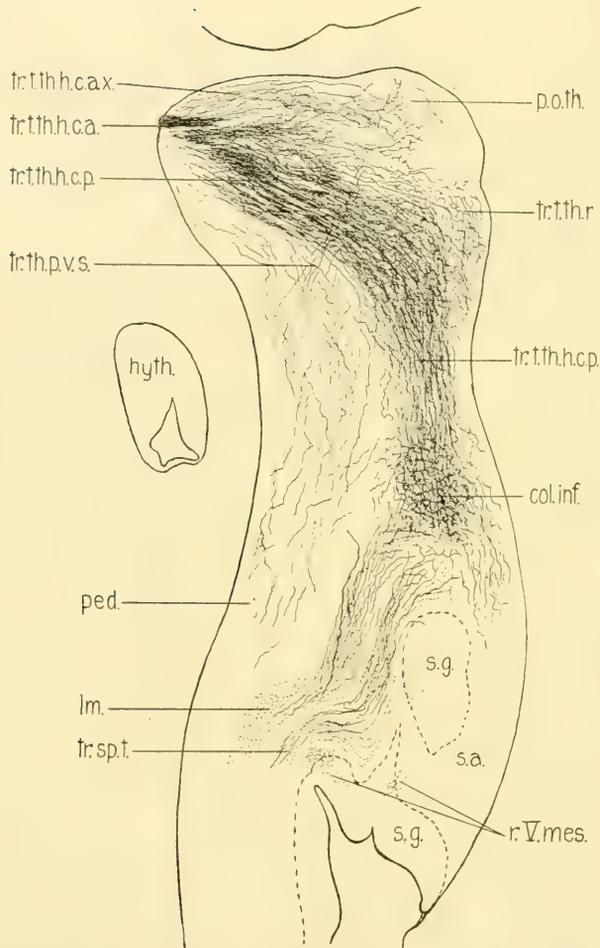
Fig. 56 Section from a different specimen and in a slightly different plane, the ventral side being somewhat farther lateral than the dorsal. The rostral end of the section lies at a level between those of figures 53 and 54; the caudal end is in about the same plane as figure 55. The optic tract is not impregnated. The free endings in the pars optica thalami (*p.o.th.*) are not optic fibers. They apparently belong to the tractus tecto-thalamicus et hypothalamicus cruciatus anterior after its decussation (*tr.t.th.h.c.a.x.*). The tractus tecto-thalamicus et hypothalamicus cruciatus posterior (*tr.t.th.h.c.p.*) is well impregnated and among its fibers are some free endings in the thalamus of the tractus tecto-thalamicus rectus (*tr.t.th.r.*); cf. figure 55, in which the tractus tecto-thalamicus et hypothalamicus cruciatus posterior is very slightly impregnated. × 30 (ciiic, 2–1–1).
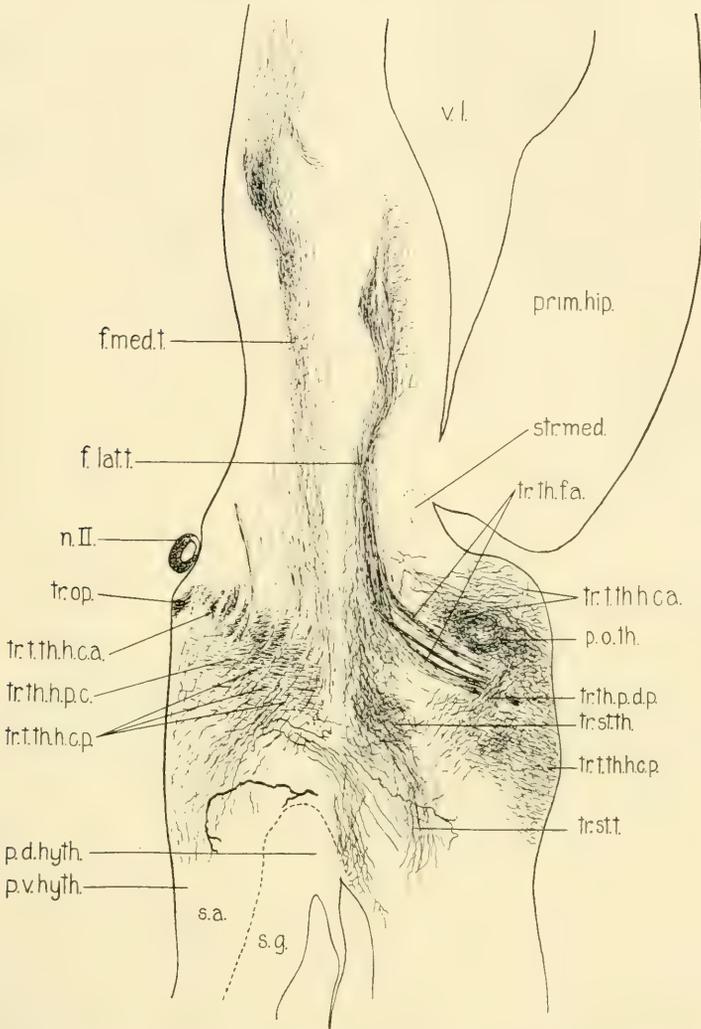
Fig. 57 Section from the opposite side of the same specimen as figure 56 and farther medial. The section is oblique to the sagittal plane, the rostral and ventral parts being farther medial. Three components of the lateral fore-brain bundle (*f.lat.t.*) can be distinguished: the tractus thalamo-frontalis anterior (*tr.th.f.a.*), the tractus strio-thalamicus (*tr.st.th.*) ending in the pars ventralis thalami, and the tractus strio-tegmentalis (*tr.st.t.*) extending into the cerebral peduncle. × 30 (ciiic, 3-2-6).
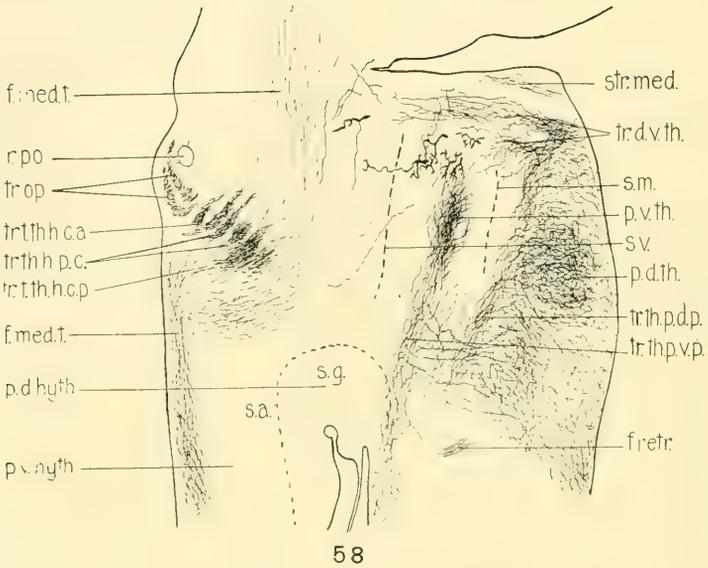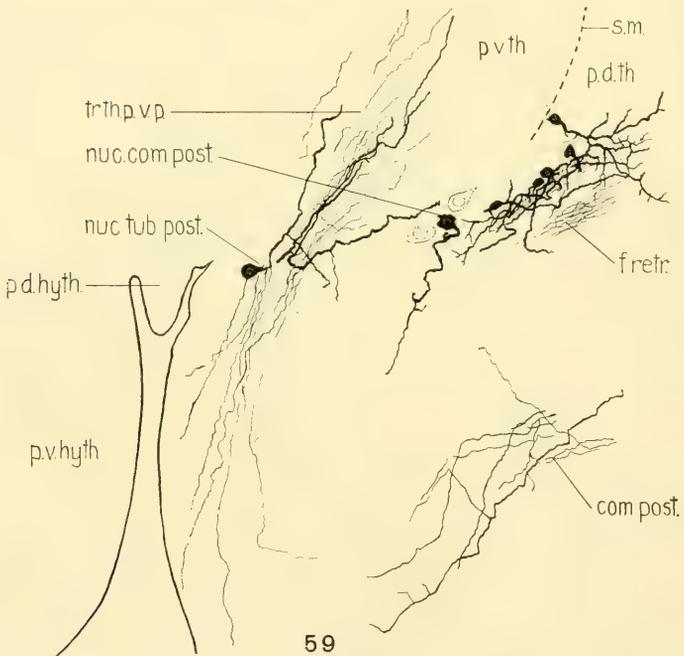
Fig. 58 From the same side of the same specimen as figure 57 and three sections nearer the median plane. In the middle of the thalamus the section passes through the boundary between the stratum album and the stratum griseum and the positions of the sulcus medius (*s.m.*) and sulcus ventralis (*s.v.*) as projected from sections farther medially are indicated by heavy broken lines. The dense

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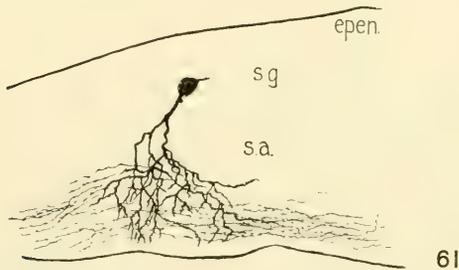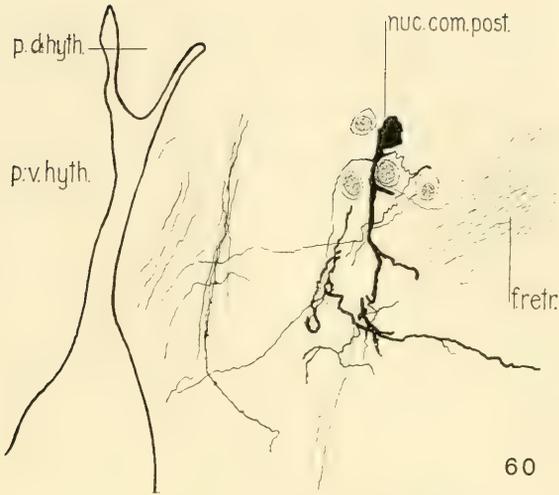
neuropil in the pars dorsalis thalami (*p.d.th.*) marks the area from which the tractus thalamo-frontalis anterior arises (cf. fig. 57). Between this neuropil and the stria medullaris (*str.med.*), which is very incompletely impregnated, are vertical fibers passing between the pars dorsalis and the pars ventralis of the thalamus, the tractus dorso-ventralis thalami (*tr.d.v.th.*). $\times$ 30 (ciiic, 3–2–3).

Fig. 59   From the same specimen as the last two sections nearer the median plane, illustrating one neuron from the ventral part of the nucleus of the tuberculum posterius (*nuc.tub.p.*), and dorsally of this an imperfect impregnation of the nucleus of the commissura posterior (*nuc.com.post.*). Dendrites of these neurons reach backward into the terminal arborizations of the commissura posterior (*com.post.*); cf. figure 60. The position of the sulcus medius thalami (*s.m.*) is indicated as projected from the adjacent section medially. Above this line are several neurons of the extreme caudal part of the pars dorsalis thalami, two of which are entered from the adjacent section laterally. Their dendrites arborize among fibers of the tractus thalamo-peduncularis dorsalis profundus which run parallel with the tractus habenulo-peduncularis (*f.retr.*). The axons of all of the neurons here figures probably enter the tegmental fascicles. $\times$ 74 (ciiicv 3–2–1).

Fig. 60   A single neuron of the nucleus of the commissura posterior from the section adjacent to the last medially. $\times$ 74 (ciiic, 3–1–9).

Fig. 61   A single neuron of the interpeduncular nucleus from a sagittal section near the median plane and a short distance caudad of the fovea isthmi. The ventral surface of the brain is below and the rostral end at the left. The

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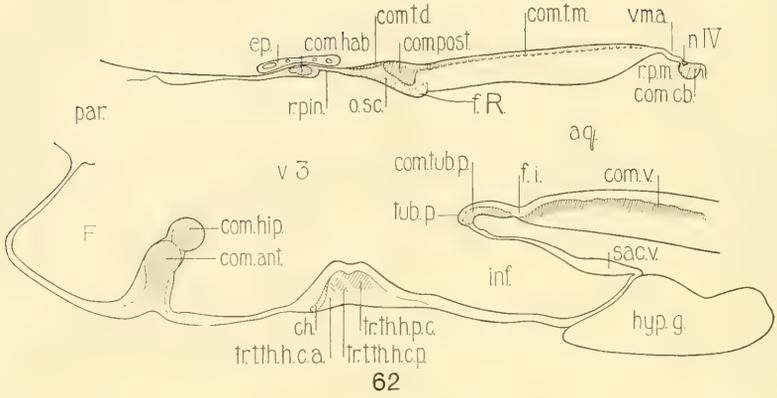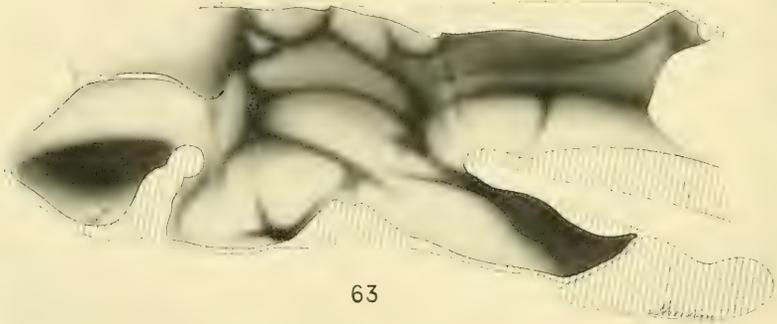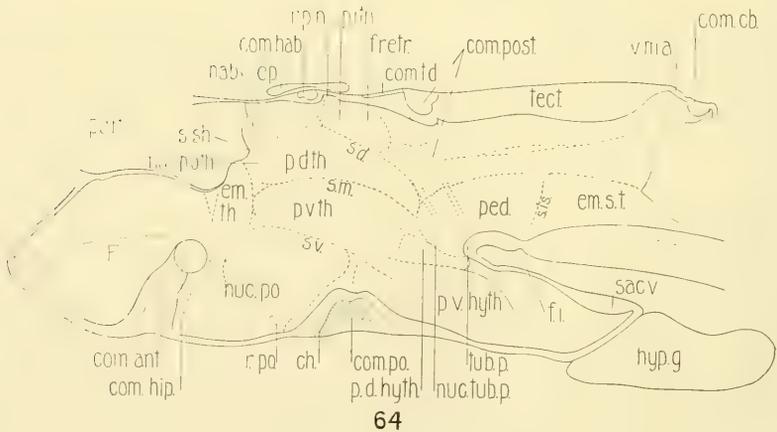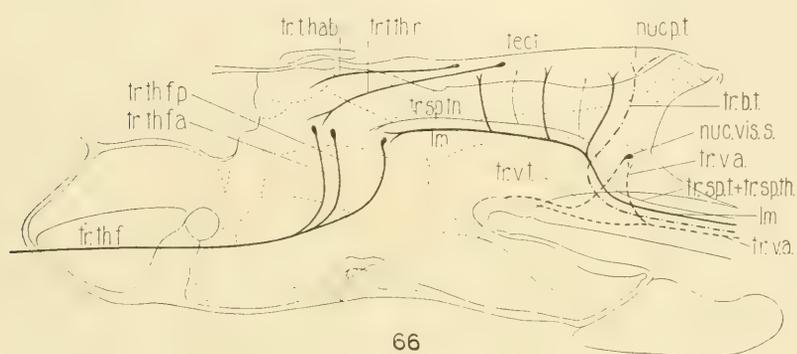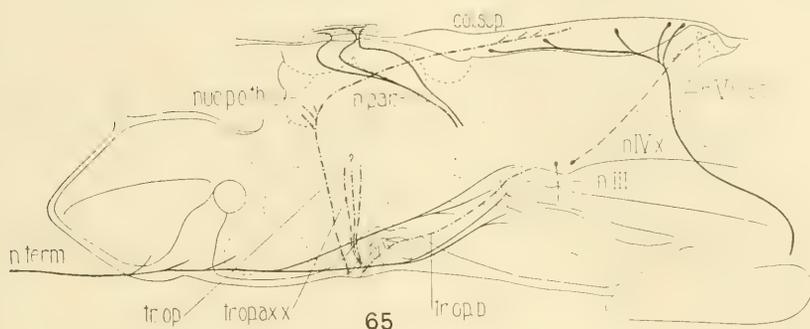
bushy and thorny dendrite is directed ventrally into the very dense neuropil of this nucleus, which occupies a ventro-medial position and is traversed by the slender varicose longitudinal axons, some of which come from the tractus habenulo-peduncularis and which descend through this neuropil far into the medulla oblongata. The axon of this neuron is not impregnated, but other preparations show that axons of this nucleus descend in the longitudinal tract shown. × 74 (cic, 2-2-2)

Fig. 62 Median section through the diencephalon and mesencephalon, reconstructed from sagittal sections by the Weigert method (series ciii). The sections from which the drawing was made are slightly oblique to the sagittal plane, which was, accordingly, reconstructed by the superposition of camera outlines of the necessary sections made on tracing paper. Comparison with gross preparations cut in the sagittal plane confirms the relations. × 13.

Fig. 63 Drawing of a graphic reconstruction of the median section of the brain. This was made on the basis of the same series of sagittal sections used for figure 62, as described on page 227. The details of the ventricular sculpturing were controlled by comparison with gross preparations cut in the median plane, and the drawing is a composite, including some slight modifications derived from the gross preparations as studied under the stereo-binocular microscope. × 13.

Fig. 64 Key drawing to accompany figure 63. × 13.
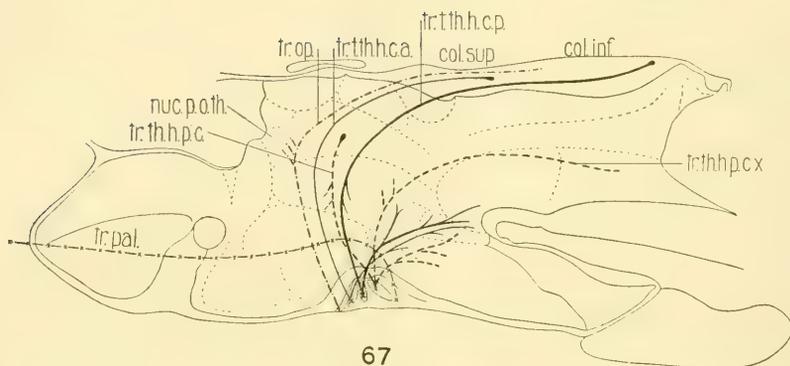
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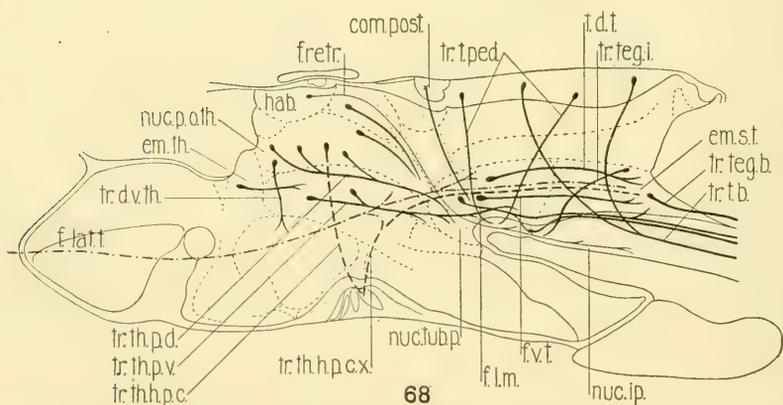
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Fig. 65   Diagram of the peripheral connections of the midbrain and thalamus.
× 13.

Fig. 66   Diagram of the lemniscus and thalamo-frontal tracts.   × 13.

Fig. 67   Diagram of the composition of the postoptic commissure complex.
The lateral bundle of the optic tract (*tr.op.*) is shown, but the other bundles of
this system are omitted; cf. figure 65.   × 13.

Fig. 68   Diagram of the chief descending tracts of the midbrain and thala-
mus, with special reference to those which enter the cerebral peduncle.   × 13.

THE PERIPHERAL DISTRIBUTION OF THE NERVUS TERMINALIS IN AN INFANT

CHARLES BROOKOVER

FOUR FIGURES

In recent years the nervus terminalis has been found in all classes of vertebrates, but the literature on its relations and structure in man is rather restricted. De Vries ('05) found a ganglion in human embryos which he suggested as being homologous with the n. terminalis in fishes, the only group of vertebrates in which it had then been found. Döllken ('09) described the ganglion cells along the olfactory nerve in human embryos of 21 mm. length and larger. Johnston ('13) gave an account of it in human fetuses as large as 47 mm. length and ('14) in older fetuses as well as its central root in adults. Contemporaneously, the author ('14) gave an account limited largely to its central relations in adult man.

The present paper is based on two series of sagittal sections of stillborn negro infants at about full term. The cells have been observed in methylene blue staining of adult material, also. The following description is based largely on a pyridine silver preparation of the right half of the nose. The modification of Ranson's method used by Huber and Guild ('13) on the rabbit was employed to permit of the section of the cribriform plate after decalcification. The material was injected with ammoniated alcohol by Dr. D. A. Rhinehart and the later treatment and embedding done by Dr. R. C. Dickinson, to both of whom I am greatly indebted.

The sections were cut 20 micra thick and measured about 35 by 20 mm., extending back of the hypophysis cerebri which was useful as an orientation mark in reconstruction. The field being too large to be covered by any of the low power objectives at hand, the ocular was removed from the microscope of
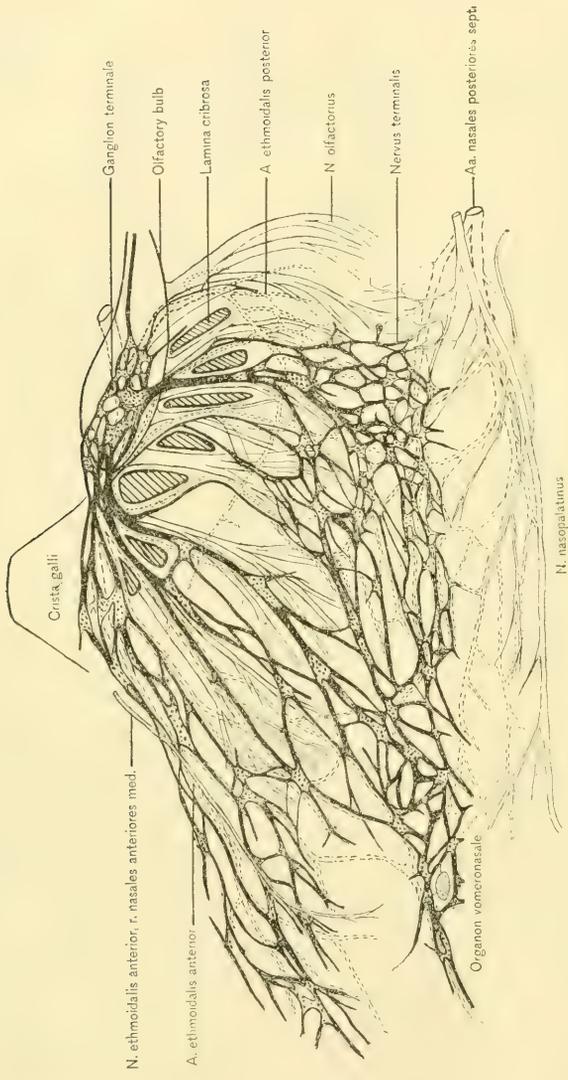
Fig. 1 Semidiagrammatic representation of the right side of the nervus terminalis of a negro infant, seen from the median plane projected upon the olfactory bulb and fila olfactoria. Ganglion cells representing location of main aggregations but not the actual number of cells. Rami of n. terminalis made robust to bring into relief from other structures. × 5.

the projection apparatus and a common hand lens substituted for the objective. The chief nerves and other prominent structures were then sketched from alternate sections and the picture completed under higher powers of the microscope for each slide at a magnification of seventy diameters. These tracings were transferred by carbon paper to a single chart from which a photograph was taken to reduce it to ten diameters. From this a drawing was made for figure 1, which is partly diagrammatic.

Figure 1 shows the ganglion terminale and the peripheral rami of the n. terminalis of the right side as seen from the median plane projected on the olfactory bulb, the sectioned lamina cribrosa and several related structures in the nasal septum and overlying mucosa. As in the rabbit (Huber and Guild '13), the peripheral rami of the n. terminalis lie next the cartilaginous septum deep to most of the vessels and other structures of the nasal mucosa. Centrally the ganglion terminale lies median to the olfactory bulb embedded for the most part in the dura lateral to the crista galli.

The ganglion terminale, as in the rabbit (Huber and Guild '13), is a nerve plexus of six or eight ganglia interlaced in all directions with nerve fibers. The overlying portion of the brain was somewhat broken and disturbed in the technique but it is thought the natural relations of the components of the ganglion terminale are preserved in the surrounding dura. A slight contraction is indicated by the wavering uncertain course of the rather fine nerve fibers as compared with the stouter peripheral fibers under the nasal mucosa which seem fairly well stretched and straight, perhaps on account of the pressure distention of the injection of this erectile-like tissue. In this instance a single strand composes the central root of the ganglion terminale which could be traced proximally about one-third the distance to the optic chiasm. The brain is displaced here in this preparation and the root was lost before it unites with the brain. The slender root was not sufficiently well impregnated to show fibers clearly. No ganglion cells were found in its course. Few of the cells in the ganglion terminale were impregnated sufficiently to follow their nerve processes. Two such are shown in figure 4.

Running distally through the cribriform plate of the ethmoid bone are three or four stout rami, each of which is larger than the central root of the nerve. These rami in their passage through the lamina cribrosa are noticeably devoid of the nerve cells characteristic of the n. terminalis elsewhere. In the posterior large branch two or three cells were found (fig. 1).

Though sagittal sections cut in the direction of the course of the main rami are not favorable for counting fibers, it was estimated that the large posterior ramus contains about two hundred. Perhaps all the other peripheral rami together have an equal number. In the plane of the sections the large posterior ramus measures about 200 micra in transverse diameter. It would appear that these rami here and elsewhere peripherally, are somewhat flattened into the sagittal plane since they are usually comprised within three sections of 20 micra. Peripheral to the lamina cribrosa these rami become slightly broader with open interlacing fibers (fig. 2) and where well impregnated could be seen with the naked eye when the sections were held over a white surface. This flat shape of the nerve rami gives undue prominence in sagittal sections and this is slightly exaggerated by the heavy shading in figure 1. In sections cut perpendicularly to the nasal mucosa it is frequently difficult to find any trace of the n. terminalis.

However, the n. terminalis in man is of so considerable bulk that it would seem strange that it should have been so long overlooked in sections of the nasal mucosa. It was somewhat surprising to find, on counting the total nerve cells found in the sections of the right side peripheral to the lamina cribrosa of this infant, that they number fifteen hundred and seventy-eight. It might be thought that, since the nuclei did not show for the count in all instances, this number is too great. But the short diameter of the nerve cells which generally have their long axes in the plane of these sections, is about the same as the thickness

Fig. 2 The posterior ventral portion of figure 1 to show distribution of the cells in the n. terminalis and in outline a possible posterior sympathetic connection through the nasopalatine to the sphenopalatine ganglion. Seen from the lateral side. × 40.
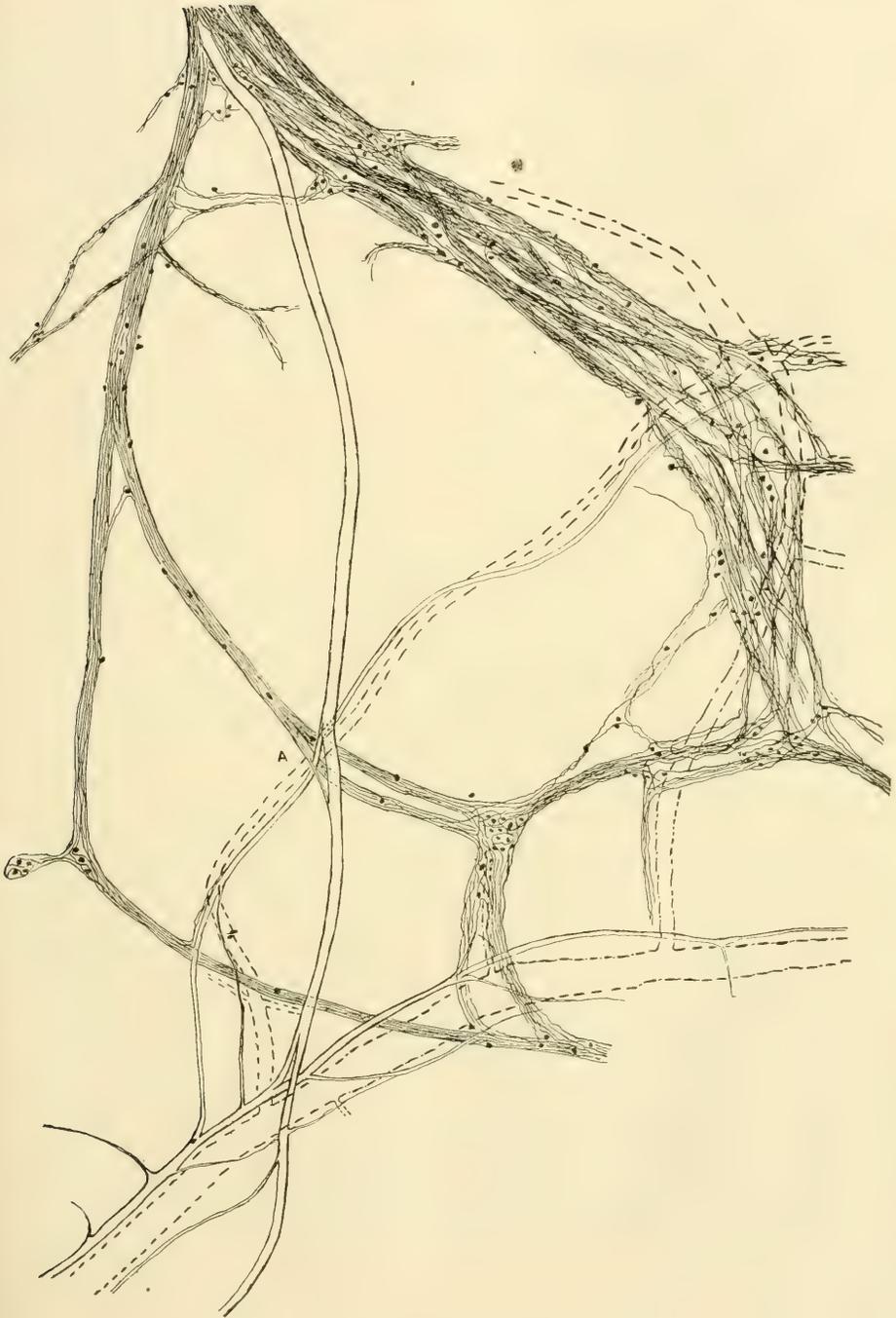
of the sections and the probabilities are that not more than half
the cells would have been enumerated twice. This would give
a minimum of one thousand, but there are reasons for believing
that the number is not far from the count as made. There are
two regions, one anterior not well impregnated and another pos-
terior where too black (blank as to n. terminalis in fig. 1), where
cells should have been found. Then too, there is probability
of overlooking some isolated scattered cells. In making the
count there was a tendency to follow the main rami of the nerve.

Figure 1 shows fairly accurately, we think, the field of dis-
tribution of the cells of the n. terminalis. As has been found to
be the case in other vertebrates in the main, the territory of the
peripheral cells is confined to the septum and generally fairly
accurately corresponds to the area covered by the fila olfactoria
in their distribution to the muscosa. This point could not be
accurately determined in this preparation. Although fibers
that were thought to belong to the nervus terminalis were in a
few instances found accompanying the blood vessels and fila to
the turbinated bones, no cells were found along the course of these
fibers to make the matter certain. Our preparations were not
favorable for the determination of this point. That the nerve
may be independent of the olfactory nerve is indicated by its
presence in the porpoise where the olfactory fila are supposed
to be absent according to Johnston ('14).

Although the cells are more numerous at intersection points
in the whole peripheral n. terminalis, the semidiagrammatic
representation in figure 1 gives only the position of the chief
groups of cells. From figure 2 it will be seen that there are occa-
sional cells along the fibers. In this drawing there is a careful
representation of all the cells in position, as nearly as could be
done in construction from several sections. Perhaps there are
more cells than shown in the main ramus where cells are rather
frequent all along the nerve. The fibers in this drawing are
somewhat conventional and too few, but give an idea of the
open character of the fibrous network in the n. terminalis.
This is in contrast with the more compact arrangement of other
nerves encountered in the nose. This character, along with

the interspersed nerve cells, serves to distinguish it. The fibers of the fila olfactoria are not impregnated except perhaps rarely in isolated spots deeply overstained.

Huber and Guild ('13) have mentioned the similarity of the peripheral nervus terminalis to an enteric plexus. Perhaps figure 1 gives too much emphasis to the anastomosis of fibers,
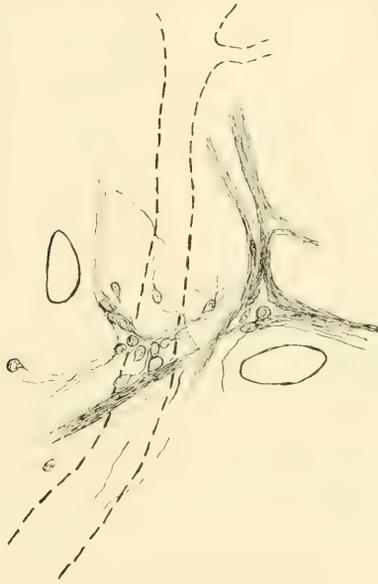
Fig. 3 Sketch from the lower right margin of figure 2 taken from a single section, shows relations of cells to the fiber plexus at nodal points. Artery of this and next shallower section in broken lines. Two other vessels cut transversely. × 80.

since in the reconstruction all fibers crossing at a common point were made to inosculate. Figure 2, drawn with more care with regard to this point, leaves little doubt that there is frequent anastomosis with a tendency to cell aggregations at the nodal points. There was seldom any evidence of bifurcation of fibers at such points although some were seen in rami of the V nerve encountered in the preparation. Figure 3 represents the appearance of a single section taken from the lower right margin

of figure 2. We may mention again the grouping of cells and fibers such as might be found in the myenteric or submucous intestinal sympathetic. The proximity of nerve and arteries (here represented in broken lines) is noticeable everywhere peripherally. A close study of figure 1 will show how general this is beyond the lamina cribrosa. I have noted instances where the fibers of the nerve trail over the surface of arteries but the method is not one suited to determination of the fine twigs of nerve endings.

Some study has been made of such cells as were favorably impregnated in the series to learn whatever is possible of their structure and relations. Drawings of some characteristic shapes are shown in figure 4. There are very few cells that appear of the primitive bipolar type with broad gradually tapering end (dendrite) on the opposite side of the nucleus from the axone. Perhaps all that were encountered were drawn. Rarely is there a bifurcation of the dendrite (fig. 4) but that is possibly on account of the short distance from the perikaryon that the impregnation of dendrites extends. Attention was paid to the direction of the dendrite in the nerve rami but there are about as many extending centrally as peripherally. The arrows in the drawings point distally. Many of the cells appear to be monopolar, though if there were two processes the sections were cut in such a plane (longitudinal to the nerve rami) that one would think both would be displayed. In some instances it was not certain whether we had to deal with a bipolar cell or an unipolar cell with a pericellular net. The nuclei of sheath cells frequently showed in the preparations, especially about the ganglion cells, but rarely were much in evidence in the rami if these were of small calibre. As well as could be judged, the fibers are nonmyelinated and it may be that many of them are devoid of the primitive sheath, and this may be the reason why they are impregnated more deeply than any other nerves in this region. It may be remarked also, that the fibers seem rather coarse. Oftentimes the cells appeared to be located at some little distance from the nerve ramus (fig. 4) and sometimes from examination of the adjacent sections this is seen to be really so.
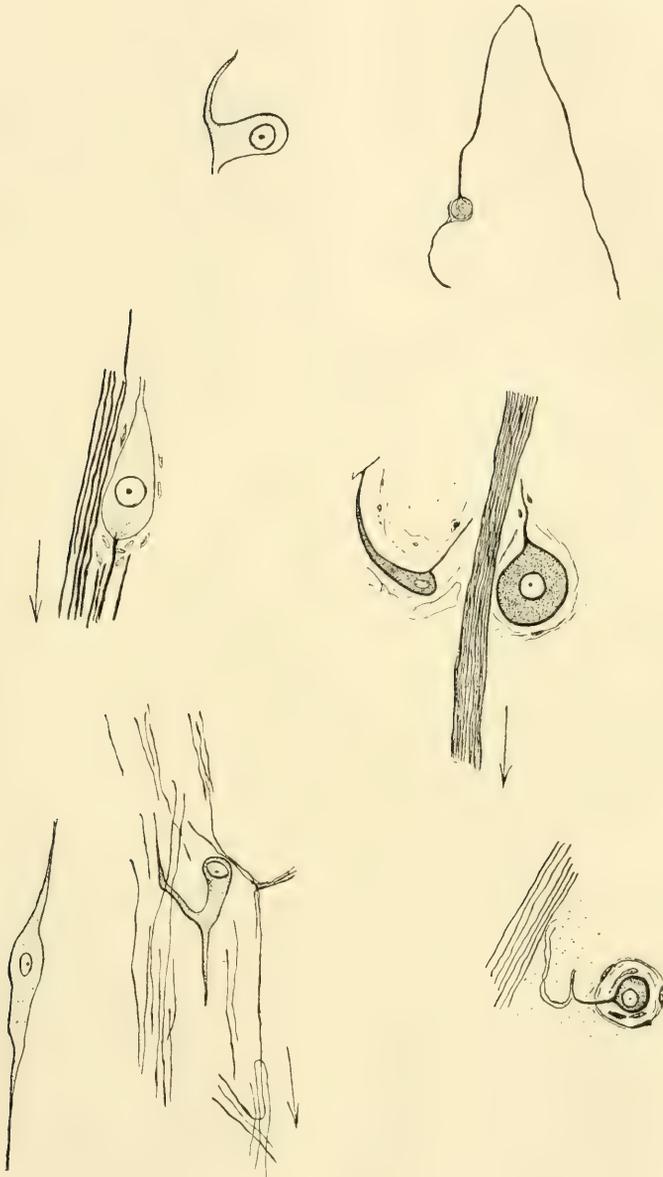
Fig. 4 Sketches of cells from various localities. The two at top from the ganglion terminale. The remainder show cells under the nasal mucosa. The arrows point distally. × 340.

Other workers have noted the presence of other fibers than those of fila olfactoria and the n. terminalis in the region occupied by the nervus terminalis. These nerve rami have been found in these preparations and come from two main rami of V, viz., the ophthalmic division sending the ethmoidal nerve into the anterior dorsal part of the nose (fig. 1) and the maxillary division sending the nasopalatine onto the septum by way of the sphenopalatine nerve and ganglion. The septal branches from this latter source are numerous and conspicuous in this series of sections (fig. 1). Some of the most posterior fibers represented are deeply impregnated and seem to end in the cancellated bone which apparently takes the place of ethmoidal and sphenoidal sinuses at this age. Farther rostrally (fig. 1) other rami pass dorsad from the main sphenopalatine nerve as it takes its course toward the foramen incisivum.

A peculiarly different nodal point marked A (fig. 2) in the anastomosis of nerve rami attracted attention in studying the sections, and on closer study seems to offer a possible posterior sympathetic connection with the sphenopalatine ganglion. When traced dorsally from this point it leads undiminished in size into the bifurcating ventral end of the main large posterior emerging ramus of the nervus terminalis as it issues from the cribriform plate (figs. 1, 2). Here there is blackening in the preparation and it can not be determined what disposition is made of the fibers in the main posterior ramus of the nervus terminalis which is composed of two or three bundles in its course through the cribriform plate. Examination of the other side and of the other series in my possession does not clarify the matter, though this ramus seems constant in position and larger than the other branches of the n. terminalis in the cribriform plate and made up of two or three bundles. There are about thirty or forty fibers in the connecting nerve which measures 35 microns in diameter in the part of its course so far described. Traced ventrally some distance beyond point A it measures 25 microns and when carefully examined shows fibers from three rami of the nasopalatine entering it. One

joins it at the nodal point A and the others more ventrally near the main sphenopalatine artery and nerve (septal division).

The fibers of this anastomosis of the nasopalatine nerve with the n. terminalis are more compacted than the fibers in the n. terminalis and no nerve cells were found in its course except perhaps one marked by a dot in figure 2, well ventrally in its course in this field. This seems well beyond the known territory of the nervus terminalis. These rami of the nasopalatine nerve lie in a similar position to those of the n. terminalis deep to the arteries and most other structures of the mucosa, so that there is no reason for confusion with the fila olfactoria. In fact, most of the details at nodal point A were made out in a single section. There is but a single cell in the double ramus of the n. terminalis crossing near nodal point A, but with the most ventral of these there is an exchange of fibers (fig. 2). A study of the various rami given off from the septal portion of the nasopalatine in this region (fig. 2) reveals an intimate interlacing of fibers with the n. terminalis. A further study of this region by various methods is needed to make these points more certain.

It has been noted in study of the sections that there is a slight tendency of the peripheral rami of the n. terminalis to converge distally and rostrally (fig. 1) around a point of slight depression which is probably the organon vomeronasale (Jacobson's organ). This point in the behavior of the nervus terminalis is in correspondence with the findings of others, viz., that there is an intimate connection of the n. terminalis with Jacobson's organ. The preparations were not well impregnated in some parts of this region.

We may summarize briefly by saying that the peripheral nervus terminalis is so large in man that it may be said to be hypertrophied as compared to the known development in other mammals, without appreciably increasing its central root. In addition to many cells in the ganglion terminale it contains about fifteen hundred cells peripherally under the nasal mucosa. Though disposed in three or four chief rami emerging from the lamina cribrosa there is a vast network of interlacing bundles

deep to the main arteries. Some of the fibers trail over the walls
of the arteries but the method of treatment by the pyridine
silver technique does not reveal ultimate endings. There is
considerable evidence that the interlacing rami of the naso-
palatine nerve send a bundle of considerable size through the
cribriform plate to establish a sympathetic chain connection
posteriorly via the sphenopalatine nerve and ganglion. More
work needs to be done to make the last point secure.

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METABOLIC ACTIVITY OF THE NERVOUS SYSTEM

I. AMOUNT OF NON-PROTEIN NITROGEN IN THE CENTRAL NERVOUS SYSTEM OF THE NORMAL ALBINO RAT

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ONE CHART

The object of this paper is to present data on the relative amount of various nitrogenous organic extractives normally present in the central nervous system of the albino rat. There has been much speculation from time to time by various writers concerning the metabolic activity of this important organ, but on account of either imperfect data or lack of the necessary information, the results have been entirely unsatisfactory. Recent advances in biochemical technique enable us now to make precise quantitative determinations of various chemical components in the nervous system, and it was the hope of the present writer to obtain by methods of analysis data which would assist in the study of the metabolic phenomena in this organ. The data so far obtained are not very extensive, nevertheless the results show several interesting relations which have not been fully appreciated heretofore and I have decided therefore to publish the present observations with some comments.

MATERIAL

For the present investigation albino rats were used. Since this animal is small, it is disadvantageous when a large quantity of material is required. However the extra labor needed to obtain the desired quantity of material is well repaid, since by thus taking a large number of rats one can reduce the effect of individual variations to a minimum. Another reason for choosing the rat was the fact that we now possess very complete

physical data on the growth changes of the body and organs, and also a complete record of the percentage of water in the brain and of the composition of the entire body (Donaldson, '15) all of which data can be advantageously utilized for the purpose of cross reference. The rats employed in this study were all in good condition, any appearing at all unhealthy having been discarded. I have also used the sheep's brain for the study of the white matter and the gray matter. These brains were secured at a local slaughter house, immediately after the animals had been killed.

TECHNIQUE

I have used 2.5 per cent trichloracetic acid for extracting water soluble organic substances from nervous tissue. This reagent was first used by Greenwald ('15) to precipitate proteins as well as small quantities of fat which are present in the blood. I have also found that this reagent not only precipitates proteins but that it also precipitates completely the lipoids. It is remarkable that the reagent gives a clear watery filtrate which is entirely free from the lipoids even in the case of the nervous tissue which contains more than 50 per cent of lipoids in its solids. I have found it convenient to take about seven grams of moist material for an analysis. The material is well ground in a mortar and then well mixed with a small quantity of water (5 cc.). Then with an aid of 45 cc. of water the entire material is transferred to a bottle. To this 100 cc. of 2.5 per cent trichloracetic acid is added, and the mixture shaken well and frequently for the first hour. The material remains in the solution for twenty-four hours (two or three hours are sufficient for a complete extraction, but for convenience in carrying out my working schedule I have left it for twenty-four hours). The filtration is very easily made and one obtains a perfectly clear solution. The following amounts of filtrate were taken for the various determinations; 5 cc. for non-protein nitrogen, 20 cc. for amino acids, 30 cc. for urea and ammonia and 40 cc. for ammonia alone. Altogether 95 cc. are used and the remainder kept for emergency purposes.

For amino-acid determination, I have followed the procedure of Bock ('17) using also Van Slyke's micro-apparatus. For ammonia nitrogen, the solution was evaporated to nearly 10 cc. in a large test tube (25 x 200 mm.) and after cooling was nearly neutralized with 0.1 N NaOH. To this was added anhydrous sodium carbonate to saturation and also 1 cc. of kerosene to prevent foaming, and the solution was then aerated for thirty minutes. For the urea determination the solution was also evaporated to 10 cc. and cooled. The solution was then made slightly alkaline by means of 0.1 N NaOH and then 1 cc. of 15 per cent arlco-urease together with 1 cc. kerosene were added. The urease was allowed to act for 25 minutes at about 23°C. At the end of 25 minutes anhydrous sodium carbonate to saturation was added and the solution aerated for 30 minutes. For determination of the non-protein nitrogen I have used a modification by Bock and Benedict ('15) of the micro-method of Folin and Farmer ('12). In all cases the nitrogen was estimated by means of the DuBoscq colorimeter.

CONTENT OF NON-PROTEIN NITROGEN IN THE ENTIRE BRAIN OF THE ALBINO RAT AT DIFFERENT AGES

The rats at thirty-five days of age and older were fed with regular laboratory diet twenty-four hours before they were examined. We, however, kept plenty of dog biscuit in the cage and therefore the rats had always enough to eat. The rats younger than thirty-five days were kept with the mother and were either exclusively nourished with the milk or partially. The dog biscuit was also kept in the cage. It seems therefore that so far as feeding was concerned they were all in the same nutritional state. Altogether 142 rats of different ages were used.

From table 1 we see that the percentage of total nitrogen in solids (column (g)) diminishes steadily with advancing age. This is to be expected since the brain during the first 350 days of life rapidly acquires the so-called myelin substance, which is poor in nitrogen. Therefore this diminution of the total nitrogen indirectly indicates the degree of myelination. The non-protein nitrogen content (column (i)) in relation to the total solids also

TABLE 1

Showing the content of total nitrogen as well as non-protein nitrogen in the entire brain of the albino rat at different ages

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)
BODY WEIGHT	BRAIN WEIGHT	WATER IN BRAIN	SOLIDS	TOTAL NITRO-GEN IN ENTIRE BRAIN	NON-PROTEIN NITRO-GEN IN ENTIRE BRAIN	TOTAL NITRO-GEN TO SOLIDS	NON-PROTEIN NITRO-GEN TO TOTAL NITRO-GEN	NON-PROTEIN NITRO-GEN PER GRAM OF SOLIDS	MEAN AGE	NO. OF RATS USED
grams	grams	per cent	grams	mgm.	mgm.	per cent	per cent	mgm.	days	
4.8	0.231	88.30	0.027	3.07	0.38	11.40	12.33	14.1	1	45
7.1	0.459	88.75	0.052	5.73	0.71	11.08	12.38	13.7	5	32
9.5	0.598	88.13	0.071	7.70	0.91	10.86	11.81	12.8	7	17
17.9	1.175	84.90	0.177	18.28	1.63	10.30	8.89	9.2	15	12
26.7	1.284	80.86	0.246	23.70	2.11	9.65	8.91	8.6	24	6
34.8	1.379	80.26	0.272	26.21	2.22	9.63	8.48	8.2	35	6
66.6	1.508	79.58	0.308	28.00	2.42	9.09	8.64	7.9	54	6
156.6	1.762	78.89	0.372	33.72	2.63	9.07	7.81	7.1	116	6
161.4	1.803	78.43	0.389	34.13	2.59	8.78	7.58	6.7	274	6
185.5	1.858	78.00	0.409	35.94	2.89	8.79	8.04	7.1	382	6

diminishes with advancing age as does the total nitrogen. However the rate of reduction is plainly more rapid in the non-protein nitrogen than in the total nitrogen. Indeed the brain at 382 days of age gives a non-protein nitrogen value which is only one half of that at one day. If non-protein nitrogen were closely related to the total nitrogen content we might anticipate a parallel fall of their value at given ages. This, as the table shows, is not the case. It is therefore possible that the greater reduction of non-protein nitrogen compared with the total nitrogen might be due to an accumulation of the myelin nitrogen which in turn may not be closely related to the formation of the non-protein nitrogen. Therefore the two sets of data might show changes which are more nearly parallel if we should calculate the total nitrogen for the solids from which the myelin has been removed. This test can not be made at this moment on account of a lack of necessary data. It will be seen later however that this supposition that the non-protein nitrogen is quantitatively related to the non-lipoid fraction in the brain is well supported.

We shall discuss this subject more fully in connection with the studies on the parts of the central nervous system.

The graphic representation of the content of non-protein nitrogen is given in chart 1.
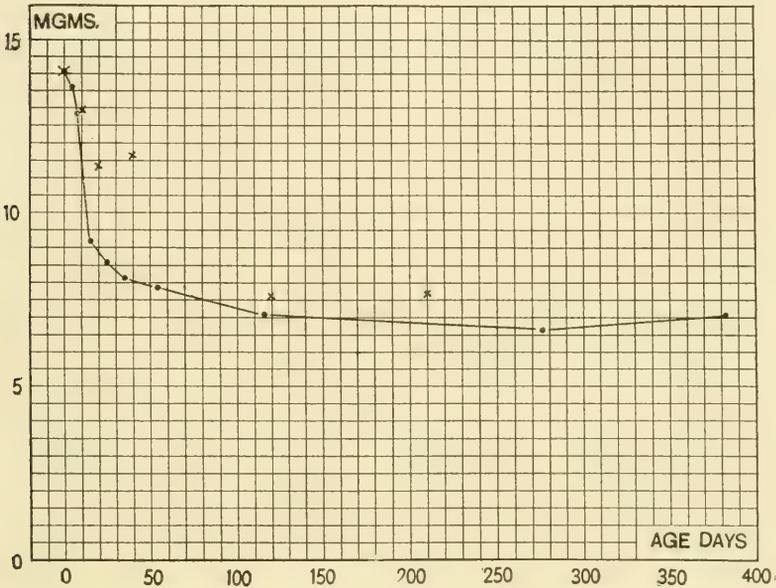
Chart 1   Showing the number of milligrams of non-protein nitrogen per gram of the dried brain at different ages.   The chart shows also the proportional value of organic extractives together with inorganic salts in the brain of the albino rat, as determined by W. Koch and M. L. Koch ('13).   ·—· Non-protein nitrogen, × organic extractives and inorganic salts.

One striking relation which is well shown in the chart is the rapid fall of the content of non-protein nitrogen within the first fifteen days of life.   At the end of this period the rate of reduction becomes much less till the animal reaches about one hundred and twenty days.   After one hundred and twenty days the subsequent changes are almost negligible.   The general appearance of the curve is similar to that for the percentage of water in the brain at different ages (Donaldson, '15).   One difference between these two cases lies in the fact that the non-protein

nitrogen shows its greatest reduction within the first fifteen days, while in the case of water, the approximate end of a rapid fall is not reached until about twenty-five days after birth. It is interesting to note that the percentage of water in the visceral organs also falls off rapidly, while the ligamentous skeleton (Lowrey '13) shows the water content similar in its time relations to that of the brain. Whether the difference noted between the fall in the percentage of water and the fall in the non-protein nitrogen in the brain has any important physiological meaning, or is merely due to a comparatively small number of observations made on the non-protein nitrogen, is not entirely clear, though the latter explanation does not seem very probable.

ORGANIC EXTRACTIVES AND INORGANIC SALTS IN THE BRAIN

The so-called 'extractives' consist of both nitrogenous and non-nitrogenous fractions. My present determinations were exclusively made on the nitrogenous fraction. Nevertheless it is of interest to determine whether or not the non-nitrogenous fraction also varies with the age of the rat in a way similar to that of the nitrogenous fraction. The data given by W. Koch and M. L. Koch ('13) were utilized to test this point, and are given in table 2, and seem to show such a concomitant variation.

TABLE 2

Showing the quantitative relations existing between the total dry substance of the brain and the organic extractives plus the inorganic salts, in the albino rat at different ages. W. Koch and M. L. Koch 1913

	AGE IN DAYS (ALBINO RAT)					
	1	10	20	40	120	210
Dry weight of one brain in milligrams (A)....	26	107	224	281	347	365
Organic extractives and inorganic salts in milligrams (B).............................	4.65	16.16	32.6	41.7	33.8	35.8
Milligrams of (B) per 100 milligrams of (A) (C).	17.9	15.1	14.5	14.8	9.7	9.8
2.12[1] per cent taken from (C) gives.............	14.1	12.9	11.4	11.7	7.6	7.7

[1] 2.12 per cent were taken off from the value given in 'C' in order to equalize the two initial values (one day old); one given by these data, and the other the number of milligrams of non-protein nitrogen per gram total brain solids for a one day old rat (table 1, column (i)).

When the data given by W. Koch and M. L. Koch were computed as indicated in table 2, and the results plotted (chart 1) we at once find that the organic extractives as a whole (including the inorganic salts) diminish with advancing age, and furthermore all the observations, except one case, fall very close to the curve described by the non-protein nitrogen. We infer from this result that the non-nitrogenous fraction as well as salts show, during the growth of the brain, changes similar to the nitrogenous fraction.

THE NITROGEN VALUES GIVEN BY NON-PROTEINS, THE AMINO ACIDS, AND THE UREA AND AMMONIA

So far I have dealt with the changes in the relative amount of the total nitrogen, as well as of the non-protein nitrogen during the growth of the brain. I now wish to consider the extent of the normal variation of various extractive bodies found in the adult brain. The data showing the nitrogen content of these four components are given in table 3.

The data given in table 3 were obtained during the course of investigations connected with other problems. The rats used for these investigations had been without food with the exception of water, for forty-eight hours before killing in the hope of putting them in a uniform nutritional state. It was found that a period of forty-eight hours was about long enough to remove the contents from the entire digestive tract. The rats at the end of the forty-eight hours were as vigorous and active as the rats fed in the ordinary way. In this case I have given the nitrogen in milligrams as found in 100 grams of fresh tissue. This manner of presentation has some advantage when the water content of tissue is nearly constant, and in fact this procedure has been widely adopted by most bio-chemists.

NON-PROTEIN NITROGEN

The number of milligrams of non-protein nitrogen per 100 grams of moist brain is exceedingly uniform. The average value (157 mg.) is slightly lower than that given by some other organs of the same animal (table 4) as well as those given by the

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TABLE 3

Showing the nitrogen content of non-proteins taken together, as well as the nitrogen of the amino-acids, the urea and the ammonia in the brain of the adult albino rat

BODY WEIGHT	AGE	NUMBER OF RATS USED	BRAIN WEIGHT	NITROGEN PER 100 GRAMS OF FRESH BRAIN			
				Non-protein	Free amino acid	Urea	Ammonia
grams	days		grams	mgm.	mgm.	mgm.	mgm.
71	89	3	1.569	148	67		
82	85	5	1.560	145	72	13	13
94	116	3	1.574	162			
96		5	1.557	159		13	16
99	89	3	1.686	161	67		
109	120	3	1.727	169			
110	108	3	1.651	157	68		
111	108	3	1.700	158	65		
114	97	3	1.661	156	51		
118	85	5	1.674	164	70	6	14
122	116	3	1.632	154	63		
124		3	1.727	159	69		
126		5	1.711	164		9	14
131	118	3	1.776	139	64		
133		3	1.616	145	62		
134		5	1.655	161	56	6	14
141		3	1.759	161	66		
146		3	1.810	145	68		
152		3	1.765	153	66		
157		3	1.694	164	66		
167	312	3	1.734	165	64		
121	120	73	1.678	157	65	9	14

organs of other mammals. This low value is probably due to the fact that the brain solids contain 50 per cent or more lipoids, which very likely has no close relation to the nitrogenous organic extractives.

AMINO-ACID NITROGEN

The amino-acid nitrogen is also quite uniform as in the case of the non-protein nitrogen. The average value of 65 mg. is very close to that given by the muscles, the pancreas and the spleen of the dog (Van Slyke and Meyer, '13–'14).

However, the amino-acid nitrogen in terms of myelin free solids (active cell substance) must be considerably higher in the brain than in these other organs.

UREA AND AMMONIA NITROGEN

Although the observations on the urea and ammonia nitrogen are not numerous, yet it appears certain that the content of ammonia is far more constant than that of urea. The amount of ammonia here found is slightly higher than that found by Levene and Stookey ('03) in the dog's brain, in which they report 10 mg. ammonia nitrogen per 100 grams of moist brain weight. Recently Marshall and Davis ('14) determined by their urease method the urea content of various organs and tissues in the dog. The brain gave 28 mg. urea nitrogen per 100 grams of fresh tissue or practically the combined values of urea and ammonia nitrogen found in the rat brain. Since Marshall and Davis did not attempt to eliminate the ammonia nitrogen from the materials previous to urea determinations, the value given by them as the urea nitrogen may have been in fact that combined values of the urea and the ammonia nitrogen. If such were the case my own results on the rat agree with those for the dog very closely. I have made also a short series of observations on the non-protein nitrogen content in other organs than the brain of the adult rat and the results thus obtained are given in table 4.

TABLE 4

Showing the total nitrogen as well as non-protein nitrogen in several organs of the adult albino rat

	TOTAL NITRO-GEN IN FRESH TISSUE	NON-PROTEIN NITROGEN PER 100 GRAMS FRESH TISSUE	NON-PROTEIN NITROGEN TO TOTAL NITRO-GEN	AGE
	per cent	mgm.	per cent	days
Brain......................	1.953	159	8.168	143
Testes.....................	1.729	170	9.806	143
Liver......................	3.435	182	5.332	143
Kidneys...................	3.243	229	7.572	143
Blood.....................	3.093	35	1.134	241

As is shown in table 4, with the exception of the blood, the non-protein nitrogen in the brain is least as compared with the other three organs. However, when the comparison is made between total nitrogen content of the organ and content of non-protein nitrogen, the brain gives a higher figure than either the

liver or kidneys and comes very close to the testes, which gives the highest value. The meaning of the higher non-protein nitrogen value found in the brain, when compared with the total nitrogen in either the liver or kidneys, can not be readily explained, and we merely record the fact as one of interest. We may also note here that the amount of non-protein nitrogen found in the blood is very close to that found in the blood of other mammals (see Bock, '17).

GRAY AND WHITE MATTER IN THE BRAIN

Since the central nervous system is composed of the so-called gray and white matter, structures which are widely different from each other, we thought it highly desirable to determine the non-protein nitrogen, as well as the nitrogen of the several organic extractives, in these two structures. For this purpose the sheep's brain was chosen on account of the ease with which fresh material could be obtained. I may here emphasize the fact that although the white matter can be obtained free from nerve cell bodies, the gray matter can not be secured without some admixture of white matter owing to the constant presence of the myelinated nerve fibers within it. We can however obtain sufficiently pure gray matter for the purpose of studying the chemical differences existing between these two fractions.

The procedure adopted was a simple one. The corpus callosum was exposed and removed, then, working from within, by means of a sharp scalpel the white matter was scraped from the gray matter of the cortex at the demarcation line which separates them. The gray matter therefore represents the entire thickness of the cortex. The method, though simple, requires practice in order to separate the two fractions quickly, so as to avoid an evaporation of water. The results of the analysis are given in table 5.

As will be seen from table 5 the percentage of nitrogen to the total solids, and also the other nitrogen values given by various organic extractives per gram of the brain solids, are always higher in the gray than in the white matter. Furthermore, the values are practically twice as great in the gray as in the white. This 2:1 relation here shown is extremely interest-

TABLE 5

Showing nitrogen content in terms of the non-proteins, the amino acids, the urea and the ammonia in the brain of sheep

SHEEP'S BRAIN	PERCENTAGE OF		MILLIGRAMS OF NITROGEN PER GRAM SOLIDS			
	Water	Nitrogen in solids	Non-protein	Free amino acids	Urea	Ammonia
Gray................	82.73	9.13	8.11	3.16	0.61	0.71
White...............	70.69	4.35	4.02	1.17	0.33	0.34

ing when we recall that in the white matter the relation between the lipoids and non-lipoids is nearly 1:1 or in other words one-half the weight of the solids given by the white matter is represented by the lipoids. Donaldson and Hoke ('05) found that in the cross surface of the medullated nerve the relation of the area of the axis cylinder to the area of the sheath is practically 1:1 in all the vertebrates studied. Recently Greenman ('13) found a similar relation in the fibers of the peroneal nerve of the albino rat and reports more recently ('17) a somewhat higher value for the lipoids. Thus my own chemical observations agree with the histological findings of others very nicely. It appears therefore certain that the nitrogen values in these two forms of nerve tissue would agree if the lipoids free solids were alone compared. This seems to show quite clearly that non-protein nitrogen is quantitatively related to proteins or active cell substance. We shall later find further evidence to support this hypothesis of a quantitative relation of non-protein nitrogen to the active cell substance as revealed by an analysis of different parts of the central nervous system.

THE NON-PROTEIN NITROGEN IN DIFFERENT PARTS OF THE CENTRAL NERVOUS SYSTEM

When we divide the central nervous system into four parts, cerebellum, cerebrum, stem and spinal cord, we are also dividing it into parts in which the content of lipoids is graded in the order named. The object of this determination was therefore to determine the quantitative distribution of the nitrogenous extractives in these four different parts in order to see whether the

relations between nitrogen values and the solids—with different lipoid contents—varies widely. Altogether seventy rats, one hundred and six days old, and twenty-five rats, one year old, have been used for this study.

Table 6 shows clearly that the number of milligrams of nitrogen per gram of solids is higher in both the cerebellum and the cerebrum than in the stem or spinal cord. Again the stem shows a higher figure than that given by the spinal cord. The same relations are found in the nitrogen values given by the amino-acids, the urea and the ammonia. We further notice that the corresponding values given by the one year old brains are definitely lower than those given by the brains one hundred and

TABLE 6

Showing the nitrogen content in the non-proteins, the amino acids, the urea and the ammonia in four different parts of the central nervous system of the albino rat

ALBINO RAT	MILLIGRAMS OF NITROGEN PER GRAM OF TOTAL SOLIDS					
	106 days old				One year old	
	Non-proteins	Amino acids	Urea	Ammonia	Non-proteins	Amino acids
Cerebellum............	8.61	3.62	0.98	0.93	7.08	2.74
Cerebrum.............	8.53	3.67	0.57	0.77	6.98	3.06
Stem.................	6.04	2.91	0.65	0.58	4.86	1.74
Spinal cord..........	5.26	2.38	0.47	0.53	4.08	1.48

six days old. If the relations already found in connection with the studies on the gray and white matter are true, and without doubt they are, we should expect that the nitrogen values should be higher in the cerebellum and cerebrum than in the stem or spinal cord; again the stem should give a higher value than the spinal cord; since the amount of the lipoids contained in these four parts relatively increases from cerebellum to spinal cord in the order given in the table. In support of this assumption the actual amount of lipoids in these four parts was determined by repeated extractions with alcohol. The extraction was carried on with boiling 95 per cent alcohol repeatedly for several hours in each case and until the alcohol showed no more the typical lipoid color, even after two hours of continuous boiling.

We find in table 7 that the percentage of lipoids to solids (at 106 days) is lower in the cerebellum and cerebrum than in the stem or spinal cord as anticipated, and furthermore, in the rats one year old there is a higher content of lipoids than in the one hundred and six days old rats, as can be inferred from the lower percentage of water and of total nitrogen.

TABLE 7

Showing the water content, the total nitrogen in solids, lipoids in solids and also nitrogen content in lipoids in different parts of the central nervous system

ALBINO RAT	106 DAYS OLD				ONE YEAR OLD	
	Water	Nitrogen to total solids	Lipoids to total solids	Lipoid nitrogen to total nitrogen	Water	Nitrogen to total solids
	per cent	per cent	per cent	per cent	per cent	per cent
Cerebellum..........	79.36	10.10	42.13	17.63	78.41	9.18
Cerebrum............	79.38	9.51	42.36	17.22	78.38	9.09
Stem................	75.16	7.75	55.03	19.90	73.14	7.06
Spinal cord..........	72.23	6.38	62.03	20.11	69.00	5.90

It is thus clear that any part of the central nervous system where the myelin, as indicated by the lipoids, is abundant, is poor in the nitrogenous extractives. It appears from this conclusion, and also from the various relations shown already in the case of the white and gray matter, that the relative content of non-protein nitrogen to the solids, from which the lipoids have been removed, may give a constant value in the several parts of the central nervous system which have been compared. To test this point table 8 was formed, based on the data given in tables 6 and 7.

As was anticipated, the relation between the protein nitrogen and nitrogen content of extractives is nearly constant. From this we may safely conclude that the lipoids probably have no relation to the nitrogenous organic extractives, and therefore we infer that the organic extractives are an index of the activity of cell substance so far as nitrogenous metabolism is concerned.

In table 8 we notice one interesting fact, that is the percentage of water is practically constant in the four different parts of the

Showing percentage of nitrogen in the various nitrogenous organic extractives in relation to the lipoid free solids (protein) in different parts of the central nervous system

ALBINO RAT 106 DAYS OLD	PERCENTAGE OF NITROGEN TO PROTEIN NITROGEN				PERCENTAGE OF WATER TO LIPOID-FREE BRAIN
	Non-proteins	Amino acid	Urea	Ammonia	
Cerebellum.................	10.34	4.35	1.17	1.11	86.92
Cerebrum...................	10.49	4.51	0.70	0.94	86.99
Stem.......................	9.72	4.68	1.05	0.94	87.06
Spinal cord................	10.33	4.67	0.93	1.04	87.26
Averages..................	10.22	4.55	0.96	1.01	87.06

central nervous system, when the water content is compared with the brain weight from which the lipoids have been removed. The percentage of water as thus determined shows a slight tendency to increase from the cerebellum to the spinal cord. This may be due to the difficulty of extracting all the lipoids from the substantia grisea—which substance in turn is relatively more abundant in the upper members of the series. The value for the water is nearly as high as that in the brain of the new born rat. Thus the water-solids relation is not significantly altered throughout life if the lipoids are excluded from consideration. This interesting phenomenon has already been noted by Donaldson ('16) in connection with the water content of the entire brain of the albino rat during growth. Donaldson came to this conclusion by comparing the water content of the brain with the myelin free solids. This constancy of water is highly interesting since the water content appears to have intimate relation to, or at least its abundance seems to indicate—the greater activity of this organ—the nervous system—in which the water content is high as compared with other organs.

DISCUSSION

The nervous system is unique when compared with other organs or tissues in its ability to hold an enormous amount of lipoids. From Koch's ('11) paper on "recent studies on lipoids" which illustrates graphically quantitative relations between

lipoids and protein in various organs and tissues, one can not fail to notice the disproportionate abundance of lipoids in the central nervous system as compared with other parts of the body.

Another equally unique character of the nervous system is its ability to accumulate the greater fraction of the lipoids outside of the cell bodies as so-called myelin substance. In most other organs the lipoids are accumulated within the cell body, and in the skeletal system also in which the accumulation of salts resembles somewhat the accumulation of lipoids in the nervous system, the deposition takes place within the osseous cells. Donaldson ('16) found that the loss of water in the nervous system is due to an accumulation of myelin, and thus the water-solid relation within the cell body is practically unaltered throughout the entire life cycle. I have also shown a like relation in the four different parts of the central nervous system. This constancy in the water-solid relation is made possible only by the unique ability of the nerve cells constantly to throw the lipoids out of the cell body and to the periphery of the axon.

Just as the percentage of water is constant when the lipoids were disregarded, so I have found a constant value for the nitrogen content given by non-proteins, the amino acids and the urea and ammonia in the different parts of the central nervous system when the lipoids are excluded from the solids. From these two facts it appears that in the central nervous system there is (1) a relatively much higher content of nitrogenous extractive bodies per gram of lipoid-free solids than in the other organs or tissues, and (2) that a constant quantitative relation exists in four different parts of the central nervous system between the nitrogen values given by various nitrogenous extractives and the protein nitrogen. We may conclude therefore that the central nervous system is always fully saturated with metabolic products and furthermore, that the substance constituting the active portion of every cell in these several parts shows a quantitative relation to the metabolic products indicating an equality in activity so far as nitrogen metabolism is concerned.

If the chief site of metabolic activity is located in the non-lipoid fraction, then what is the function of the lipoid fraction

or myelin? Mathews ('16) considers that the function of myelin is probably nutritive and certainly there is some indirect evidence favoring this view. I have however at the present moment no direct evidence on the basis of which to discuss this question, but it is my hope to extend my research into this obscure field in the future.

CONCLUSIONS

1. The number of milligrams of non-protein nitrogen per gram of solids decreases rapidly in the course of the first fifteen days of life. After the fifteenth day, the approximate time when myelin appears, and up to about one hundred and twenty days, the rate of decrease becomes much slower. After one hundred and twenty days the change is hardly noticeable. This decrease is interpreted as the result of the accumulation of myelin, a substance which probably has no direct relation to the nitrogenous metabolism (table 1 and chart 1).

2. The relative amount of organic extractives and inorganic salts to the total solids changes with advancing age in a way similar to the non-protein nitrogen. This is interpreted as meaning that the change in the non-nitrogenous organic extractives is similar to that in the nitrogenous fraction (table 2 and chart 1).

3. The number of milligrams of non-protein nitrogen per 100 grams of fresh tissue is much less in the brain than in other organs so far examined (testes, liver and kidneys). The nitrogen values given by the amino acids, the urea and the ammonia are very similar to the values given by several other organs and tissues of other mammals (tables 3 and 4).

4. Although the amount of nitrogen given by the non-proteins, the amino acids, the urea and ammonia in relation to the solids is higher in the cerebellum and cerebrum than in either the stem or the spinal cord, these nitrogen values become constant in all the four parts when they are computed in relation to the protein nitrogen. This is interpreted to mean that the nitrogenous organic extractives are intimately related with the

active cell substance and not at all with the lipoid substance (tables 6, 7 and 8).

5. The percentage of water to solids varies considerably in these four parts. However the percentage of water in the lipoid-free solids is constant in these four parts. This confirms Donaldson's conclusion that the diminution of water in the brain is due to an accumulation of the water-poor myelin, and the active protoplasm maintains its water-solid relation little altered by the age of the rat (tables 7 and 8).

6. Studies on the gray substance and the white substance of the sheep's brain show that the nitrogen values given by the total solids, non-proteins, amino-acids, urea and ammonia are practically twice as much in the gray as in the white. Since the white matter contains 50 per cent of lipoids or more we conclude that these nitrogen values should become similar in both the gray and the white, should we compare the nitrogen values with lipoid-free solids in these two structures. This assumption has not been directly tested in the sheep's brain (table 5).

7. The results of the present investigation show that the protein fraction of the central nervous system is well saturated with the metabolic products, and furthermore that the relation between metabolic products and active cell substance is quantitatively similar in all parts of the central nervous system and in both parts of the neuron. It appears possible that the degree of metabolic activity in the nervous system is greater than hitherto has been supposed.

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THE MOTOR NUCLEI OF THE CEREBRAL NERVES IN PHYLOGENY. A STUDY OF THE PHENOMENA OF NEUROBIOTAXIS

II. AMPHIBIA

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ELEVEN FIGURES

CONTENTS

INTRODUCTORY

The aims and scope of the present investigation have already been set forth in my first communication on this subject (10), of which this paper forms the second part.

After the completion of Part I, I received a copy of a most interesting thesis by H. Van der Horst (54). This author has carried out a careful research upon the motor roots and nuclei of the cerebral nerves, more especially upon the taxonomic value of these structures in the lower vertebrates. Though his extensive investigations in this field have been chiefly concerned with the true fishes, yet in addition he has studied the arrangement of the motor roots and nuclei of the brain stem in two cyclostomes (viz., Bdellostoma dombeyi and Petromyzon marinus) as well as in two amphibians (viz., Rana catesbeana and Molge cristata). Charts of the motor nuclei and roots of the cerebral nerves in all the forms studied have been reconstructed by this author with great attention to detail and are reproduced as figures I to LXXXI in his thesis. His description and chart of the motor nuclei and roots in Bdellostoma dombeyi differ in no essential way from my own, and the same may be said also with regard to his work on Selache maxima, Polydon spathula and Ameiurus nebulosus. In the case of Rana catesbeana the reconstruction chart by Van der Horst corresponds closely with my own, though some slight differences appear and will be noted subsequently in the present paper. Unfortunately a detailed description of the motor roots and nuclei of the cerebral nerves has been omitted by Van der Horst in the case of the amphibians which he studied, so that his reconstruction charts only are available for comparison in the present connection.

The motor nuclei and roots have already been studied and reconstruction charts have been made in the following anuran amphibians: Rana esculenta (Kappers, 35); Rana fusca and Bufo sp.? (Röthig, 50).[1] These charts are reproduced in the present paper in figure 9, p. 395.

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[1] Rana fusca (Rösel), appears to be but another synonym for R. temporaria, the common European Grass Frog (v. Gaupp, 25; Gadow, 22). Röthig does not give the species of Bufo which he studied but it was most probably B. vulgaris.

Of the urodele amphibians, reconstruction charts of the motor roots and nuclei have been made in the following forms: Triton (Molge) vulgaris and Siren lacertina (Kappers, 35); Crypto-branchus japonicus and Necturus maculatus (Röthig, 50). Of these, the first three (Triton, Siren and Cryptobranchus) are reproduced here in figure 10, p. 395.

MOTOR ROOTS AND NUCLEI IN RANA CATESBEANA

Second spinal nerve (hypoglossus)

The motor nucleus of the second spinal nerve (first of the adult spinal series) in Rana catesbeana shows evidence of considerable specialization and is very definitely divisible into two sub-nuclei, viz., (a) a dorso-medial cell group, and (b), a ventro-lateral cell group.

The latter cell group is directly continuous caudally with the motor cell columns of the anterior horn, of which it represents the unmodified rostral extremity. The ventro-lateral cell group does not extend rostrad as far as the exit level of the first motor rootlet of the second spinal nerve.

On the other hand, the dorso-medial cell group, though its caudal end lies in the gray reticular matrix of the somatic motor column of the cord, is throughout its extent easily distinguished from the other elements of this column. Its large multipolar cells form a compact nucleus which extends from slightly below the exit level of the last motor rootlet of the second spinal nerve, to a point some distance rostrad of the exit level of the first motor rootlet of this series (figs. 1 and 2). In figure 9 A the sagittal extent of this nucleus is indicated diagrammatically.

The dorso-medial cell group described here without doubt corresponds to the nucleus centralis of Stieda (52). The nucleus gives rise to certain motor fibers which are evidently restricted in their emergence to the ventral rootlets of the second spinal nerve, as Gaupp long ago considered probable (26).

From a study of central relations alone, it is not possible to decide whether the most rostral of the motor rootlets noted above represent remnants of the ventral root of the first spinal

nerve of the embryo. Fürbringer (21) found the first spinal
nerve (first occipito-spinal) absent in all adult opisthoglossal
anurans examined, and Gaupp (l. c.) confirms this observation
for the Ranidae which he studied. For this reason I do not
understand why Van der Horst has labelled the most rostral
rootlet of this series, the first spinal nerve in his reconstruction
of this region in Rana catesbeana (l. c., fig. LXXXI).
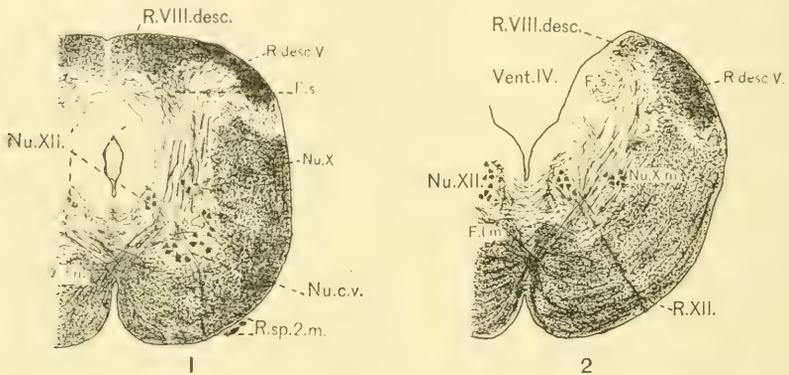
Fig. 1 Rana catesbeana. Transverse section through the medulla caudad
of the calamus. Abbreviations: *F.l.m.*, fasciculus longitudinalis medialis;
F.s., fasciculus solitarius; *Nu.c.v.*, nucleus cornu ventralis; *Nu.X.*, two cells of
the caudal end of the motor vagus column; *Nu.XII.*, dorso-medial cell group
(nucleus hypoglossus); *R.desc.V.*, descending trigeminal fibers in dorsal funiculus;
R.sp.2 m., motor rootlets of second spinal nerve; *R.VIII. desc.*, descending acustic
fibers in dorsal funiculus.

Fig. 2 Rana catesbeana. Transverse section through the medulla at the
level of the rostral end of the hypoglossal nucleus. Abbreviations: *Nu.X.m.*,
motor vagus nucleus; *R.XII.*, hypoglossal rootlet. Other abbreviations as in
figure 1.

It is evident from the above description of the extent of the
dorso-medial nucleus, that it must represent the chief if not the
sole place of origin for the most rostral motor rootlets of the
second spinal nerve. The more caudal motor rootlets of this
nerve take their origin from both dorso-medial and ventro-
lateral cell groups of the cervical somatic motor column.

The dorso-medial cell group is believed to represent the hypo-
glossal nucleus of higher forms for reasons which will be consid-
ered subsequently. Thus in figure 2, the nucleus in question is

labelled *Nu.XII* (hypoglossal nucleus) and the rootlet arising from it is labelled *R.XII* (hypoglossal root), while in figure 1 the corresponding rootlets are labelled simply *R.sp. 2 m.* (motor root of second spinal nerve), since at this level they have their origin in both dorso-medial and ventro-lateral areas.

A similar arrangement of cell groups obtains in Rana esculenta (Kappers) and in Bufo (Röthig), though these authors have not indicated the extent of the dorso-medial cell group in their reconstruction charts as I have done in Rana catesbeana (cf. fig. 9). The relations of the cell groups described above are essentially similar in the brain stem of Rana pipiens, of which I have recently examined several series stained by different methods.

The arrangement of the elements in the rostral end of the somatic motor column of the cord in urodele amphibians presents a marked contrast to the conditions obtaining in this region in Bufo and Rana. In adult urodeles the motor roots of the first spinal nerve (first occipito-spinal) are regularly represented (Fürbringer, l.c.) and the rostral end of the cervical motor column shows but slight, if any signs of differentiation from the more caudal segments of this region (cf. Kappers, 35 and Kingsbury, 40).

Nerves IX and X

In describing the IX-X complex in late larval stages of frogs, Strong has distinguished five roots (53). A motor component was lacking in the first of these roots (most rostral). The second and third roots were attached very close together and sometimes an intermediate rootlet could be distinguished. Motor components were present in both these roots but in addition to the visceral sensory fibers, Strong identified a well marked general cutaneous component in the third root. The fourth root was separated from the third by a considerable interval and contained but two components, viz., visceral sensory and motor. The fifth and last root emerged some distance caudal to the fourth and "seems to derive its fibers from one source only" (l. c., p. 137). Strong considered this root to be purely motor in character.

In the adult medulla of Rana catesbeana the most rostral
motor root of the IX-X series was taken to represent the motor
component of the IX nerve and has been so named in figures 3
and 9 A. It should be noted, however, that a well marked gen-
eral cutaneous component is to be observed entering the me-
dulla on the exit level of the motor IX root in figure 3. In this
respect the relations which obtain here would seem to differ
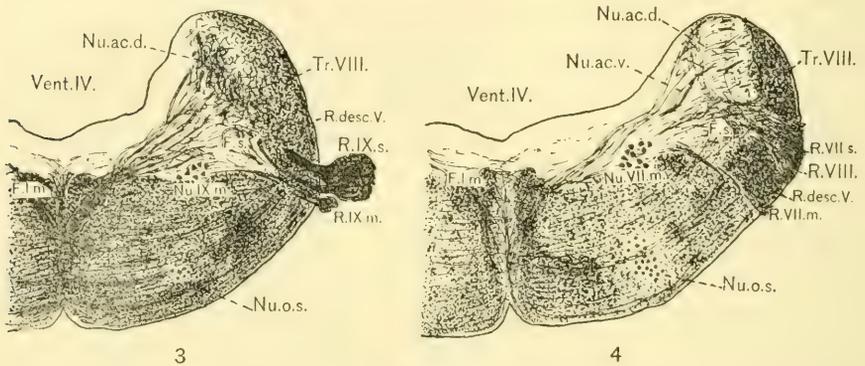
Fig. 3 Rana catesbeana. Transverse section through the medulla at the
exit level of the motor glossopharyngeal root. Abbreviations: *Nu.ac.d.*, dorsal
acustic nucleus; *Nu.o.s.*, superior olive; *Nu.IX.m.*, motor glossopharyngeal nu-
cleus; *R. desc.V.*, radix spinalis trigemini; *R.IX.m.*, motor glossopharyngeal root;
R.IX. s., sensory glossopharyngeal root; *Tr.VIII.*, area acustica alba; *Vent. IV.*,
fourth ventricle. Other abbreviations as before.

Fig. 4 Rana catesbeana. Transverse section through the medulla at the
exit level of the motor facial root. Abbreviations: *Nu.ac.v.*, ventral acustic
nucleus; *Nu.VII.m.*, motor facial nucleus; *R.VII.m.*, motor facial root; *R.VII.s.*,
sensory facial root fibers; *R.VIII.*, acustic root fibers. Other abbreviations as
before.

from those described by Strong in larval forms, probably as the
result of a fusion of the roots which he has designated second and
third.

The most caudal vagus rootlets in R. catesbeana are wholly
motor and emerge as fine strands to reach the periphery along a
line somewhat more ventrally placed than the line of emergence
of the rostral roots.

The motor X nucleus lies on the periphery of the periependy-
mal gray beneath the sulcus limitans in the caudal third of the

medulla. Rostrally its limit is not sharply defined but it certainly terminates some distance caudal of the exit level of its first emergent rootlet. Caudally the motor X nucleus extends well below the calamus but in this region its continuity is interrupted and it is represented by scattered cell clusters which may be traced backwards to the end of the second cervical segment. In figure 9 A the rostral and caudal limits of the motor X nucleus have not been definitely marked on account of these peculiarities. The relations of the motor X nucleus at the exit level of the first motor rootlet of the second spinal nerve are indicated in figure 2. At the level indicated in figure 1, two cells apparently belonging to this nucleus can be distinguished just dorsal to the ventro-lateral cell group of the anterior horn.

Between the motor IX and X nuclei a very definite hiatus occurs. In transverse section the former nucleus occupies a position exactly analogous to that of the caudally placed motor X nucleus. The motor IX nucleus extends approximately from the exit level of its own motor root, caudally to the level of emergence of the first vagus motor rootlet. The emergent motor IX fibers pass laterad and make their exit below the corresponding sensory root (cf. figs. 3 and 9 A).

The hiatus between the motor IX and X nuclei in R. catesbeana has also been recorded by Van der Horst (l. c., fig. LXXXI). In R. esculenta on the other hand, these two nuclei appear to be continuous with one another (Kappers). According to Röthig the latter condition also obtains in R. fusca and in Bufo (cf. fig. 9).

It is of interest to note at this point that in the 38 mm. larva of Amblystoma tigrinum, Herrick has shown that the nucleus ambiguus (motor IX-X nucleus) is placed in relation to its emergent roots in a position practically identical with that obtaining in R. esculenta, R. fusca and Bufo (v. Herrick, 29, fig. 1).

Nerve VII

The motor VII nucleus in R. catesbeana is situated on the periphery of the periependymal gray and extends partly over the level of its root exit and for a short distance caudal to this

point.   This small nucleus lies below the poorly defined sulcus limitans in a situation similar to that of the more caudally placed motor IX nucleus (figs. 4 and 9 A).

The motor VII fascicles first pass slightly rostrad from their nucleus of origin and then curve latero-ventrad to emerge below the radix spinalis trigemini.   These relations are indicated in figure 4, in which the arrangement of the acustic and the sensory VII roots are also to be seen.

It is of interest to note that the acustic fibers enter the brain stem for the most part dorsad of the sensory VII root as Strong (53) has shown to be the case in the tadpole stage.   In the larval Amblystoma on the other hand the reverse is the case and the two VIII roots enter the medulla chiefly ventral to the visceral sensory VII (Herrick, 29), while in the adult Amblystoma the visceral sensory VII enters the medulla midway between the dorsal and ventral VIII roots (Coghill, 16).

The relations of the motor VII nucleus described above closely approximate those of this nucleus in R. esculenta, R. fusca and Bufo, though in the last mentioned animal the motor VII nucleus lies wholly caudad of its root exit level.   On the other hand, in all adult urodeles examined the motor VII nucleus forms the rostral end of a cell column which includes within its limits all the motor perikaryons of the VII-IX-X nerves (figs. 9 and 10).

The arrangement of the motor VII-IX-X muclei in adult urodeles is thus strikingly similar to the arrangement of these nuclei in selachians, ganoids and dipnoans (cf. figs. 10 and 11). On this account the members of each of these animal groups are characterized by the presence of a relatively long ascending emergent motor VII root whose relations in Necturus were first adequately described by Kingsbury (40).   In contrast to this, the situation of the motor VII nucleus approximately on the level of its root exit as in adult anurans, is not paralleled elsewhere among the Ichthyopsida except in cyclostomes.

In this connection it is important to note that the motor VII nucleus in the 38 mm. larva of Amblystoma tigrinum, though situated approximately on the level of its root exit, is separated

by but a very slight interval from the motor IX-X column
(v. Herrick, l. c., fig. 1).

Nerve VI

In R. catesbeana three very fine roots emerge in series from
the ventral periphery of the brain stem, approximately midway
between the exit levels of the motor IX and VII roots. Three
rootlets of this nerve were also distinguished by Kappers in R.
esculenta but Röthig has only indicated two rootlets of this
nerve in his reconstructions of R. fusca and Bufo (fig. 9). The
superficial attachment of the abducens rootlets appears to lie
nearer to the exit level of the motor VII root in R. catesbeana
than in any of the other anurans thus far recorded. In Van der
Horst's reconstruction, however, the three abducens rootlets
are represented in a position very similar to that which they
occupy in R. esculenta. As I am at present unable to verify
my records by an examination of the original material, this
point must remain in doubt but some individual variation in the
superficial origin of this nerve would not be surprising. Gage
(23) long ago noted that the superficial origin of the abducens
nerve appeared to be variable in different amphibians.

The nucleus from which the abducens rootlets arise is very
diffusely arranged, and is with difficulty distinguished from the
neighboring coördination elements of the motor tegmental
nucleus. For this reason the outline of the nucleus in figure
9 A represents only the approximate area in which its cells are
scattered. In his reconstructions Kappers has already drawn
attention to this point (33).

The cells of the abducens nucleus are arranged around the
fasciculus longitudinalis medialis and are intimately associated
with its fibers. The nucleus is also traversed by many fine and
coarse medullated fibers from the homolateral acustic area. A
similar intimate association of the acustic area and the abducens
nucleus is evident in urodeles as well as in all anurans, and in
the larval Amblystoma Herrick has described the course of a
special fascicle from the area acustico-lateralis via the fasciculus
longitudinalis medialis to the abducens nucleus.

In all adult amphibians examined the abducens nucleus occupies a position in close association with the elements of the periependymal gray. In Amblystoma at least, this is a larval character (Herrick, 1. c.) and there is no reason to suppose that the nucleus in question is more ventrally situated at any time in other amphibian larvae. Thus, though the adult topographical relations of the amphibian abducens nucleus closely resemble those of selachians (cf. figs. 9, 10 and 11), yet the ontogenetic history of the nucleus appears to differ in these two vertebrate groups; for Kappers has already pointed out in this connection that for a time during the development of selachian larvae the abducens nucleus in these animals is quite ventral in its position (33).

Note on the nucleus olivaris superior

In R. catesbeana a circumscribed nuclear mass appears in the ventral area of the reticular formation of the medulla and extends in this position over the area included between the exit levels of the motor IX and VII roots (figs. 3, 4 and 9 A). In anurans this nucleus has been considered as the representative of the superior olive of higher forms by Gaupp (26), Kappers (33) and others. The nucleus in question has been represented in the reconstruction charts in all forms in which it occurs in order to increase the number of landmarks for comparison.

The connections of this nucleus are as yet imperfectly understood, but Kappers has pointed out that it is a derivative of the acustic area which secondarily becomes displaced ventrally. It is significant also to note that the area in which the anuran superior olive becomes differentiated, corresponds exactly to that in which Herrick's tractus octavo-tectalis et thalamicus (bulbar lemniscus) courses in the larval Amblystoma brain.

Of further interest is the fact that the superior olive is not differentiated as a definitely circumscribed nuclear mass in any vertebrate in which the lateral line organs are functionally developed in the adult. Thus among amphibians the superior olive is present only in anurans.

In addition, in the latter animals the lagena becomes further constricted off from the sacculus than in urodeles, and the papilla acustica basilaris lagenae which appears for the first time in phylogeny in amphibians, attains a higher stage of development in anurans than in urodeles (Retzius, 48; Wiedersheim, 58).

Nerve V

The motor V nucleus in R. catesbeana lies a short distance rostrad of the motor VII nucleus and occupies a similar position
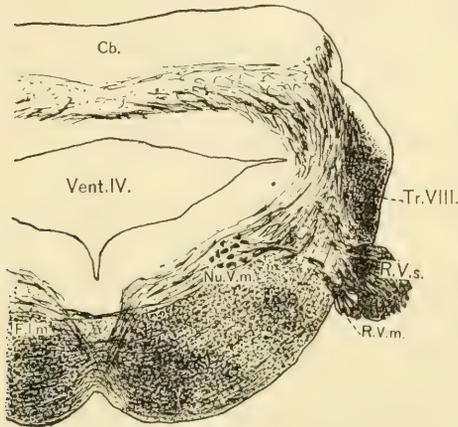
Fig. 5 Rana catesbeana. Transverse section through the medulla at the exit level of the motor trigeminal root. Abbreviations: *Cb.*, cerebellum; *Nu.V. m.*, motor trigeminal nucleus; *R.V.m.*, motor trigeminal root; *R.V.s.*, sensory trigeminal root. Other abbreviations as before.

in the periependymal gray. The motor V nucleus is at least double the size of the motor VII nucleus and its limits are more sharply circumscribed. It extends approximately from the rostral border of the emergent motor V root to that of the motor VII root, so that its chief bulk lies caudad of its root exit. The motor fibers arising in the caudal part of the nucleus course a short distance rostrad as separate strands through the nucleus and then, together with those arising further forward, pass laterad and slightly ventrad in small fascicles to emerge below the large sensory trigeminus root (fig. 5).

The relations of the motor V nucleus and its emergent root in R. esculenta and R. fusca are essentially similar to those obtaining in R. catesbeana. In Bufo, however, the rostro-caudal extent of the motor V nucleus is somewhat greater than in the Ranidae examined, though its position relative to the motor VII nucleus remains practically unchanged (fig. 9).

In urodeles the motor V nucleus is apparently not so large nor so sharply delimited from the motor tegmental elements as in anurans. The relation of the nucleus to the exit level of its root is subject to some variation among urodeles (v. fig. 10). In all cases, however, in both urodeles and anurans, the motor V nucleus is situated dorsally and lies in the periphery of the periependymal gray beneath the poorly defined sulcus limitans.

Nerves III and IV

In R. catesbeana the trochlear nucleus consists of a well marked and definitely circumscribed collection of multipolar perikaryons which lie beneath the periependymal Sylvian gray in a trough-like excavation of the fasciculus longitudinalis medialis, on a level rostrad of the ganglion interpedunculare. The trochlear nucleus lies immediately caudad of the oculomotor nucleus, from which it is separated however by a small but distinct space. In the specimen from which the reconstruction chart figure 9 A was prepared, the trochlear nucleus was slightly more than half the length of the oculomotor nucleus, and the motor perikaryons which compose both nuclei were of similar size and morphology.

The fibers of the trochlear nerve are collected on the lateral aspect of the nucleus and pass first laterad and then dorsocaudad to reach the superior medullary velum. In the latter structure the trochlear decussation occurs and the nerves then emerge on either side of the mid-dorsal line (v. figs. 6 and 7). It is possible that some of the fibers entering the trochlear nerves at their emergence are uncrossed and are derived, as McKibben (43) has suggested in Necturus, from the mesencephalic nucleus of the trigeminal nerve.

The oculomotor nucleus in R. catesbeana is a large well developed structure which extends over the level of its root exit and for some distance rostrad of this. The motor perikaryons are placed for the most part dorso-mediad of the bundles of the fasciculus longitudinalis medialis, though many of them may be seen lying between separated bundles of this tract
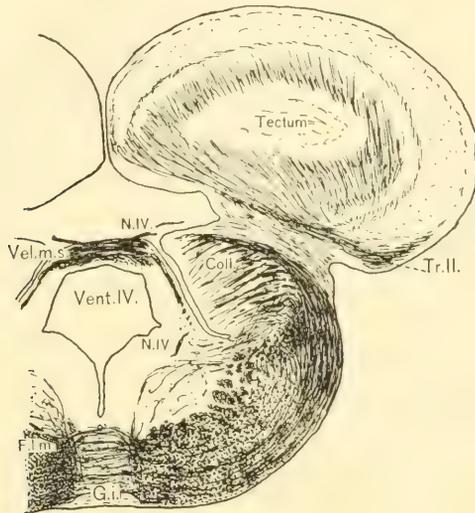
Fig. 6 Rana catesbeana. Transverse section through the brain stem at the level of the trochlear root exit. Abbreviations: *Coll.*, colliculus; *G.i.*, interpeduncular ganglion; *N.IV.*, trochlear nerve; *Tr. II.*, posterior root of optic tract; *Vel.m.s.*, superior medullary velum. Other abbreviations as before.

(v. fig. 8). The nucleus extends laterally for some distance and a distinct tendency toward subdivision into medial and lateral cell groups is evident. This phenomenon has already been noted by Kappers in R. esculenta (35).

The oculomotor fibers emerge in numerous fascicles which pass ventrad and slightly laterad through the base of the midbrain. Some of the most caudal oculomotor fibers appear to arise from the heterolateral nucleus, but this relation was not demonstrated with certainty.
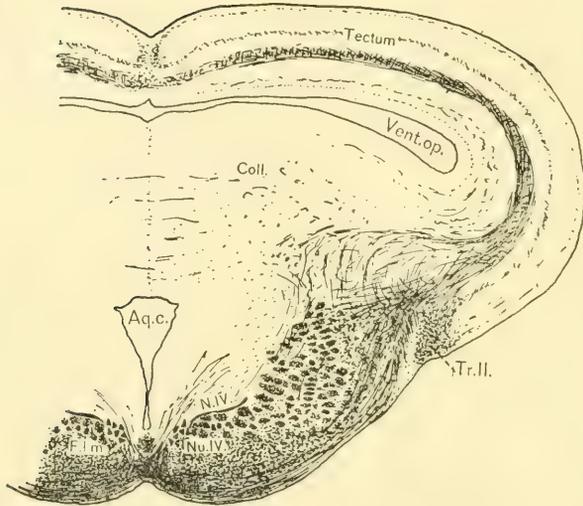
Fig. 7 Rana catesbeana. Transverse section through the brain stem at the level of the trochlear nucleus. Abbreviations: *Aq.c.*, iter; *Nu.IV.*, trochlear nucleus; *Vent. op.*, optocoele. Other abbreviations as before.
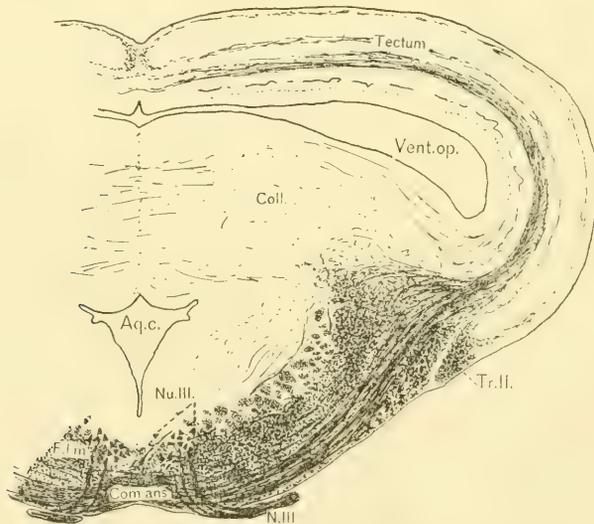
Fig. 8 Rana catesbeana. Transverse section through the brain stem at the level of the oculomotor root exit. Abbreviations: *Com.ans.*, ventral tegmental commissure; *N.III.*, oculomotor root; *Nu.III.*, medial and lateral moieties of the oculomotor nucleus. Other abbreviations as before.

In general it may be said that the relations above described in R. catesbeana are characteristic of all anurans yet examined. In modification of this statement, however, it must be noted that the size of the trochlear nucleus among these forms is subject to some variation, although in this its relation to the oculomotor nucleus remains practically unaffected.

The condition of the oculomotor and trochlear nuclei and roots in urodeles presents a marked contrast to that obtaining in anurans. In the adult Triton examined by Kappers (35), though the small nuclei and roots in question present relations essentially similar to those in anurans, yet the nuclear differentiation in this animal was much inferior to that in Rana. Further, Van der Horst found that the trochlear nucleus in Molge cristata was placed far behind the oculomotor nucleus and lay about midway between the exit levels of the oculomotor and trochlear roots, in the Sylvian gray.

In Siren, Kappers (l. c.) could discover neither trochlear root nor nucleus, though subsequently Norris (46) has been able to trace the peripheral course of this much reduced nerve in all his specimens. In Necturus and Cryptobranchus, Röthig (50) was unable to distinguish with certainty the trochlear nucleus, though he identified the nerve at its point of emergence in both these animals.

In Siren (Kappers), Cryptobranchus and Necturus (Röthig), the oculomotor nucleus is very small and poorly differentiated from the neighboring periependymal gray, and it lies on the level of its root exit. In Siren the oculomotor root emerges but a very short distance rostrad of the exit level of the motor V root. In general, among the urodeles examined the distance between the rostral border of the emergent oculomotor root and that of the motor V, tends to be reduced when compared with anurans (cf. figs. 9 and 10).

DISCUSSION

1. Hypoglossal complex

In the foregoing description of the nucleus of the second spinal nerve in R. catesbeana, and in the subsequent comparison of the morphology of this area in anurans and urodeles, one very striking feature became apparent, viz.: that in all opisthoglossal anurans examined there is developed a very well circumscribed dorso-medial cell group in the rostral end of the cervical somatic motor column, while in all urodeles examined no such circumscribed nucleus is to be distinguished in this area.

In an enquiry into the significance of the difference in the development of this cell group in the members of these two amphibian orders, it is necessary to consider first whether or not the plan of peripheral distribution of the motor fibers of the first two spinal nerves is fundamentally different in anurans and urodeles. In this connection Rana is taken as representative of opisthoglossal anurans. Triton, Siren, and Cryptobranchus have been selected among urodeles because in these forms the motor nuclei have been studied from the point of view of the present investigation and reconstruction charts are available for comparison (fig. 10).

Oculomotor root and nucleus

Trochlear root and nucleus

Trigeminus motor root and nucleus

Abducens nucleus

Facial motor root and nucleus

Glossopharyngeus motor nucleus

Vagus motor nucleus

Spino-occipital motor roots and nucleus

Nu.olivaris superior

Nu.paramedianus
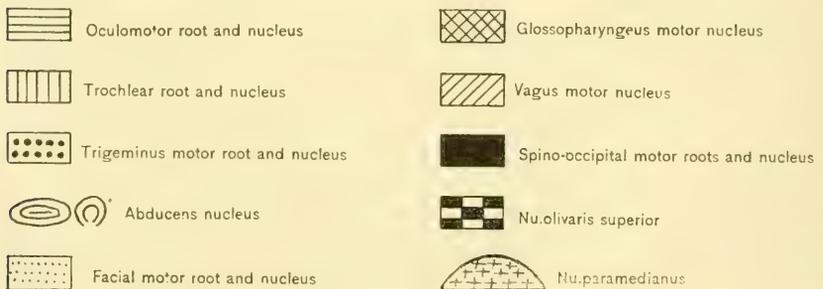
Fig. 9   Reconstruction charts of motor roots and nuclei in anurans.   A, Rana catesbeana; B., R. esculenta (after Kappers); C, R. fusca (after Röthig); D, Bufo (after Röthig).   Abbreviations: *VI*, abducens roots; *IX*, motor glossopharyngeal root; *X*, motor vagus root; *XII*, most rostral cervical somatic motor rootlet (hypoglossus).   The arrow indicates the site of the calamus.   See diagram above for explanation of signs.
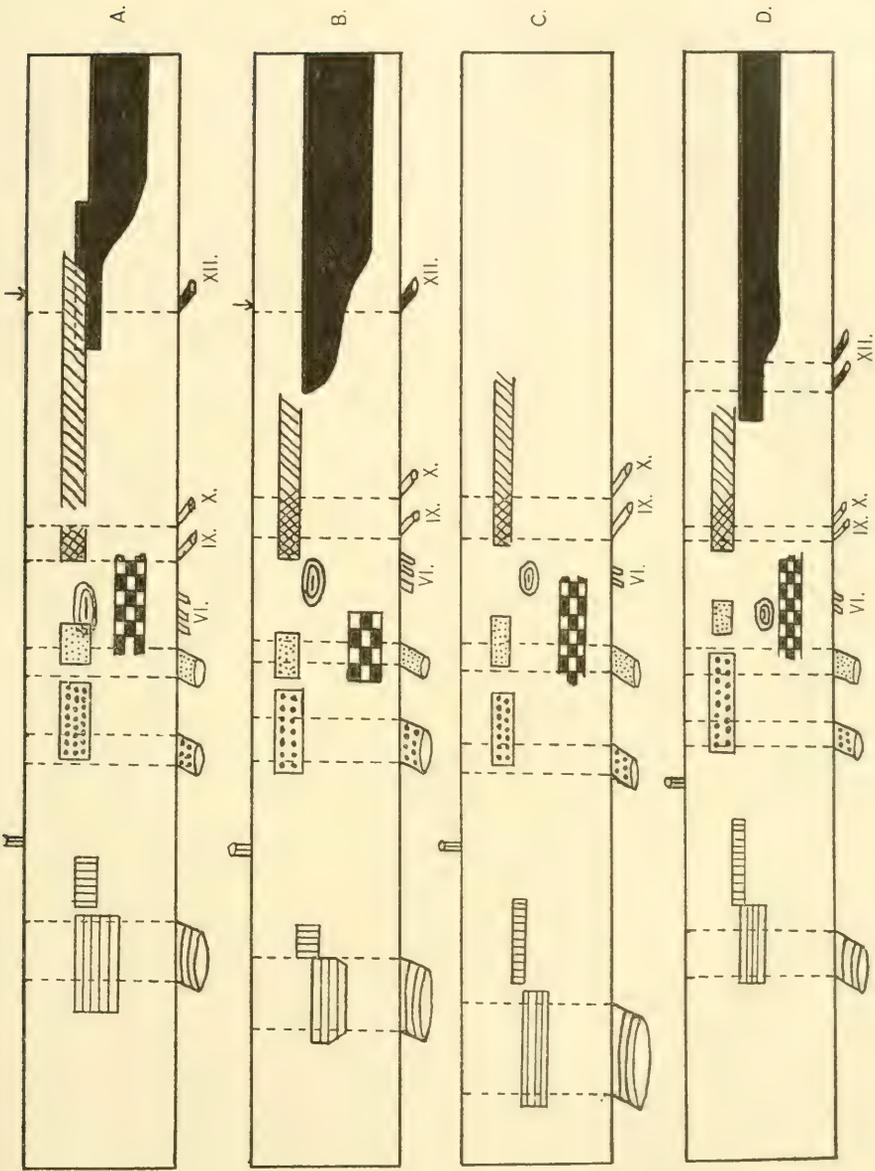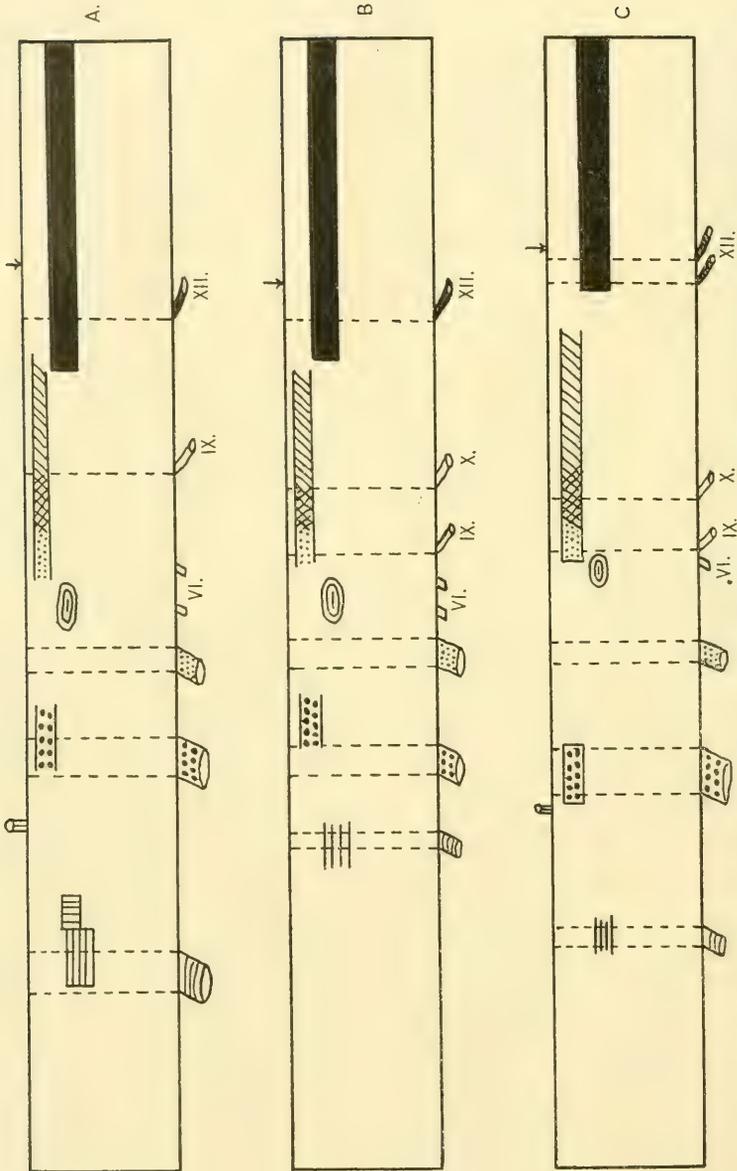
Fig. 10 Reconstruction charts of motor roots and nuclei in urodeles. A, Molge (Triton) vulgaris (after Kappers); B, Siren lacertina (after Kappers); C, Cryptobranchis japonicus (after Röthig). Signs and abbreviations as in figure 9.
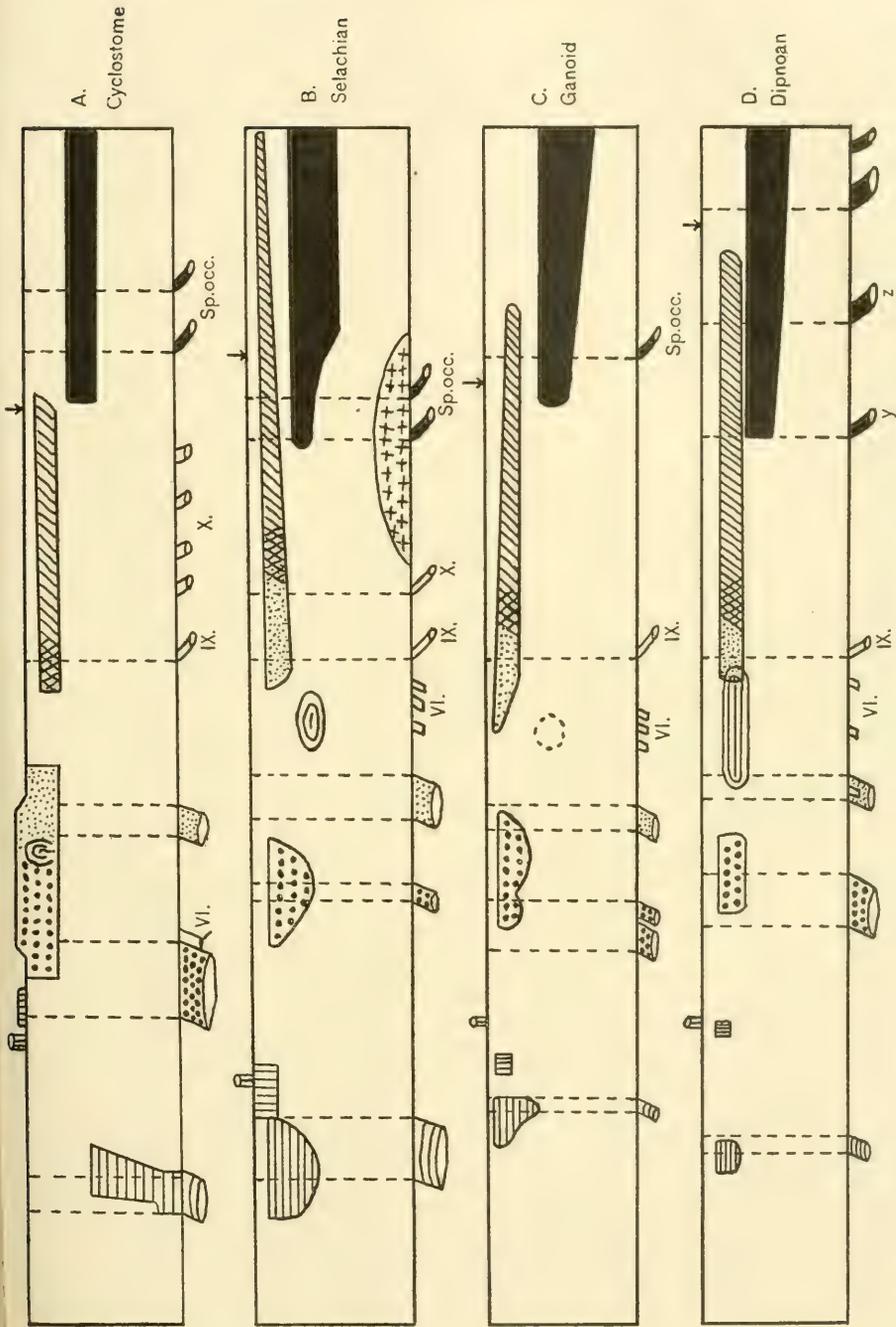
Fig. 11 Reconstruction charts of motor roots and nuclei in lower ichthyopsidans for comparison with amphibians. A, Petromyzon fluviatilis (after Kappers); B, Selache maxima (10); C, Polydon spathula (10); D, Neoceratodus forsteri (after Van der Horst). Except in the caudal position of the trochlear nucleus in this form, the motor nuclear pattern in Neoceratodus is almost identical with that in the crossopterygians, Polypterus and Calamoichthys (v. Van der Horst, 54). *y, z* and *sp.occ.,* = spino-occipital rootlets. Other signs and abbreviations as before.

The first spinal nerve is absent in adult Rana and according to Gaupp the dorsal ramus of the second spinal nerve sends motor branches to the m. intertransversarius capitis superior and to the m. longissimus. The ventral ramus of this nerve has a wide distribution by means of five chief branches: (1), communicating branch to the sympathetic; (2), large branch to the m. intertransversarius capitis inferior; (3), ramus thoracicus superior anterior to m. levator scapulae superior, m. levator scapulae inferior and sometimes to m. rhomboideus anterior; (4), communicating branch to brachial plexus; and (5), a large trunk termed the nervus hypoglossus which innervates the following muscles: sterno-hyoideus, hyo-glossus, genio-hyoideus and genio-glossus.

It is unfortunate that Coghill (17) has not given a detailed description of the distribution of the first two spinal nerves in Triton taeneatus (s. Molge vulgaris), but in his paper it is implied that the arrangement of these nerves is similar to that obtaining in Amblystoma. In the latter animal (Coghill, 16) the dorsal ramus of the first spinal nerve sends motor branches to the m. extensor dorsi communis, while its ventral ramus gives off numerous fibers to the m. intertransversalis, fibers to the m. basiscapularis (levator scapulae inferior) and then apparently fuses with the ventral ramus of the second spinal nerve. The motor fibers of the dorsal ramus of the second spinal nerve are distributed in a manner essentially similar to those of the first dorsal ramus. The ventral ramus of the second spinal nerve gives off fibers to the m. intertransversalis, m. thoraci-scapularis and sterno-hyoideus before its junction with the corresponding ramus of the first nerve. The resulting trunk innervates the remainder of the m. sterno-hyoideus and the m. genio-hyoideus. No further mention is made of the distribution of this hypoglossal trunk to other muscles, but Drüner (18) states that all pre-hyal hypobranchial spinal muscles are innervated from this source and Kallius (32) has described both a genio-glossus and a small hyo-glossus muscle in Triton.

Drüner (l.c.) has noted the presence of a well developed spino-occipital nerve in Triton which emerges through a sepa-

rate foramen below the occipital condyle and fuses ventrally with the ventral ramus of the first spinal nerve. Spino-occipital nerves were also observed by this author in Menopoma (Crypto-branchus alleganiensis) and Salamandra. Fürbringer (21) noted a similar condition in a specimen of Cryptobranchus japonicus, though according to this author spino-occipital nerves are not commonly present in adult urodeles. With the exception of the occurrence of a spino-occipital nerve in some species, Coghill concluded that the formation of the hypoglossal nerve by the junction of the ventral motor rami of the first two spinal nerves, is the usual arrangement among urodeles (16).

In Siren lacertina (Norris, 46) the ventral ramus of the first spinal nerve joins that of the second spinal nerve to form the hypoglossal trunk. The latter nerve is distributed posteriorly to the three anterior segments of the m. abdomino-hyoideus, to the m. sterno-hyoideus and to the m. omo-hyoideus. Anteriorly the nerve innervates the m. genio-hyoideus. No genioglossus muscle was found in Siren and no mention is made of a m. hyo-glossus. A branch from the ventral ramus of the second spinal nerve to the brachial plexus is described.

From this short summary it appears that the hypoglossal trunk is formed in much the same way in each of the urodeles described, though in the case of Amphiuma, Norris (45) notes that the hypoglossal nerve is formed entirely by the ventral ramus of the first spinal nerve. On the other hand, the hypoglossal trunk in anurans is derived solely from the ventral ramus of the second spinal nerve.

Whether the amphibian hypoglossus is formed by the ventral ramus of the first spinal nerve alone, by the union of this trunk with that of the second spinal nerve or only by the ventral ramus of the second spinal nerve, the plan of peripheral distribution is very similar in all cases and the musculature which it innervates is represented with but few exceptions in both urodeles and anurans.

Thus, the differentiation of a circumscribed dorso-medial nucleus in the rostral end of the somatic motor column of the cord in opisthoglossal anurans cannot be due to the possession

by these animals of a musculature which is unrepresented in urodeles. Rather would the nucleus in question appear to be the direct expression within the central nervous system of opisthoglossal anurans of the peculiar specialization of certain muscles (i.e., m. genio-glossus and m. hyo-glossus) which in other amphibians are more primitively arranged.

Kallius (32) has shown that the anuran tongue in development is primarily muscular and secondarily glandular, while in the urodele tongue the reverse is the case. In both orders the genio-glossus and hyo-glossus muscles are represented, but in anurans these muscles develop within the substance of the tongue to form a relatively complex intrinsic prehensile mechanism.

According to Gaupp (28) the genio-glossus muscle, by means of its specialized arrangement, is responsible for the complex protractor and forward rotator prehensile movements of the frog's tongue, while the hyo-glossus muscle is concerned only in the relatively simple act of retraction of this organ. On the other hand, among urodeles the genio-glossus muscle is primarily developed as a mechanism to aid in the prompt secretion of the tongue glands and not to produce movements of the whole organ. For this reason in origin, insertion and in disposition of fibers the intrinsic tongue musculature of urodeles is more simply arranged than in the frog (Kallius, 32).[2]

For the first time in phylogeny the tongue in certain amphibians (e.g., Rana) has become developed as a muscular prehensile organ. Further, a definite sub-nucleus (the dorso-medial cell group) appears within the motor segment from which the tongue musculature receives its innervation only in those amphibians in which the intrinsic prehensile muscle complex is highly developed (e.g., opisthoglossal anurans). It is therefore reasonable to regard the dorso-medial cell group of Rana as a

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[2] I do not know whether the intrinsic musculature of the tongue in Spelerpes is relatively more highly developed than in other urodeles, and I have been unable to find an adequate description of the tongue mechanism of this form in the literature at my disposal. It would appear, however, that aside from the specialized protractor and retractor mechanism, the action of the intrinsic tongue musculature does not play such an important rôle in prehension as does the viscid glandular secretion of this organ.

nucleus especially concerned in the innervation of the intrinsic tongue musculature. According to this interpretation the dorso-medial cell group would consist of motor perikaryons whose axones innervate the protractor, rotator and retractor muscles of the tongue, together with certain coordination neurones, and the whole complex would be essentially homologous with the hypoglossus nucleus of higher forms.

Though the position of the hypoglossal nucleus in Rana is in many respects similar to that of the hypoglossal nucleus of higher forms, the factors which determine its location would appear to differ radically in the two cases.

Kappers (34) has already pointed out that the ontogenetic dorsal migration of the hypoglossal nucleus in mammals is probably to a great extent due to the fact that in these forms the tongue has become the chief organ bearing taste buds, and for this reason its reflexes are largely dominated by sensory impulses from the IX-X terminal nuclei. Thus in mammals the ontogenetic migration of the hypoglossal nucleus is in the direction of the most important center acting upon it reflexly, in accordance with the first concept of neurobiotaxis (v. 10 and 39).

Since the function of the taste organs upon the frog's tongue manifestly cannot be exercised until the food reaches this organ, the influence of taste impressions in initiating the prehensile tongue reflex in this animal need not be considered. On the other hand, the whole complex series of movements by which the frog catches his prey (of which the reflex action of the intrinsic tongue musculature is an integral part) is almost exclusively initiated as the result of visual impressions (v. Yerkes, 62, 63). Further, the protractor and retractor action of the tongue musculature is brought into play in the closing phase of every complete respiratory cycle in the frog, so that these muscles act in a coordinate manner with the other muscles of the respiratory mechanism (vide infra). The reflex action of this mechanism is to a great extent initiated by afferent impulses from the mucosa of the bucco-pharyngeal area (v. Baglioni, 5) and the hypoglossal nucleus of the frog receives many

fine internal arcuate fibers from the communis area. Thus, though many arcuate fibers reach this nucleus from other sources (especially from the acustic area of the medulla), it would appear probable that its dorsal position is chiefly determined through the influence exerted upon it by the tecto-bulbar constituents of the fasciculus longitudinalis medialis and by arcuate efferents from the homolateral fasciculus solitarius.

In addition to the specialization of a hypoglossal nucleus in the most rostral segment of the cervical motor column in the frog, evidence is also to be adduced indicating a rostral displacement of the whole somatic motor column in opisthoglossal anurans as compared with urodeles.

In opisthoglossal anurans, the first spinal nerve of the adult is in reality the second nerve of the series, the first having been lost at metamorphosis (v. Fürbringer, l.c.). In urodeles, however, this is not the case and the first nerve of the larva is retained in the adult, while in not a few of these animals the last spino-occipital nerve is also functional in the adult.

Notwithstanding the reduction of the rostral cervical nerves, the first motor cervical rootlet in the adult opisthoglossal anurans examined emerges either on the same level as the first motor cervical rootlet in the urodeles charted, or even on a more rostral plane. Thus, in Triton where the last spino-occipital nerve is frequently retained in the adult, the distance between the exit level of the first cervical motor rootlet and that of the motor VII root is practically the same as this distance in R. catesbeana and R. esculenta, and even greater than the corresponding distance in Bufo (v. figs. 9 and 10).

It is evident therefore that the reduction of the spino-occipital and occipito-spinal elements in the ontogeny of anurans is accompanied by a rostral migration of the whole cervical motor column, together with a rostral displacement of its emergent motor roots, so that in this respect these amphibians form no exception to the general rule observed elsewhere in other ichthyopsidans examined (10).

Visceral motor nuclei

In amphibians all the viscero-motor nuclei (V, VII, IX and X) are dorsally placed within the brain stem. A dorsal position is also characteristic of these nuclei in petromyzonts, selachians, ganoids and according to Van der Horst (54) in dipnoans also (cf. figs. 9, 10 and 11). This condition as already noted (10), is to be considered a primitive feature in the motor nuclear pattern of these forms.

In respect to the grouping of the viscero-motor nuclei in their relation to one another and to their emergent roots, two major types of nuclear pattern evidently obtain among adult amphibians. These types are apparently of ordinal importance, for in all anurans examined the motor VII nucleus is much more closely related to the motor V nucleus than to the motor IX-X complex, while in all adult urodoles examined the reverse is the case and the motor VII nucleus is placed far caudad of the motor V nucleus and forms the rostral component of the caudal viscero-motor column. In the case of the larval Amblystoma, however, it has been already noted that the motor VII nucleus, although situated immediately rostrad of the motor IX-X nucleus, is not directly continuous with the latter (Herrick, l.c., fig. 1).

In considering the causes underlying the arrangement of the motor nuclei in cyclostomes and fishes, it became evident that the most important influences determining the relations of the motor VII-IX-X nuclei were exerted respectively by the visceral effector mechanism of the jaws and branchial region and by the visceral sensory centers. It will be of interest therefore to inquire whether these factors exert an equally important influence upon the viscero-motor nuclei in amphibians also.

Kappers (35) has already noted the striking similarity of the motor VII-IX-X nuclear pattern in Siren and Triton to that obtaining among sharks. He was inclined to ascribe the similarity of pattern in Siren and sharks to the fact that, since both are gill respiring forms without opercula, the caudal position of the motor VII nucleus and its fusion with the motor nuclei of

the gill arch nerves had in each case been similarly determined by the influence of the communis center. However, in the case of Triton, though gill respiration is lost in the adult just as completely as it is in anurans, yet the motor VII-IX-X nuclei are arranged in essentially the same manner in this animal as in Siren. Kappers concluded that in spite of the absence of gill respiration, the selachian arrangement of the motor VII-IX-X nuclei in Triton was determined by the position of the visceral sensory VII nucleus within the communis area, and he drew further attention to the equal development of this area in both Siren and Triton. In addition he pointed out that the VII gustatory components in Siren and Triton were of greater importance than in Rana, and considered that this circumstance might furnish an explanation of the strong influence of the communis area upon the motor VII nucleus in urodeles.

In comparison with ganoids and teleosts, the number of taste organs in amphibians is much reduced and, though it is probable that taste buds do exist on the skin in some larval amphibians (v. Coghill, 16, 17; Drüner, 18), they are entirely confined to the mouth and pharynx in adults. In considering the distribution of taste buds in different classes of vertebrates, Kappers (38) has again drawn attention to the fact that, while the buccopharyngeal mucosa is richly supplied with taste buds in all amphibians, the number of these organs especially in the anterior part of the mouth is greater in urodeles than in anurans.

The visceral sensory branches of nerve VII reach the mucosa of the mouth in urodeles by way of the palatine and alveolar rami, and in anurans by the palatine and internal mandibular rami (Coghill). The greater part of the buccal mucosa, however, receives its visceral sensory innervation from the IX nerve (Kallius), chiefly by way of the ramus lingualis. All of these branches, whether conducting gustatory or general visceral sensory impulses, enter the fasciculus solitarius centrally and terminate in the communis area. The communis area is not especially large in amphibians, and in both urodeles and anurans reaches its maximum size about the level of the entering IX root. Further, there is little if any difference to be observed in

the relative development of the fasciculus solitarius and its associated gray in urodeles and anurans.

It it be difficult to determine whether the communis area is relatively more highly developed in one amphibian group than in the other, then this would appear to indicate that the presence of additional taste buds within the mouth area of urodeles as compared with anurans cannot be of very great functional importance. But a large number of visceral sensory fibers must enter the fasciculus solitarius which are not concerned in gustatory conduction, and it is conceivable that the impulses they conduct should also exercise an influence on the reflex action of the musculature innervated by the motor VII-IX-X nerves and thus be important factors in determining the motor nuclear pattern.

In general it may be said that the extent of the peripheral area inervated by the communis components of the VII-IX-X nerves among metamorphosed amphibians varies directly with the number of persisting gill clefts. In adult anurans, in the adult Cryptobranchus japonicus and in all the adult Salamandridae, gill clefts are lacking, so that in these forms the area innervated by general visceral sensory fibers should be approximately equal. On this basis of reasoning, the representation of these fibers within the fasciculus solitarius should also be approximately equal in the animals mentioned, and appreciably less than among perennibranchiate forms. One might expect, if the influence of these communis fibers upon the reflex action of the VII-IX-X musculature and consequently upon the motor VII-IX-X nuclei be in proportion to their numerical representation within the communis area, that such a considerable reduction in their number would lead to some alteration of motor nuclear pattern away from the type obtaining in perennibranchiate amphibians. Since both Cryptobranchus japonicus and Triton exhibit the typical urodele motor pattern after metamorphosis is complete, it becomes evident that in determining this pattern the simple numerical representation of the general visceral sensory components in the communis area cannot be an important deciding factor, though the greater

importance of gustatory components within this area in urodeles may no doubt in part account for the caudal position of the VII motor nucleus in these animals, as Kappers has suggested.

Mechanics of respiration. A study of the mechanics of respiration brings out the fact that neither the acquisition of the pulmonary type of respiration nor the retention of the aquatic type of respiration appears in itself to be a factor of importance in determining the motor nuclear pattern.

Baglioni's observations (4, 5) on the respiratory movements in the adult frog may be briefly summarized as follows: Two varieties of respiratory movements occur, (a), laryngeal movements of a rythmical oscillatory nature which by alternate elevation and depression of the bucco-pharyngeal floor provide for constant renewal of the air within this cavity, and (b), 'proper' respiratory movements at longer intervals which bring about the renewal of the pulmonary air. In the 'proper' respiratory movements three distinct phases may be recognized. The first phase (aspiration) begins at the end of one of the rhythmical laryngeal movements when the floor of the mouth is depressed, and results in a further active dilation of the bucco-pharyngeal cavity. Towards the close of this active dilation, the glottis is opened and air is forced out of the lungs into the bucco-pharynx while at the same time the nares are closed. The passive enlargement of the bucco-pharynx by the expulsion of the pulmonary air marks the close of the second phase of the respiratory act (expiration). Immediately following expiration the tongue is thrust against the roof of the mouth, the choanae are closed by the hyoid cornua, the bucco-pharyngeal floor is strongly elevated and air is forced through the glottis into the lungs (inspiration). On the completion of the inspiratory act the glottis is closed and remains in this condition while the oscillatory laryngeal movements begin anew.

Among urodeles pulmonary respiration takes place in a manner essentially similar to that obtaining in anurans, and the same three phases (i.e., aspiration, expiration and inspiration) may be recognized in each complete respiration (v., Brüner, 12, 13, 14; Camerano, 15; Wilder, 60, 61). Further, aquatic

respiration in urodeles is carried on by means of the same bucco-pharyngeal pump mechanism, and in anuran larvae by but a slightly modified one, so that the act of aquatic breathing differs in these forms from that of pulmonary respiration only in the absence of the third phase, viz: Inspiration (Babák und Kühnová, 3; Brüner, l.c.; Gaupp, 24; Schulze, 51). In this connection Gaupp has pointed out that the phases of aquatic respiration are aspiration—inspiration—(expiration) and that in carrying out these movements the bucco-pharynx acts first as a suction pump and then as a force pump just as it does during pulmonary respiration.

Gaupp was led to the conclusion that, since there occurs no fundamental change in the mechanics of respiration at meta-morphosis, it was to be expected that the morphology of the 'respiratory center' would be the same in larval and adult anu-rans. Baglioni (5) has pointed out that this thesis cannot be accepted in its entirety because at metamorphosis, though the respiratory effector mechanism may not be greatly altered, yet a very fundamental physiological change must occur, since at this time air takes the place of water in furnishing the 'adequate' stimulus initiating normal respiratory reflexes.

In the urodeles in the case of Cryptobranchus and Triton, though physiological changes may have occurred during the evolution of their exclusively pulmonary type of respiration, the visceral motor nuclear pattern of these animals is practically identical with that of the perennibranchiate form Siren. Whatever the 'respiratory center' in amphibians may be anatomically, it would seem that the visceral motor nuclei of the brain stem in these forms as in fishes, must be very intimately concerned in its make-up. Viewed in this light the phylogeny of the visceral motor nuclear pattern in urodeles points to the essential truth of Gaupp's conception that in itself a change from aquatic to pulmonary respiration need involve no change in the morphology of the 'respiratory center' (vide infra).

Thus among urodeles the same visceral motor nuclear arrangement that obtains in perennibranchiate forms (continuity of VII-IX-X motor nuclei) is retained also in exclusively air

breathing members of the group while in adult anurans, though also exclusively air breathing forms, an essentially different type of visceral motor nuclear pattern is evident. If Gaupp's conclusion be correct, a typically anuran nuclear pattern should begin to be manifest in these forms early in ontogeny and the circumstances combining to produce this pattern must likewise have arisen early in larval life.[3] With this in view it will be of interest to inquire whether or not any correlation is evident between the arrangement of the bucco-pharyngeal effector mechanism and that of the motor nuclei from which it receives its innervation,

Motor V nucleus. The position of the motor V nucleus with reference to the exit level of its root is subject to relatively little variation among amphibians. In anurans the motor V nucleus is relatively larger than in urodeles and its cells are more easily distinguished from the neighboring tegmental elements,

Reflex connections both crossed and direct are evidently established between the motor and sensory nuclei of the V nerve on the level of the entrance of the latter, and Herrick (29) has shown further that in Amblystoma, individual fibers of the mesencephalic V root send collaterals into the homolateral motor V nucleus. Herrick's observations would indicate that this relation of the mesencephalic V root to the motor V nucleus is an important one for proprioceptive reflexes of the head muscles.

The muscles innervated by the trigeminal nerve in the amphibians under discussion are as follows: in Rana, mm. pterygoideus, temporalis, masseter, submentalis and submaxillaris; in Siren, mm. pterygoideus, temporalis, masseter, intermandibularis anterior and posterior, anterior part of the m. interhyoideus and in addition, two small muscles which have been termed by Norris the levator and retractor muscles of the ant-

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[3] It may be recalled again in this connection that in the 38 mm. Amblystoma larva, a typical urodele visceral motor nuclear pattern is already evident, although at this stage the motor VII nucleus is not in direct continuity with the motor IX–X (v. fig. 10, also Herrick, 29, fig. 1).

orbital cartilage; in Triton and Cryptobranchus, with the exception of the absence of the antorbital muscles, the motor distribution of the V is essentially similar to that in Siren.

It is evident on comparing the motor nuclear pattern in amphibians and sharks that the position of the motor V nucleus in relation to its emergent root is very similar in the two groups (cf. figs. 9, 10 and 11B). It has already been pointed out that "the rostral position of the motor V nucleus in sharks may be said to express a function of the negative influence of the communis center upon the reflex action of the jaw musculature in these forms" (10, p. 499). In amphibians likewise the importance of the trigeminal musculature in respiration is relatively slight, and its function during this act is in most cases chiefly that of tonic contraction (Gaupp, 24). It would thus appear that in both selachians and amphibians the primitive dorsal position of the motor V nucleus approximately on the level of its root exit, is to a large extent an indication of the relative independence of the trigeminal musculature in the respiratory activities of these forms (vide infra).

In this connection it is of interest to recall that the motor V nucleus in Siren, unlike any other amphibian examined, is situated almost wholly caudad of the exit level of its root. Norris and Bruner have found antorbital muscles developed only in Siren and Amphiuma. These muscles direct the activity of the choanal openings during respiration (Bruner, 13, 14) and their nerve supply is derived from the pterygoid branch of the ramus mandibularis V (Norris, 45, 46). Though these relations may be quite fortuitous, it is not impossible that the caudal situation of the motor V nucleus with reference to its root exit may be correlated with the respiratory function of the antorbital elements.

Facial musculature. The peculiar differences in the relations of the motor VII nucleus in anurans and urodeles which have been described above, assume a new significance when the functional development of the facial musculature is considered in the two orders.

In anurans (Rana) only two muscles are innervated by the
VII nerve: the m. depressor mandibulae and the m. subhyoideus.
The former muscle acts as its name implies, as a depressor of
the lower jaw and also, by traction on the tympanic ring, as a
tensor of the tympanum. The m. subhyoideus arises from the
posterior portion of the cornu principale of the hyoid close to the
skull, and extends ventro-mediad just caudal to the m. sub-
maxillaris to be inserted into a tendinous raphé common to it
and the latter muscle. It acts as a weak levator of the caudal
part of the buccal floor and in common with the m. submaxil-
laris, assists in deglutition and inspiration. Neither of these
muscles under normal curcumstances plays an important part
in respiration, and the action of both may be dispensed with
without seriously interfering with the respiratory mechanism.[4]
It becomes evident that in anurans the facial and trigeminal
musclature together form a complex which is primarily con-
cerned in effecting movements of the lower jaw and plays but
a relatively unimportant accessory rôle in purely respiratory
movements in these forms.

In urodeles the facial musculature is more extensively rep-
resented and more primitively arranged than in anurans. In
Siren lacertina an unusual and primitive condition obtains in
the arrangement of the m. depressor mandibulae (m. cephalo-
dorso-mandibularis) so that some of the fibers of this muscle
retain their insertion on the hyoid arch and constitute the m.
levator hyoidei. The facial nerve is distributed also to the
following muscles in Siren: mm. interhyoideus, interbranchialis
and cerato-hyoideus externus (v. Norris, 46; Drüner, 19). In
Cryptobranchus (McGregor, 42; Drüner, l.c.) the facial mus-
culature is represented as follows: mm. depressor maxillae in-
ferioris, mylohyoideus posterior and sphincter colli (cephalo-
dorso-pectoralis). In Triton (Coghill, 17; Drüner, l.c.) the

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[4] Langendorff (41) has observed that rhythmical respiratory movements con-
tinue in R. esculenta after the isolation of a segment of the medulla about 5 mm.
in length between the exit level of nerve VII and that of the caudal X rootlet.
Reference to fig. 9 B makes it evident that within such a segment only the motor
IX-X nuclei of the efferent centers will be retained intact.

facial musculature is represented as follows: mm. depressor mandibulae, interhyoideus, cerato-hyoideus externus and sphincter colli (quadrato-pectoralis).

Not only is the facial musculature in urodeles more extensively represented than in anurans, but with the exception of the m. depressor mandibulae, all these muscles in urodeles are intimately asssociated with the IX-X musculature and play a relatively important rôle in the inspiratory movements of the hyo-branchial region. Before the possible significance of this association can become fully apparent, it is necessary to consider the arrangement and innervation of the aspirator and inspirator muscles in anurans and urodeles.

Aspirator musculature. In both anurans and urodeles the depression of the bucco-pharyngeal floor in aspiration is chiefly due to the action of the sterno-hyoid and omo-hyoid muscles.[5] These muscles by their aspiratory action also aid in expiration. Owing to the aspirator action of these powerful somatic muscles, the branchial musculature aside from the intrinsic laryngeal complex, plays chiefly an inspiratory rôle.

Inspirator musculature. In anurans the chief muscles concerned in this action of inspiration are the mm. petro-hyoidei anterior and posterior, innervated respectively by the IX and X nerves. Inspiration is also strongly assisted by the contraction of the mm. genio-hyoidei, hyo-glossi and genio-glossi (N. XII) and in addition is aided somwhat by the tonic action of the mm. submentalis, submaxillaris and subhyoideus (N. V and VII) (v. Gaupp, l.c.). Thus in anurans the chief center from which the respiratory movements of the visceral musculature are directed, is the motor IX-X nucleus, which is placed in close relation both to the somatic motor center governing the chief aspirators (mm. sterno-hyoidei and omo-hyoidei) and to that of the most important accessory inspirators (mm. genio-hyoidei, hyo-glossi and genio-glossi), but which is relatively far removed from the center concerned in innervating the essentially non-respiratory jaw musculature (i.e., motor V and VII nuclei).

[5] It is of interest to recall that in ganoids and teleosts the m. sterno-hyoideus is also one of the chief inspirator (i.e., aspirator) effectors (v. 10).

In urodeles the musculature concerned in the act of inspiration is derived both from the hyoid and branchial segments. Thus in Siren the cerato-hyoideus externus, interbranchialis and levator hyoidei muscles which are innervated by the VII nerve, act as inspirators, together with the levators of the branchial arches, the cerato-hyoideus internus and branchial constrictors, innervated by the IX–X nerves. Similarly in Triton the m. quadrato-pectoralis (m. constrictor colli) acts in common with the branchial levators and constrictors, and in Cryptobranchus the m. cephalo-dorso pectoralis is associated in the same way with the branchial inspirators.

Hyo-branchial skeleton. It is obvious from this brief review that, though the mechanics of respiration may be essentially similar in both anurans and urodeles, yet the units of the musculature upon whose action the respiratory movements depend are fundamently different in their arrangement in the two orders. The immediate cause of the muscular arrangement peculiar to anurans is undoubtedly to be found in the characteristic morphology of the hyo-branchial skeleton in these forms. Thus the fusion of the hyoid with the broad basilingual plate and the final almost complete obliteration of branchiomeric arrangement in the adult anuran cartilago hyoidea has resulted in the specialization of the mm. levatores arcuum branchialium to form the mm. petro-hyoidei, and in the marked reduction in the respiratory importance of the facial musculature. On the other hand, in urodeles the absence of such extensive fusion of the hyoid and branchial arches has favored the retention of the hyoid and branchial levator muscles as individual units, and has even made possible the retention in some cases of a primitive superficial hyoid constrictor musculature (e.g., Triton and Cryptobranchus). It is apparently owing to the retention of this modified branchiomeric arrangement of the visceral musculature in urodeles, even in the absence of gill slits, that the primitive selachian character of the VII–IX–X motor column has not been lost.

It should be noted here that the peculiar arrangement of the hyo-branchial skeleton becomes early manifest in anuran larvae

(Ridewood, 49) by the fusion of the broad cerato-hyals with the basi-hyal and hypobranchial elements.[6] During larval life the chief aspirator muscle is the diaphragmato-branchialis from which is developed the sterno-hyoid muscle; but the most important levators of the bucco-pharyngeal floor are the mm. orbito-hyoidei and sub-hyoidei which are innervated by nerve VII (Schulze, 51). Thus during larval life, the muscles of the hyoid arch are important inspiratory effectors while in the adult this function is almost completely lost by the rearrangement of these muscles. On account of this arrangement of the facial musculature it is rather to be expected in view of the preceding observations, that the VII and V motor nuclei in larval anurans will not be so closely associated with one another as in adults of this order, and it would not be surprising to find the motor VII-IX-X nuclei associated in larval anurans in a manner very similar to that obtaining in larval Amblystoma (v. Herrick, l.c.).

The oldest larval anuran material at present available to me are 9 mm. tadpoles of Rana sp.? cut in series transversely at 6 micra. As none of this material is stained by Cajal or Golgi methods no charts of the motor nuclear pattern can be reconstructed with accuracy, but it can be determined with reasonable certainty that the motor VII nucleus of the 9 mm. frog tadpole lies relatively further caudad of the motor V nucleus than in any adult anuran examined. In the tadpole the distance between the motor V and VII nuclei is greater than the total length of the motor V nucleus itself. Strong's observations (53) would also indicate that the nucleus of origin of the motor VII root is differently arranged in the tadpole than in the frog. This author describes a motor root which emerges at the most

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[6] According to Ridewood (l. c.) the development of this region in Pelodytes is in general very similar to that obtaining in other tadpoles. In the 13 mm. Pelodytes the ceratohyals and basihyal (basibranchial) together with the hypobranchial plates form a ventro-median complex from which the distal ends of the ceratohyals and the bar-like ceratobranchials radiate outwards. This complex by the fusion of its elements forms in the 23 mm. larva a structure which in its shield-like arrangement is essentially similar to the adult anuran basilingual plate.

caudal part of the VII + VIII complex and which apparently derives some of its fibers from the motor V nucleus while others arise from the posterior longitudinal fasciculus. The conclusions indicated here are evidently opposed to those of Gaupp, and since his deductions are also largely based on the observations of Schulze, further study is desirable to decide this point.

Note on the fasciculus solitarius. Kappers (38) has pointed out that in contrast to other ichthyopsidans, a predominatingly descending character of the IX-X portion of the fasciculus solitarius is to be observed for the first time in amphibians. He came to the conclusion that the descending course of the IX-X sensory roots in amphibians was evidently not due to the neurobiotactic influence of any gustatory centers, but has probably resulted from the necessity for general visceral sensory correlation in the upper cervical segments on account of the presence in this region of the motor nuclei innervating the tongue musculature and especially those innervating certain important respiratory muscles. On account of its importance as a respiratory correlation tract, Kappers pointed out that the fasciculus solitarius apparently merited its old name 'fasciculus respiratorius'. Wallenberg (57) has noted that descending fibers of the fasciculus solitarius may be traced as low as the third cervical segment in the frog, and since this is below the level of the motor segment innervating the aspirator musculature, its important respiratory nature may be doubted.

In amphibians certain parts of the pulmonary mechanism, in addition to their primary respiratory function, are also concerned in the production of sound. The sound producing apparatus in amphibians is most highly developed in anurans though by no means restricted to these forms. It consists essentially of a vibrating mechanism in the larynx and a mechanism providing for the forcible expulsion of air from the lungs. In the frog, where this apparatus has been most extensively investigated, it has been found that certain muscles of the body wall (pars scapularis of the obliquus externus and the rostral part of the m. transversus) are especially fitted to aid forced

expiration (Gaupp). These muscles are innervated from the III and IV spinal segments and are ordinarily not called into special play during respiration, since the tonicity of the muscles of the body wall together with the natural elasticity of the lungs is sufficient to expel the pulmonary air when the glottis is opened. The mucosa overlying the functional vocal cords is innervated by sensory branches of the R. laryngeus longus and brevis X, while the mucosa of the bucco-pharyngeal cavity with its accessory vocal sacs is innervated by visceral sensory branches of both VII and IX nerves. Since the degree of tension of the walls of the resonator cavity, as well as that of the vocal cords must be factors of great importance in regulating the action of the accessory expiratory muscles during sound production, the descent of fibers in the fasciculus solitarius to the level of the III cervical segment may well have been determined during the perfection of this method of sound production, whose principle has been universally adopted by all air breathing vertebrates.

The probability of the importance of such a factor in determining the descending course of certain elements of the fasciculus solitarius is increased when it is realized that the evolution of a cervical motor center of a purely respiratory function must have occcurred in phylogeny after the stage represented by modern amphibians.

N. accessorius and m. trapezius. In the descriptive part of this communication attention has been drawn to the fact that the most caudal vagus rootlets in R. catesbeana are made up entirely of motor fibers, and emerge from the medulla on a more ventral plane than the rostral rootlets of this series. According to Strong (l.c.) a similar condition obtains in the tadpole. In Rana the motor fibers of the caudal vagus rootlets are distributed by two branches (R. accessorius and R. scapularis) respectively to the m. cucularis and m. interscapularis (Gaupp, 26). Among urodeles also the accumulated evidence goes to show that the caudal vagus rootlets are chiefly if not entirely motor, and that from these roots the innervation of the m. cucularis is derived (v. Coghill, 16, 17; Norris, 45, 46; Kingsbury, 40).

Though the peripheral relations of the motor fibers distributed to the trapezius are subject to considerable variation among amphibians, the essential features of innervation remain the same in both urodeles and anurans, so that there can be no doubt that the branch of the X nerve supplying the amphibian cucularis is the true homologue of the so-called external ramus of the N. accessorius of mammalian anatomy. From this it must follow that in amphibians as in sharks, the caudal end of the motor vagus column represents the nucleus accessorius of higher forms.

In considering the possible significance of the apparent absence of a trapezius muscle in many bony fishes (10), the important influence of the operculum upon the development of the levator musculature of the shoulder girdle became again emphasised. However, in view of the fact that a levator muscle innervated by the X nerve and quite definitely homologous with the selachian trapezius is developed in some bony fishes but not in others, the presence of a bony operculum cannot be the sole factor determining the absence of such a muscle in some teleosts, as Gegenbaur originally thought. The extensive development of a bony operculum such as obtains in many modern teleosts must certainly place a restricting influence upon the growth of the levator musculature of the shoulder, so that if a trapezius muscle innervated by the caudal X roots be retained in any of these animals, a proportionate reduction or suppression of muscular levator elements of somatic origin may be looked for, while if all the latter components of this complex be retained, a complete suppression of visceral trapezius elements might well result.

The cucularis muscle of sauropsidans and mammals is characteristically innervated from two sources, and the elements thus innervated are inextricably mixed within the fascial sheath of the muscle in question. In fishes such an intermixture of elements within the substance of the trapezius is not known, so that either somatic (shoulder levator) elements and visceral (trapezius) elements are both present as anatomically independent units, or one or other of them may be suppressed. Thus

the homologue of the cucularis muscle of higher forms must be looked for not in the piscian trapezius alone, but in this muscle plus certain closely associated and synergic effectors derived from the somatic musculature and innervated by spinal nerves. For these reasons the elements referred to were designated visceral and somatic components respectively of the trapezius complex (v. 10, p. 546).

If the above conception of the phylogeny of the sauropsidan cucularis be correct it may be expected that, in view of the absence of a bony operculum in amphibians, both the somatic and the visceral components of the trapezius complex will be retained in these forms. Further, in contrast to sharks, the functional differentiation of the somatic components from the general trunk musculature as well as the specialization of the visceral components should be more extensive especially in anurans, while in the more primitive urodeles some indication of a saurospidan prostadium may be looked for. It will be of interest therefore to consider briefly certain anatomical facts bearing upon these questions.

In Rana (Gaupp, l.c.) the so-called visceral components of the trapezius complex may be recognized without difficulty in the mm. cucularis and interscapularis. On the other hand, the levator musculature derived from the trunk segments has undergone such extensive differentiation in anurans that it is difficult if not impossible to select any one of the muscles associated in this function as the sole representative of the somatic component of the trapezius complex of higher forms. Dorsally the m. rhomboideus anterior, innervated from the III spinal segment, acts synergically with the m. cucularis and was even misnamed m. cucularis by Ecker on account of its relations (v. Gaupp, 25 p. 103). Ventrally the m. levator scapulae inferior, innervated from the II spinal segment, is also closely associated with the m. cucularis, with which its action is synergic. Fortunately, however, it is of no moment in the present connection to attempt to decide which of the many possible muscles may represent the somatic component of the trapezius complex in these forms; it is sufficient merely to indicate that in the ab-

sence of the operculum, the somatic levator musculature is plentifully represented and highly specialized. Similarly, in the absence of the operculum no apparent hindrance has been imposed in the way of development of the visceral component of the trapezius complex, and it is significant to note that in the most highly specialized amphibians (i.e., anurans) this visceral component has become further elaborated to form two distinct muscles (mm. cucularis and interscapularis).

With regard to the possible recognition of a sauropsidan prostadium in any amphibian condition of cucularis development, it is of interest to record that in the tadpole Strong detected an anastomosis between vagus and hypoglossus fibers passing to what he considered to be the m. diaphragmato-branchialis medialis of Schulze (v. 53, p. 141). This condition was absent in the tadpole in which metamorphosis had begun and in view of the fact that Schulze (l.c.) considered that the m. diaphragmato-branchialis develops into the m. sterno-hyoideus, its significance in the present connection may be questionable. It provides very suggestive evidence, however, that in anuran ontogeny there may be an admixture of muscular elements derived from both splanchnic and somatic sources within a single effector.

Among urodeles Fürbringer (20, p. 265) has denied the existence of a double innervation of the m. capiti-dorso-scapularis (the cucularis muscle in salamandrids) or of the m. dorso-scapularis of perennibranchiate forms. On the other hand, McGregor (42) recorded the occurrence of an anastomosis between vagus and hypoglossus fibers in the adult Cryptobranchus alleghaniensis, but his observations were incomplete and no mention was made of the innervation of the trapezius muscle.

It has remained for the exact observations of Norris (46) upon the distribution of the vagal and spinal nerves in Siren lacertina to demonstrate beyond a doubt that in this animal, in which so many oddly mixed primitive and specialized characters appear, the trapezius muscle receives its innervation from two sources, viz., from a branch of the R. intestino-accessorius X, and from small dorsal branches of the ventral rami of the

first and second spinal nerves. The latter "shortly before they recurve to their point of union . . . . . . give off a few small branches, *some of which anastomose with the branch of the ramus intestino-accessorius X supplying the trapezius muscle*, and others innervate the basi-scapularis" (46, p. 331).[7] The m. basi-scapularis is apparently the homologue of the anuran m. levator scapulae inferior.

Thus in the double innervation of the m. trapezius, a condition obtains in Siren which is essentially similar to that which becomes characteristically developed only in higher vertebrates. Whether such a condition obtains elsewhere among urodeles is at present uncertain. In Norris' earlier account of these nerves in Amphiuma means he states "we may confidently deny the occurrence of any anastomosing between hypoglossal and vagal nerves" (45, p. 553). However, in Plate IV the branch of the R intestino-accessorius to the trapezius is labeled 'acc,' and the abbreviation 'acc' is explained as "branch of X nerve supplying the anterior part of the trapezius muscle." If this implies a different innervation for the posterior part of the trapezius of this animal, it is possible that spinal nerves may be involved without anastomosing with branches of the vagus.

In view of the above observations, the relations of the caudal part of the motor X nucleus in Rana are significant. It has already been pointed out that in the caudal part of this nucleus the cells become scattered, so that it is difficult to set an exact caudal limit to this column. Further, the condition obtaining here presents an interesting contrast to that obtaining in the corresponding region in sharks. In the latter animals, rostrad of the calamus (10, fig. 10) the motor X nucleus lies just beneath the ventricular floor, some distance laterad of the spino-occipital nucleus and on a more dorsal plane. Caudad of the calamus in the upper cervical cord the motor X nucleus in sharks lies in the central gray completely dorsal to the spino-occipital motor column. In Rana (figs. 1 and 2) the motor X nucleus above the calamus is separated by a relatively great distance from the ventricular floor on account of the thick layer of peri-

[7] The italics are my own.

ependymal gray. In this position it is placed laterad to but on the same level as the hypoglossal nucleus. Below the calamus the motor X nucleus in Rana is even more ventrally placed, so that in its caudal end it becomes associated with the most dorsal elements of the ventro-lateral nucleus of the anterior born. In this relation the motor perikaryons giving origin to the accessory nerve come to lie almost side by side with those from which fibers pass out to innervate the somatic shoulder muscles. It is evident that the increased differentiation of the levator musculature of the shoulder in Rana as compared with selachians has been accompanied by a corresponding specialization in the arrangement of the elements of the motor nuclei from which these muscles receive their innervation. This closer association of vagal and spinal motor nuclei in Rana represents what may be considered as the second stage in the phylogenetic development of the accessory nucleus, the first stage of which is seen in the condition obtaining among sharks (v. 10, p. 495).

Eye-muscle nerves

Nerve VI. But little remains to be said concerning the abducens nucleus and its emergent roots. The diffuse nature of the nucleus and its relation to the fasciculus longitudinalis medialis and octavo-motor fibers have been noted above, and Kappers has remarked already upon the primitive relation evident in the emergence of the VI rootlets caudad of the motor VII root exit. The chief bulk of the anuran superior olive is also located between the exit levels of the motor VII and IX roots but whether this nucleus establishes such direct reflex connection with the abducens cells as obtains in higher forms is not known.

Nerves III and IV. In most amphibians in which the trochlear nucleus has been identified, it lies almost directly behind the oculomotor nucleus and on a level rostrad of the trochlear root exit. In the latter relation these amphibians differ from all petromyzonts and from some selachians and teleosts; on the other hand, the position of the trochlear nucleus in amphibians

rostrad of its root exit recalls the relations obtaining in all ganoids and most teleosts (cf. figs. 9, 10 and 11). A more primitive arrangement of the trochlear nucleus has been recorded in Molge cristata by Van der Horst (l.c., fig. LXXX). Here the nucleus lies a considerable distance caudad of the oculomotor nucleus and about midway between the exit levels of the trochlear and oculomotor roots.

In describing the oculomotor nucleus in fishes (10) it became evident that in both ganoids and teleosts this complex showed distinct indications of division into dorsal and ventral cell groups. It should also be noted that in petromyzonts a peculiar specialization of the oculomotor nucleus into dorsal and ventral moieties has been described by Huet (31) and Kappers (35), but this nuclear differentiation evidently differs from that obtaining among the true fishes.

Attention has already been drawn to the tendency among anurans toward subdivision of the oculomotor nucleus into subgroups, but in contrast to the conditions obtaining in other ichthyopsidans, the components of the anuran oculomotor complex are arranged to form medial and lateral moieties.

The differentiation of the oculomotor complex in ganoids and teleosts away from the condition obtaining in sharks has probably been correlated with specialization of intrinsic effectors in the eyes of the former animals (10), and this differentiation appears to have reached its maximum in modern teleosts (e.g., Pleuronectidae, Lophidae). The relatively simple undifferentiated condition of this nucleus which obtains in selachians as well as in dipnoans and crossopterygians (Van der Horst, l.c.) indicates, however, that the arrangement of the oculomotor elements so characteristic of modern ganoids and teleosts cannot be regarded in any sense as representing a prostadium of the amphibian condition.

It would thus seem probable that the factors which determined the anuran oculomotor cell arrangement must have appeared comparatively late in phylogeny. The eyes of most fishes are normally focussed for near objects when at rest, and since the eyes of most amphibians, reptiles, birds and mammals

are normally focussed for distance (Beer, 6, 7, 8, 9), it is not improbable that the chief original cause of the amphibian oculomotor nuclear specialization may be seen in the readjustment of the mechanism of accommodation which must have taken place in phylogeny during the evolution of the amphibian type.

Finally it should be observed that, as Kappers has pointed out, in the arrangement of the elements of the anuran oculomotor nucleus a first stage in the evolution of the more highly specialized sauropsidan condition may be recognized (35).

CONCLUSION

In the foregoing pages attention has been drawn to the striking correlation that exists between the arrangement of the visceral motor nuclei in amphibians and the functional development of the musculature which they innervate. It has been shown that in correlation with the more primitive arrangement of the branchial musculature in urodeles, the visceral motor nuclear pattern in these animals conforms in all essentials to the type of nuclear arrangement characteristic of selachians and crossopterygians (cf. figs. 10 and 11).

On the other hand, the evidence here produced makes it strongly probable that the apparently primitive arrangement of visceral motor nuclei obtaining in anurans is in reality the result of nuclear rearrangement completed late in ontogeny, and that the anuran nuclear prostadium must closely approximate the permanent urodele condition. It thus emerges that in correspondence with the specialized development of their branchial skeleton and musculature, the anuran visceral motor nuclear pattern is a specialized one which however has come to resemble that of petromyzonts as the result of analogous or convergent evolution (cf. figs. 9 and 11A).

In the present connection it will be of interest to compare the conditions which obtain in two such widely separated forms as Rana and Bdellostoma. In the general organization of Bdellostoma many primitive as well as regressive characters are evident, and the animal has manifestly been derived from a type very low in vertebrate phylogeny. Notwithstanding these

facts, the arrangement of the cerebral motor nuclei in this animal is undoubtedly a specialized one (10). In the case of Rana however, conditions of an apparently opposite nature exist. The anuran type has certainly been evolved comparatively recently in vertebrate phylogeny, and the Ranidae represent the highest stage in the evolution of the Anura (22), but within the brain stem in Rana a motor nuclear pattern obtains which on first examination would seem to be much more primitive than the motor nuclear pattern in selachians.

The fundamental difference between the anuran and urodele visceral motor nuclear pattern is essentially due to the different position of the motor VII nucleus in the two groups. In Rana during ontogeny this nucleus apparently retraces the path along which it travelled in phylogeny. The neurobiotactic influences which determine the phylogenetic migrations of the motor VII nucleus have been considered in detail by Kappers (l.c.), but unfortunately with regard to the factors which are directly responsible for the rostral migration of this nucleus during development in Rana but little can be said as yet. The observations of Herrick (l.c.) on the mesencephalic V root in Amblystoma make it plain that many fibers from this source establish connections with the motor VII nucleus in this form and probably also in other urodeles. If similar connections obtain in Rana, where descending mesencephalic root fibers are known to exist (55, 56), the rostral migration of the motor VII nucleus in this form may well have taken place along this path.

Not only does the general arrangement of the anuran motor nuclear pattern differ from that of urodeles but also in certain anuran motor nuclei a higher degree of intrinsic specialization has been acquired than is evident in the corresponding nuclei of urodeles. Especially is this true in the rostral part of the somatic motor column of the cord, so that in Rana two very definite cell groups are to be distinguished here, from which arise fibers emerging by the most rostral cervical motor roots. One of these cell groups has been identified as the homologue of the hypoglossal nucleus of higher forms, and the dorsal position of this cell group is most probably the result of neurobio-

tactic influences derived from two main sources, viz., the fasciculus longitudinalis medialis and the arcuate fibers from the overlying communis area.

In addition to the evolution of a hypoglossal nucleus as an individual cell group of the cervical motor column, there is also evidence of intrinsic nuclear differentiation to be seen in the arrangement of the oculomotor perikaryons.   Here however it is not possible to indicate as yet any individual fiber systems by whose direct influence the cellular arrangement may be determined, but as already noted, these changes are in all probability indirectly determined by the evolution of the amphibian mechanism for accommodation.

Finally it may be noted that such evidence as can be adduced from the study of the cerebral motor nuclei in amphibians is in accord with the conception of the derivation of these animals from crossopterygian-like ancestors.

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THE CENTRAL NERVOUS SYSTEM OF SIMPLE CRUSTACEA

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THIRTEEN FIGURES

Although the general form of the nervous system of branchiopod Crustacea is well known, there has been very little recent work. Most papers deal with the general form and arrangement of the ganglia and not at all with the structure and arrangement of cells. Zaddach, '41, wrote on Apus. Leydig, '51 and '60, considered Artemia. In 1853 there is the paper of Grube on Limnetis. The work of Claus on Branchipus, Daphnea, Estheria and Apus appeared in 1873, 1876, and 1876, and that of Weismann on Leptodora in 1874. Spangenberg's publication on Limnadia was in 1878. Packard has something of the general anatomy of Estheria and Branchipus in 1883. The well known and often copied work of Lancaster on Apus, '81, was followed by that of Pelseneer on the nervous system of the same genus in 1884. Spencer in 1902 discusses and figures the anterior nerves of the brains of Artemia and Branchipus.

From the various observations the general ladder-like type of nervous system has been described and figured in this group of Crustacea. There is the supraesophageal ganglion with its marked region of optic nerves, while the two other pairs of nerves to the antennae and antennules are less marked and come from more caudal portions of the brain or on or near the esophageal connectives. From the cephalic margin of the brain are the median eye branch and the two small pairs of nerves lateral to it, at least in Branchipus and Artemia, as described by Spencer, '02. Each segment of the body below the brain is ordinarily represented by a pair of ganglia connected across the middle line

by two commissures. The number of pairs of ganglia depends largely upon the degree of segmentation of the body of the crustacean.

The fortunate opportunity to obtain a large number of living Crustacea gave much of the material for this study. Methylen blue was tried without success as long as the animals could be obtained alive, afterwards dissections and sections were made from preserved material. Mercuric chloride fixation seemed most advantageous. The whole nervous system was dissected out and lightly stained with a carmine solution or a clear alcoholic hematoxylin. Later the specimens were mounted in balsam. This method had many advantages because all parts of the simple nervous system could be seen at once The cells and fibers were not numerous enough to greatly interfere with the clearness of the preparations. Especially was it noted that the cells were not distorted as is usually the result after sectioning methods. Some serial sections of whole animals or parts were prepared for comparison.

GENERAL FORM OF THE GANGLIA

In the forms studied, in general no new features of external morphology were noted. Artemia and Branchipus were practically the same except for the larger size of the nervous system in Branchipus. In these the brain has connected with it laterally the two large optic nerves which expand into the optic ganglia (not shown in figures). The antennulary nerves come off from the brain where it joins the esophageal connectives and the larger antennal nerves come off a little farther down From the cephalic side of the brain a median nerve is connected to the median eye and two pairs of nerves lateral to this supply upper parts of the head.

The first three pairs of ventral ganglia supplying the head, mouth parts and upper portions of the body are much smaller than the more caudal ganglia. The last ganglion or pair of nearly fused ganglia change somewhat as they terminate in abdominal branches (figs. 1 and 2).
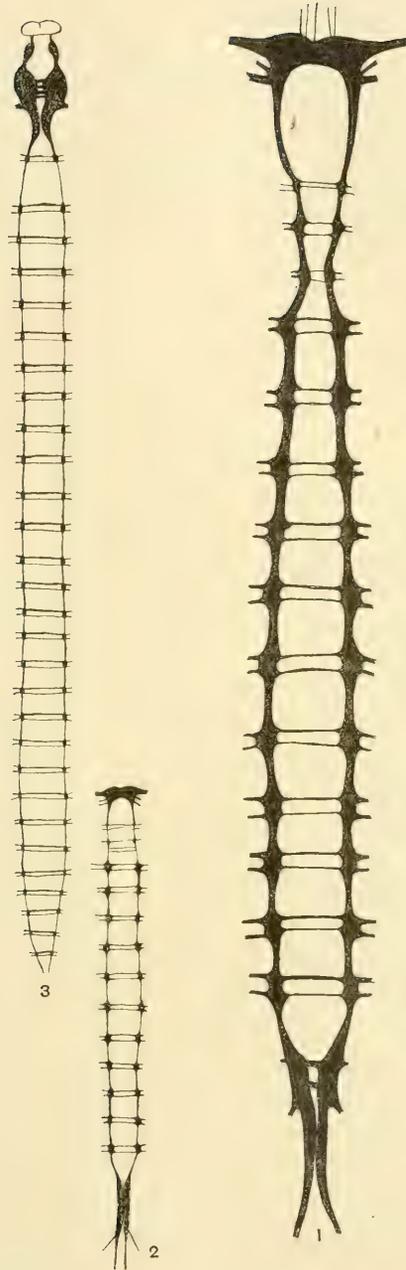
Fig. 1. The central nervous system of Branchipus venalis.   × 10.
Fig. 2. The central nervous system of Artemia sp.   × 10.
Fig. 3. The central nervous system of Estheria californica.   × 10.

In Estheria it was very difficult to remove the ganglia intact, so the sketch given is from the nervous system *in situ* for the most part. The brain is of quite a different shape, the optic ganglia are shown in the drawing connected with the compound eyes, which nearly touch each other. Only one pair of antennal nerves is shown in the drawing. The brain is more decidedly
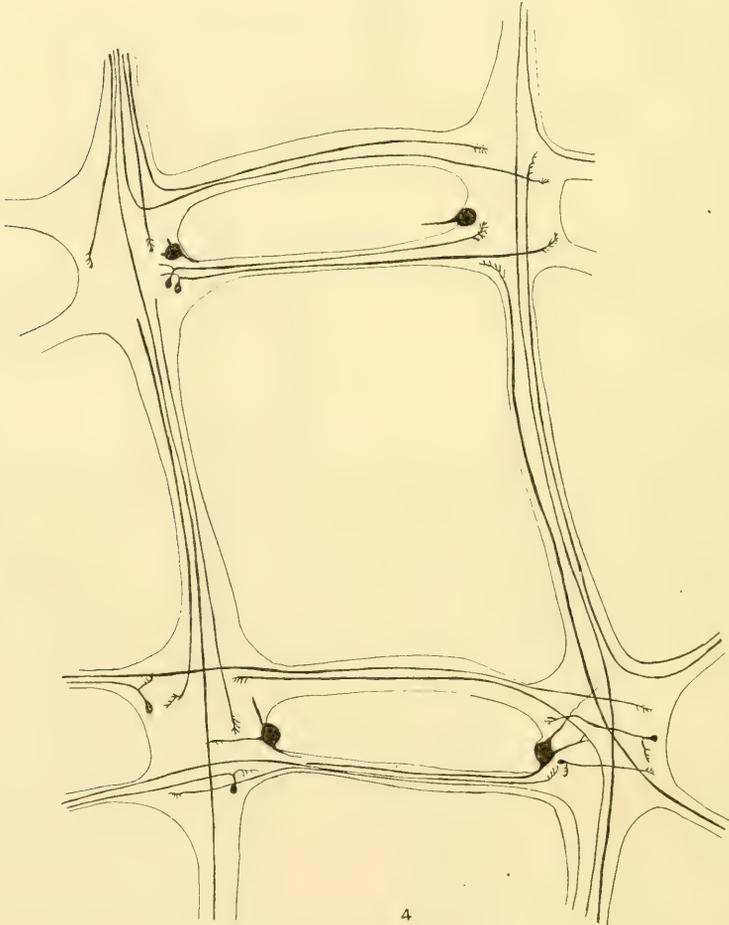
Fig. 4 Diagrammatic plan of cell arrangement in the ventral ganglia of Branchipus. × 75.

made up of two lateral masses than in the other genera studied. Four commissures may be clearly seen connect'ng the two lateral parts. Four were also seen in Artemia and Branchipus but not so clearly. The ventral ganglia of Estheria begin with the mandibular and extend to the end of the body with a pair of ganglia to each segment and with two commissures connecting each pair (fig. 3).

Cells of a number of types were found. In Branchipus they are from 0.01 mm. to 0.05 mm. in diameter. The much smaller but similar Artemia has smaller cells, the largest being about 0.02 mm. and the smallest about 0.005 mm. Two divisions of cells may be made, the neuroglia cells and the nerve cells. The neuroglia cells, small or large, were not so deeply stained in the fibrillar area; they usually have granular nuclei. From whole mounts and sections it was evident that the neuroglia nets are much as described in other invertebrates. It is possible that some of the small cells which seem to be nerve cells are neuroglia cells.

CELLS

Practically all the nerve cells, especially of Branchipus, have well-marked cell bodies filled with dark staining material and clear nuclei containing nucleoli. A few nerve cells have much clearer cytoplasm than the others. In the large cells especially, tigroid substance may be seen even in surface views. In the larger cells also the fibrillae are quite evident. The general shape of the cells is spherical, but some are elongate. Most cells are unipolar or bipolar, but a few are multipolar (figs. 5 to 13).

Large, medium sized and small cells are found in the cellular areas with no apparent special order, but the largest cells are found at certain places at the margins of the ganglia. At least one, sometimes two or three of the largest cells are located on each side near the more caudal commissures of each ventral ganglion. These in many cases send or receive processes to or from the connectives. Perhaps they are cells in most cases with long commissural fibers.

In each ganglion of each side the cells are arranged in a characteristic manner. In all the middle body ganglia the ventro-mesal cell group is less marked than the ventro-lateral. The outer portion of the ventral-lateral group often has a number of large cells similar to those in the other group. The larger cells in most cases represent those that send their fibers longer distances, but they often have more than one branch and the external and internal mass of fibrillae connected to them seems more complex than on smaller cells. In some cases the larger cells seemed to have their cytoplasm fused, but most of the cells, although near each other, had their cell bodies distinct. Nerve fibrillae are evident between and in cells, although some of the largest cells have one or two large fibers which leave or enter the cells. Some cells of apparently the same type seem to have no very large branch, but fibrillae enter and leave the cell. Many large cells seem to be penetrated on all sides by numerous fibrillae, or if fibrillae do not all penetrate they are closely related to all the peripheral parts of the cell body.

Cells of varying numbers are found in the commissures, these are chiefly medium or small cells and some at least are nerve cells. The number of cells in the ganglia was possible to determine quite well from surface preparations and some comparisons were made between Artemia and Branchipus. The larger species has not only the larger nerve cells, but the larger number of nerve cells. The number of cells in corresponding ganglia was found to be less in the smaller animals. The average number of cells in the middle ventral ganglia on each side ran from 130 to 204 in Branchipus, while Artemia had from 120 to 160 cells in each lateral ganglion. The number of cells in the intermediate ganglia of a number of specimens was counted and, although the count cannot be considered absolute because of

Figs. 5 and 6   Sections through the abdominal ganglia of Branchipus. The dorsal side is to the top of the page.   × 75.

Fig. 7   Brain of Branchipus from a surface preparation. The cephalic side is to the top.   ×75.

Fig. 8   Upper ventral ganglia of Branchipus, surface view. The cephalic end is at the top.   × 75.
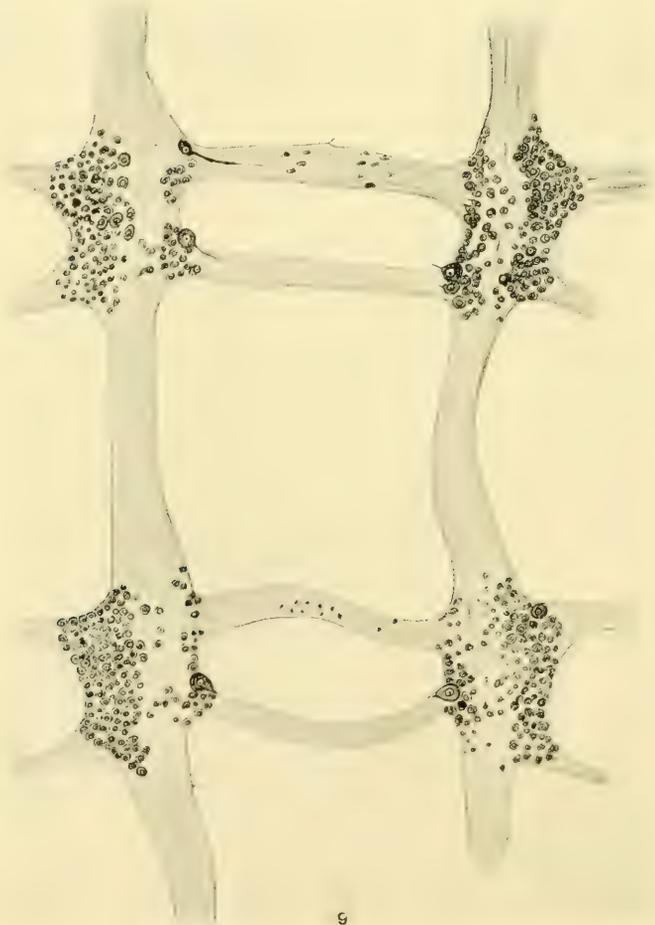
difficulty in seeing all at one focus, difference in mounting and difference in staining, yet the following seems clear:

1. The cells often differ slightly in number in different similar ganglia of the same animal, both in the same segment on each side and in different segments at various levels.

2. The number of cells is also variable in the same parts of the same ganglia in different animals.

3. The peripheral parts supplied by each of these ganglia do not differ in any way that could be determined.

4. It was even more clear that the cells in the commissures differed widely. The next to the last cephalic commissure in one specimen had 38 cells, the next 20, then, 19, 7, 10, 12, 16, 10, etc. Similar variations were found in other specimens. The lower cephalic commissures as a rule had more cells, while the upper had less.

It was also noted that some of the large cells which have quite a characteristic position are in some places represented by one cell, in others by two. In a few cases noted the large cells have an independent peripheral distribution as compared with the usual indirect distribution through a commissure. It is as though a cell which ordinarily grew out to the periphery by way of a commissure missed it in some way and left the ganglion by a single fiber.

FIBER TRACTS

Branchipus was especially studied because the material was more favorable.

The brain so far as could be determined is united from side to side by four commissures, a dorsal, two medial and a ventral. The last is below a small group of medial cells. The commissure just above this group seems the largest. The ventral commissure is partly from near-by cells and from basal parts of the con-

Fig. 9 Sixth and fifth ventral ganglia of Branchipus. Surface view. Cephalic side at the top. × 75.

Fig. 10 Section through one abdominal ganglion of Artemia. ·The dorsal side is above. × 75.

Figs. 11, 12 and 13 Sections through various levels of the brain of Artemia, from the base to the region of the optic nerve. The dorsal side is up. × 75.

nectives. Many of the medial fibers may be traced out to the optic lobes. Fibers from the largest median cephalic cells descend the connectives. Fibers from cephalic and lateral cell groups cross in the center of the brain and either run straight into commissures or cross somewhat diagonally. Fibers from the smaller cell groups on the connectives near antennal nerves descend the connectives and ascend into the central parts of the brain to the same side or the opposite side. The small cephalic branches of the brain send fibers for a short distance into the brain and cells near here supply them. The mass of the connective fibers runs straight in to the central parts of the brain. Fibers from cephalic lateral cells cross at angles to relate themselves to various cell groups, to run in the optic nerves and to run into the central part of the brain.

The connections of the optic ganglia were not studied. So far as there is a special center in the brain to which all fibers converge it would be the general region of median cephalic cells. It is from this region that the larger cells probably send their fibers long distances down the connectives to ventral ganglia.

In general the distribution of tracts in the ventral ganglia is as follows:

1. Fibers in the connectives ascending or descending.

2. Fibers from the branches or nerve trunks end, cross in commissures and ascend or descend in the connectives. Many end where they enter the ganglia or on the opposite side in the same ganglion or in the opposite ganglion.

3. Fibers in the commissures cross from cells of either group and end in relation to cells of either group of the opposite ganglion. Fibers in the commissures may also be seen to ascend or descend in the direction of the connectives.

4. Each cell area of each ganglion is probably connected as follows: a) Fibers to other cell areas of each side through the commissures. b) Fibers to cell areas of each side not from the other side through the commissures. c) Ascending fibers. d) Descending fibers.

The commissures are probably made up as follows: a) Fibers from cells in upper levels. b) Fibers from lower levels. c)

From the same level from both sides, especially from median cells. d) Probably fibers to and from lateral branches of ganglia, although this was not clearly demonstrated (fig. 4).

SOME GENERAL CONCLUSIONS

The study of these nervous systems shows certain advantages due to the nature of the material and the method. There is less distortion because with whole mounts no heat was used. There is also a more perfect picture presented than in most methylen blue preparations because all the cells show. When large ganglion cells were at the edge of the preparation quite a little could be seen of their finer structure and the fibers and fibrils were often presented with great clearness. I believe that, although the grosser processes are important, that the fibrillar connections are more important in determining the intimate relationships of cells to each other. It seems probable from these observations that any cell may have its cytoplasm penetrated by fibrils which are directly connected with other cells, while its one or two main branches carry out fibrils in larger masses, break up into fibrils and by usual methods are not followed farther.

The variation in the number of cells in similar segments suggests the probability that the nervous system acts not so much through individual innervation of special areas by special cells, as by a more general innervation by groups of cells. In the course of evolution in more specialized forms it may be that individual functions may more nearly be connected with individual cells or small groups.

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THE CELL-CHANGES IN THE HYPOPHYSIS OF THE ALBINO RAT, AFTER CASTRATION

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FIVE FIGURES

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INTRODUCTION

The operation of castration in the albino rat is followed by definite progressive alterations in the structure of the ventral glandular portion (pars anterior) of the hypophysis. Indeed, aside from the atrophy of the several related organs of the reproductive system and the diminution of the secondary sexual characters, the cytomorphic changes in this portion of the hypophysis present one of the most striking phenomena resulting from castration. This suggests that perhaps the growth changes, recorded in various organs and tissues after castration, are influenced by this alteration in the structure and physiological activity of the hypophysis. The study of the changes in the hypophysis after castration also affords evidence in regard to

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the debated question of the relation of the several cell-types seen in the ventral glandular portion; i.e., as to whether they are merely functional variations of one type, or really constitute independent types of cells, each forming a different specialized secretion.

The present investigation was begun as a study of the histological details of the interesting observations of Hatai. These were, in part, briefly, that there was a sex-difference in the weights of the hypophyses of male and female albino rats, always in favor of the female (Hatai '13), and that after gonadectomy, there was a decided increase in weight in the male hypophysis, but only a very slight increase in the female hypophysis (Hatai, '15).

It is proposed in the present paper to give a description of the normal male hypophysis of the albino rat, and of the changes consequent upon the removal of the sex-glands. Fichera ('05) was apparently the first to study castration changes, using domestic cattle, domestic fowl and European buffaloes, and his results will be discussed later. No extended study has been made of the albino rat in this respect, although a number of investigators have worked upon other animals, with results somewhat at variance. Reference will be made to these under the heading "Discussion of Observations."

In view of the discordant results, it has seemed advisable to restrict the present phase of the work, to the one form, the albino rat.

MATERIAL AND METHODS

Both young immature animals and adult animals were used. The operations in the former were performed at about the age of one month, and the experiments were all done by litters, some of the males of the litter being operated upon, and the remainder kept as controls. The hypophysis material from the experimented animals was taken at varying periods after operation, the longest period being nine months. Always the hypophysis from a control animal of the same litter was taken at the same time. In the early part of the work, the weights of the hypo-

physes were taken as recorded by Hatai ('15). For changes during the first month, material was taken at weekly, or semi-weekly intervals. For changes after the first month, material was taken at monthly intervals after operation, up to nine months. Most of the operations were done by Dr. J. M. Stotsenburg of the Wistar Institute, to whom I am greatly indebted.

The fixing fluids which proved most useful were the neutral formol-Zenker mixture of Bensley ('11), and the neutral formol-potassium bichromate fluid as employed in the mitochondrial technique of Cowdry ('16). Others used in the early part of the work were the fluids of Bouin, of Ohlmacher and the acetic-osmic-bichromate mixture of Bensley.

After embedding in paraffin, sagittal sections were cut at 3 or 4 microns. For staining were used Mallory's aniline blue-orange G, iron hematoxylin, and Altmann's acid fuchsin followed by methyl green, as detailed by Bensley and Cowdry.

NORMAL HYPOPHYSIS

In figure 1 are illustrated the four parts of the rat hypophysis, as seen in median sagittal section. These parts are named, (1) pars nervosa or infundibular portion; (2) pars intermedia, or juxta-neural portion, (3) pars anterior, or ventral glandular portion, and (4) pars tuberalis (Tilney). The pars nervosa is connected with the base of the brain by a stalk which is solid, except at its connection with the diencephalic floor, where it contains a short extension of the third ventricle. The residual pouch of Rathke (5, fig. 1) persists as a cavity between the pars intermedia and pars anterior. Passing cephalad along the hypophyseal stalk is a strand of cells (1 to 2 rows deep), constituting the pars tuberalis. Of these several parts of the hypophysis, the ventral glandular portion is the one which shows the most marked changes after castration, and is the only part which calls for detailed description here.

In the normal adult animal, the ventral glandular portion of the hypophysis presents a compact arrangement of masses of cells between the blood-vessels. The distribution of the blood-vessels is best seen in sections parallel to the greatest surface

expansion of this part of the hypophysis. Beginning cephalad where the largest vessels are seen, these branch and break up into a sinusoidal network, which extends throughout the substance of the gland.

The connective tissue framework is very slight, and there is no definite division into groups of cell masses by connective tissue septa, as is seen in man and many other species.
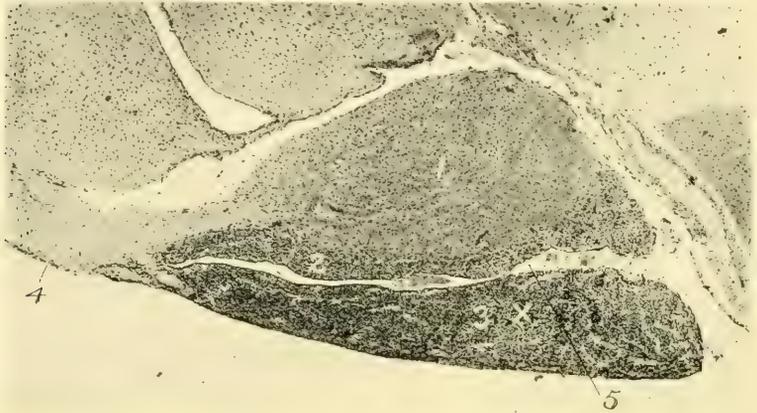
Fig. 1 Median sagittal section of hypophysis of albino rat showing (1) pars nervosa, or infundibular portion; (2) pars intermedia, or juxta-neural portion; (3) pars anterior, or ventral glandular portion; (4) pars tuberalis; (5) lumen of residual pouch of Rathke. × 40.

The differentiation of the cell-types occurs in the first month of post-natal life. At birth nearly all cells are of similar appearance, and still resemble the primitive fetal type. Here and there, examples of two other cell-types may be seen developing from the undifferentiated type. At the end of one month, however, differentiation is well advanced, and the three types are easily recognized. These three types are: (1) acidophiles, so named from containing numerous small closely-packed granules, which stain with acid dyes and especially well with acid fuchsin; (2) basophiles, larger cells than the preceding with cytoplasm moderately basophilic; and (3) unchanged descendants of the primitive fetal type, which may be termed reserve cells.

In the young mature animal (e.g., 69 days old, figure 2) two of these three types are very distinct, and the third is without such distinct characters. The two distinct glandular types are the acidophiles and the basophiles, and they together form the continuous lines of glandular cells, abutting upon the endothelium of the blood-streams. Away from the sinusoids are located the reserve cells. In the groups of reserve cells between the sinusoids are seen also acidophiles and basophiles, but it would appear that all these latter cells come into close relationship with a blood-vessel, either above or below the plane of section. The reserve cells, however, do not usually form part of the lining glandular cells of the blood-channels in the normal mature hyophysis.

Taking a survey of entire sagittal sections, the acidophiles always outnumber the basophiles, and as a whole occupy a greater proportion of the section, although the basophiles are individually usually larger. The number of the reserve cells varies with the age of the individual, being more numerous in the immature animal, and becoming as a whole less conspicuous in the full-grown. As will be seen later, their number may also vary with the functional conditions of the animal. All types are found intermingled throughout the section in a fairly uniform manner, although sections from different animals may show some definite local variation in the proportionate number of the cells. The most constant variation from the uniform grouping was found in a limited area at the attached end of the hypophysis, where the basophiles were present to the nearly entire exclusion of the acidophiles and reserve cells. The basophiles of this group become very coarsely granular as age increases. Another variation seen in sections from several different specimens, was a greater proportion of basophiles along the most ventral border of the gland, and a decreased proportion of basophiles in the marginal zone along the caudal half of the pouch of Rathke. In general, in the central part of the caudal half of the sections, the acidophiles were always present in increased proportion to the basophiles. In Mus decumanus, Tilney ('11, page 48) found the acidophiles occupying the central regions of this part of the hypophysis.

The differential staining of the cells to show the three types requires considerable care. With routine stains, the cells do not stain so distinctively as those of man, or of some of the domestic animals. Stendell ('14, p. 116) found that this part of the hypophysis of Mus and Cavia stained less intensely than that of Lepus. Good differential staining of the acidophiles so as to distinguish them from reserve cells is not easily accomplished by eosin. A better method for the acidophilic granules is the acid fuchsin method of Altmann, or as variously slightly modified. With this stain one finds that the granules of all cells are not equally deeply stained, and this is probably due to the cells being in different states of functional activity. The granules are found to be very minute and individual ones are less easily definable than in the human or cat hypophysis. In the Mallory methylene blue-orange G method, the acidophilic granules are of orange-yellow color and, when the staining is successsful, I think that by this method one can best differentiate the acidophiles from the reserve cells. By this method, too, the cytoplasm of the basophiles is stained blue, thus giving good contrast with the acidophiles. The nuclei are also stained blue but they are usually easily made out. The cytoplasm of the reserve cells is grayish and practically unstained, and the cell boundaries are often indistinct. With this stain at least six weeks old, good preparations are usually obtained after 20 minutes staining. Care must also be taken in differentiating, as there is apt to be considerable variation in the depth of color in different preparations. Wittek ('13) and Trautmann ('09) agree that, while the basophiles are to be found in all the domestic animals, they are as a rule, less distinct than the acidophiles.

While colloid substance is found characteristically in many hypophyses, there is, as a rule, very little if any to be seen in the hypophysis of the normal albino rat. As an exception, it does occur in considerable quantity and then is seen in the residual lumen of the pouch of Rathke, and in small masses in the vascular channels of the ventral glandular portion of the hypophysis but not in the other parts of the hypophysis.

TABLE 1

Average diameters of cells in ventral glandular portion of hypophyses of normal male albino rats at various ages; average of 10 in each case

| AGE | ACIDOPHILES | | BASOPHILES | | | RESERVE CELLS |
	Cell bodies	Nuclei	Cell bodies	Nuclei	Maculae	Nuclei
months	μ	μ	μ	μ	μ	μ
2	11.5 x 8.0	6.3 x 5.2	13.5 x 11.5	5.8 x 4.8	5.5 x 4.0	5.9 x 4.6
3	11.1 x 9.3	6.4 x 5.3	14.0 x 11.0	6.7 x 5.0	5.5 x 3.8	6.3 x 4.4
4	11.3 x 8.0	6.4 x 5.5	14.5 x 11.0	6.7 x 5.6	6.0 x 4.3	6.4 x 5.0
6	11.4 x 8.4	6.5 x 5.1	15.5 x 11.3	6.8 x 4.7	5.4 x 4.3	6.3 x 4.9
8	11.2 x 8.2	6.8 x 5.8	15.9 x 12.4	7.1 x 6.0	5.7 x 4.5	6.5 x 5.4

The size of the two distinct glandular cell types is shown in table 1 and also the size of the reserve cell nuclei. According to the measurements there is continued, though slight, growth of the basophiles throughout the period measured, but there is little change in the acidophiles. In one normal specimen, 136 days old, were found basophiles containing large vacuoles, and consequently of a ring shape. This has also been reported recently by Jackson ('17).

In the glandular cells is seen a cytoplasmic structure, the nature of which is at present under discussion. After the methods of fixation of Bensley and of Cowdry mentioned above in "Material and Methods," it is more evident than after fixing fluids containing acetic acid. I have referred to it in the description of the figures as the *macula*. It appears in these preparations

TABLE 2

Average diameters of normal and experimented acidophiles at varying periods after operation. Normal and experimented animals from same litter at each age. Average of ten measurements in each case

| TIME ELAPSED AFTER OPERATION | EXPERIMENTED | | AGE | NORMAL | |
	Cell bodies	Nuclei		Cell bodies	Nuclei
months	μ	μ	months	μ	μ
1	9.9 x 7.0	5.6 x 4.7	2	11.5 x 8.0	6.3 x 5.2
2	10.7 x 8.2	5.6 x 4.9	3	11.1 x 9.3	6.4 x 5.3
3	10.8 x 8.1	6.0 x 5.8	4	11.3 x 8.0	6.4 x 5.5
5	10.4 x 7.4	5.4 x 4.5	6	11.4 x 8.4	6.0 x 5.1
7	10.7 x 8.5	6.9 x 6.2	8	11.2 x 8.2	6.8 x 5.8

as a mass, sometimes rounded, sometimes elongated or irregular, separated by a lighter zone from the surrounding cytoplasm. It is always adjacent to the nucleus, and persists in the experimented hypophyses. After methods of preparation used for demonstrating the Golgi network, it shows, in favorable specimens, as a close-meshed reticulum. For this reason, I have considered it (Proc. Am. Assoc. Anat., Anat. Rec., January, 1917) to be the structure described as the network apparatus of Golgi. Gemelli ('00) has pictured a larger looser network in the cells of the hypophysis of animals other than rat, using the early method of Golgi for demonstrating this structure. Soyer ('12), however, has found a structure near the nucleus in the glandular cells of the human hypophysis, which he considers merely to be a large centrosphere, and in it he has stained the pair of centrioles. Deineka ('12) has found that in many cases where the position of the centrosphere is known, the position of the net-apparatus agrees with it (cells of Descemet's membrane, leucocytes, medullary cells of adrenal). Basile ('15) in renal epithelium finds support for the hypothesis of Barinetti, that the reticular apparatus and the centrosomes present a union, anatomical and functional.

In view of these several highly interesting observations, it has seemed best to term the body which one sees in the cells of the hypophysis, macula, until further information is forthcoming. In the basophiles the macula is much larger than in the acidophiles, and by measurement one finds that it increases with the size of the entire cell (table 3).

EXPERIMENTED HYPOPHYSIS

After castration there quickly ensues a reaction, which is discernible in a week, readily apparent in two weeks, and which is progressive throughout the life of the animal. Grossly, there is a color change which is due to the increased amount of blood in the vascular channels, and this hyperaemia no doubt contributes to the increased weight of the organ. Histologically, the first changes are seen in the basophiles, and in the reserve

TABLE 3

Average diameters of normal and experimented basophiles at varying periods after operation. Normal and experimented animals from same litter at each age. Average of ten measurements in each case

TIME ELAPSED AFTER OPER-ATION	EXPERIMENTED				AGE	NORMAL CELL BODIES
	Cell bodies	Nuclei	Maculae	Vacuoles		
months	μ	μ	μ	μ	*months*	μ
1	17.9 x 14.3	6.7 x 5.8	6.6 x 5.5		2	13.5 x 11.5
2	With vacuole 19.2 x 15.9	6.7 x 5.6	8.0 x 5.0	10.8 x 7.4	3	14.0 x 11.0
	No vacuole 14.7 x 12.9	5.9 x 5.5	7.0 x 5.6			
3	With vacuole 21.5 x 15.4	7.7 x 6.0	7.6 x 5.1	12.6 x 9.2	4	14.5 x 11.0
	No vacuole 15.7 x 12.5	6.7 x 5.8	6.2 x 4.6			
5	With vacuole 20.3 x 18.0	7.8 x 4.2	7.7 x 4.8	18.2 x 14.3	6	15.5 x 11.3
	No vacuole 13.2 x 9.7	6.3 x 4.7	4.6 x 3.8			
7	With vacuole 23.3 x 19.5	7.3 x 5.6	8.5 x 5.6	16.7 x 12.0	8	15.9 x 12.4
	23.0 x 18.8	largest vacuoles		19.0 x 14.7		
	No vacuoles 19.9 x 16.0	7.7 x 5.9	7.6 x 6.2	Coarsely granular		
	16.3 x 13.5	6.9 x 5.9	5.6 x 4.4	Finely granular		

cells. The former increase in size, and some of the latter begin to grow and to take on the staining reaction of the basophiles.

One month after castration (69 days old)

The condition arrived at one month after castration, is shown in figure 3. Comparison with figure 2, taken from the normal control animal of the same litter, shows the striking changes,

which have been produced due to the experimental procedure. The most evident effect is the increased size of the basophiles, and this, with their greater stainability, makes them much more conspicuous than in the normal sections. The basophiles, however, are not all of the same size, there being many of smaller diameter, and these are probably to be regarded as reserve cells, which have recently differentiated into basophiles. The acidophiles are numerous and very evident, and only slight changes from the normal were noted. The reserve cells show as groups of less conspicuous elements scattered throughout. Other changes, not shown in figure 2, are the increased diameter of the blood vessels and the constant presence of the colloid-like secretion in the residual pouch of Rathke.

Measurements of the basophiles showed them to average 17.9μ x 14.3μ, in contrast with the normal cells of the same age which measured 13.5μ x 11.5μ, but many individual cells in the experimented hypophysis exceeded this size considerably, and so helped to accentuate the impression of the large size of this type of cell. The nuclei of the cells showed a similar contrast between experimented and normal, measuring in the former, 6.7μ x 5.8μ, and in the latter 5.8μ x 4.8μ. By measurement it was found that the acidophiles were retarded slightly in their growth, averaging 11.5μ x 8.0μ in the normal, and 10.0μ x 7.7μ in the experimented. At 45 days of age, the normal acidophiles measure 9.6μ x 7.5μ, so that these experimented animals at 69 days of age, had acidophiles very slightly larger than the normal animal of 45 days.

Measurements of the reserve cells showed that their nuclei increased slightly (from 5.9μ x 4.6μ normally to 6.1μ x 5.1μ in the experimented). This probably has little significance, for while it may be due to a real growth of reserve cell nculei, participated in by all, it may be due to the inclusion in the cells measured of one or two cells which are beginning to differentiate and to grow into basophiles.

Two months after castration (98 days old)

At two months after castration (fig. 4) the differences already noted between normal and experimented hypophysis are increasingly apparent, and these affect all three types of cells. The basophiles are now the most conspicuous elements in the experimented hypophysis and in some of them vacuoles have appeared. These vacuoles are filled with a homogeneous secretion which stains like the colloid in the pouch of Rathke, and probably from these cells come the abundance of colloid, which is now seen in the lumen of the pouch of Rathke. There are two sizes of population recognizable among the basophiles, with, however, many of intermediate size. In general, those which have the vacuoles are the larger, but here and there is seen a cell without a vacuole, as large as those possessing it. The smaller basophiles have usually a more homogeneous cytoplasm and are again regarded as immature basophiles. The cells with vacuoles are still greatly in the minority. Some basophiles are more coarsely granular than others, and this seems to depend upon the age of the cell; i.e., the time which has elapsed since differentiation. Sometimes a cell is seen with many minute vacuoles (1 to $2\,\mu$) in one limited area. This probably represents a first stage towards the development of one or more large vacuoles.

Measurements of those with vacuoles show a mean size of $19.2\,\mu \times 15.9\,\mu$, while those without vacuoles measure $14.7\,\mu \times 12.9\,\mu$. The larger cells have slightly larger nuclei $6.7\,\mu \times 5.6\,\mu$ as compared with $5.9\,\mu \times 5.5\,\mu$ for the smaller. The vacuoles of the largest cells average $10.8\,\mu \times 7.4\,\mu$.

The acidophiles and the reserve cells of the experimented hypophysis are not so conspicuous as in the normal. Often when measuring acidophiles, there appear to be two sizes of population. Those seen along the margins of blood-streams have more cytoplasm, than those which are found in the cell-masses away from the blood vessels. It seems that this apparent difference may be, however, only a result of the cells having been cut in different planes. Measurements of the acidophiles show them to be not as large, on the average, in the experimented

hypophysis, as they are in the corresponding positions in the normal. Thus normal acidophiles of this age measured 11.1μ x $9.3\,\mu$ with nuclei $6.4\,\mu$ x $5.3\,\mu$, while the experimented acidophiles measured $10.7\,\mu$ x $8.2\,\mu$ with nuclei $5.6\,\mu$ x $4.9\,\mu$. There also seems to be a decrease in the staining of the acidophiles, and as they tend to become compressed between the enlarging basophiles, they now play a less conspicuous part in the lining of the blood channels. It should also be mentioned, however, that sometimes in the experimented hypophysis is seen an acidophile quite as large as in the normal hypophysis, and that in such a case, it is usually situated on a blood vessel.

Cell-divisions were found in some sections of both normal and experimented hypophyses of this age.

Three months after castration (135 days old)

At three months after castration, large basophiles with vacuoles are seen everywhere throughout the sections. One may observe cells showing intermediate stages in the growth of these large vacuolated cells. 1) Some cells contain one or more small vacuoles, their cytoplasm is coarsely granular, and the nucleus is of spherical shape. 2) As the colloid-like material increases, the nucleus becomes more eccentric and the main part of the cell is made up of the colloid-containing vacuole. 3) Finally, the cells become ring-shaped in section, the cytoplasm forming the periphery of the cell, and containing at some point a more or less flattened nucleus. In these cases nearly the entire cell is constituted of the colloid-like material. Although the basophiles possessing vacuoles are more numerous than before, they are still outnumbered by the non-vacuolated basophiles. As in the preceding age, just described, many of the non-vacuolated basophiles are little or no larger than normal basophiles of the control hypophyses. These are probably elements which have differentiated more recently than the large vacuolated cells.

The acidophiles are less conspicuous than in the normal, and this is due partly to their slightly diminished average size and partly to the less intense staining of the acidophilic granules.

In addition, there may be an actual lessening of the number of stainable granules in some of the cells. Along the blood vessels, the acidophiles usually still occupy more space than the basophiles, although in some localized areas they may be pressed away by the enlarging basophiles. A definite example of this was seen in sections of this age, in an area near the cephalic end, where the basophiles occupied the greatest proportion of the space, but the acidophiles were still there, although rather flattened by the pressure of the neighboring cells. Another exception to the usual cell type mixture, was seen in a limited area towards the caudal end, where there were continuous rows of acidophiles along the blood vessels, but no basophiles. This arrangement of cells was seen at other ages also, in this area. The reserve cell nuclei occur in small groups. These nuclei show a uniform distribution of chromatin, with usually a nucleolus. The cytoplasm is pale staining, finely granular and has indistinct boundaries.

Measurements of the large vacuoles in the basophiles show them to average 12.6 x 9.2 μ, as contrasted with 10.8 x 7.4 μ at two months after castration. The large cells containing these vacuoles measure 21.5 x 15.4 μ, with nuclei 7.7 x 5.9 μ. The smaller basophiles without vacuoles measure 15.7 x 12.5 μ, with nuclei 6.7 x 5.8 μ. The normal cells at the same age in the control animals measure 14.5 x 11.0 μ, with nuclei 6.7 x 5.6 μ, which is but slightly less than the small sized basophiles of the experimented hypophyses.

Measurements of the acidophiles do not show any great difference between the preceding age and this one. On the average, the experimented acidophiles measure less than the normal, but occasionally one finds a cell in the experimented hypophysis quite as large as the largest normal acidophile. One gets the impression that the stained acidophile granules are becoming fewer in many cells of the experimented hypophysis, and that these granules are not so closely arranged as in the normal.

Five months after castration (179 days old)

The vacuoles in the basophiles are still larger than at the three months after castration, and there appear to be more cells with vacuoles than before. However, there are still some of about the normal size and appearance, without vacuoles, which represent cells of younger growth, but doubtless destined to become vacuolated. There is a third variety of a basophilic type, perhaps intermediate between the two preceding varieties, which is nearly as large as many of the vacuolated cells, but is coarsely granular, or perhaps better described as filled with numerous very minute vacuoles. A definite group of these was seen near the cephalic end of the section, and individual ones scattered elsewhere. Along the blood vessels are still both basophiles and acidophiles, the former becoming progressively larger and more conspicuous, the latter becoming less conspicuous. The acidophiles really seem fewer, but this may be due in part to their separation by the large basophiles and also to being compressed by them. Sometimes when situated between several of these large cells they may be pressed into a triangular outline. At other places again, where they are seen to stain distinctly and are not compressed, they appear of about the same size as in the control. The vacuoles in the large basophiles measure 18.2 x 14.3 μ as contrasted with 12.6 x 9.2 μ at three months after operation. These cells measure 20.3 x 18 μ as contrasted with 15.5 x 11.3 μ in the cells of the normal control animal. Measurement of small non-vacuolated basophiles show them to be 13.2 x 6.7 μ, and these must be cells still growing. The acidophiles in the experimented animal measure 10.4 x 7.4 μ and in the normal animal 11.4 x 8.4 μ, and these measurements are not greatly different from those at the preceding stages.

Seven months after castration (246 days old)

The general appearance of the ventral glandular portion of the experimented hypophysis at this period (fig. 5) is dominated by the large number of vacuolated basophiles, distended with their colloid-like secretion. The presence of these large cells with the groups of smaller cells between gives it an appearance

which is very different from the more compact and uniform structure of the normal. Measurements of the largest vacuoles average 19 x 14.7 μ, and these are in ring-shaped cells, measuring 23 x 18.8 μ, which is again distinctly larger than at five months after operation. Of the other basophiles without vacuoles, two varieties were seen, some coarsely granular, others finely granular. The former measure 19.9 x 16 μ, and the latter measure 16.3 x 13.5 μ. In the normal, also, at this age the basophiles were found to be of two varieties, some having finely granular and others coarsely granular cytoplasm. Measurement of these two varieties showed the finely granular here again to be smaller than the coarsely granular (table 3). This suggests that this differentiation of the basophiles may be here an age phenomenon.

In most regions the acidophiles seem distinctly fewer and it appears that many of this type of cell have lost their characteristic staining and now appear more or less like reserve cells.

DISCUSSION OF OBSERVATIONS

In the albino rat hypophysis, as we have seen, there are three distinct types of cells,—(1) acidophiles, (2) basophiles, and (3) the remaining cells tentatively classed together as reserve cells (chief cells). These last during histogenesis and the early growth of the organ are continually being differentiated into the two functionating types. Small groups of these reserve cells are still present during mature life, and lie dormant until stimulated by the needs of the organism. The removal of the testes we have found to increase the size of the basophiles, and also the number of basophiles, and we believe these new basophiles to come from reserve cells. It is conceivable that, up to a certain time in the life history of these reserve cells, they are all similar and have an equal potentiality for becoming either acidophiles or basophiles. It is possible that this capacity persists after maturity is reached and throughout life, but it may be that at maturity each has acquired a tendency to become definitely of one or of the other type. In our observations, in addition to some reserve cells becoming basophiles, it has ap-

peared that some acidophiles lose their specifically staining granules and in consequence have the appearance of reserve cells. So that in the later stages after castration, the reserve cells may contain among their number many cells which are dedifferentiated acidophiles.

Fichera ('05), who was the first to study castration changes in the hypophysis, found increased size and weight of the entire organ, and histologically an increase in number and size of eosinophiles (acidophiles). The animals he used in his male series were domestic cattle, European buffaloes and domestic fowl. Wittek ('13) who worked with a large series of individuals of domestic cattle, agreed in finding that castration increases the weight of the hypophysis. Histologically, however, he differed. He described this part of the hypophysis as composed chiefly of two cell types—eosinophiles (acidophiles) and chief cells (reserve cells). True basophiles were only exceptionally seen. After castration he found no change in cell types and no increase of eosinophiles.

Cimorini ('08) in rabbits and dogs found increase in volume after castration, and an increase in number and size of the eosinophile cells.

Kolde ('12), made observations principally upon the female hypophysis, but as a minor part of his work also examined three normal male rabbits and one castrated rabbit. He does not indicate any different results in the two sexes, and gives his results as an increase of eosinophiles, and the appearance of special eosinophile cells, the protoplasm of which does not show the usual even stain but is filled with little red granules, perhaps colloid formation.

Marrassini ('10) and Marrassini and Luciani ('11) found no constant histological differences between normal and castrated dogs, sheep, domestic cattle, rabbits and Guinea-pigs. In domestic fowls, however, they found, after complete castration, decided changes. There was more or less increase in weight, but hyperaemia was not constant. In the normal fowl they found a relatively small number of eosinophiles. In the capons the eosinophiles had not changed in number, but in addition

there was a number of large elements present, containing spherules of varying size, sometimes larger than the nucleus. The contents of these spaces had a marked electivity for acid colors.

Kon ('08) described a human case. This was of a 32-year old man, examined three years after total castration. He found that the chromophobe cells (reserve cells) had enlarged, so that they had very abundant cytoplasm, and that these cells were present in usually large numbers.

Aside from the inconclusive experiments of Barnabo ('08), the only investigation on albino rats which we have seen reported is that of Zacherl, who worked in Biedl's laboratory. Biedl ('12) describes his work briefly. He found grossly an increase in volume of the organ, and histologically a diminution in number of acidophiles as contrasted with chief cells. But above all there was a peculiar new cell formation, which he called castration cells. This was an especially large pale cell, with pale stained nucleus, with cytoplasm finely granular and filled with very fine vacuoles. He pictures these at two months after castration, showing the beginning of vacuolization.

Consideration of these observations shows differences which may be attributed to several causes. Where several investigators obtain different results from their studies upon the same form, it is evident that the methods and carefulness of the investigations must have varied. For instance, in domestic cattle, Fichera found relative increase in weight and increase in size and number of eosinophiles; Marrassini found no increase in weight and no constant increase in eosinophiles; while Wittek found increase in weight, but no constant change in the number of eosinophiles. In such a situation, one is apt to be most favorably impressed with the most recent work, as the latest investigator has probably tried to avoid the errors of his predecessors. In domestic fowl, however, Fichera and Marrassini are in practical agreement, finding relative increase in weight and increase in size of one type of cell. They describe this type of cell somewhat differently, but it would seem that the material on which their observations are based must be very similar..

Most investigators have found hypertrophy of some type of cell. The majority call it eosinophile (acidophile). In the rat, I have considered it the basophilic type and that the new basophiles have come from the reserve cells. Kon, in his human case, thought it was the chief cells (reserve cells) which he saw enlarged, and this seems similar to what I have seen in the rat. Marrassini and Luciani, in the domestic fowl, describe the enlarged cells as having "granular protoplasm, stained fundamentally by hematoxylin and containing a variable number of globules of different sizes, strongly stained by eosin. Between these elements and others of the hypophysis, there are intermediary forms which make one think of the possibility of reciprocal transformation." From this description, it would seem that the castration changes in the domestic fowl may be somewhat similar to those in the albino rat.

Several find droplets of a secretion appearing in the enlarged cells. Thus Kolde in the rabbit, found little red granules which he thought might be colloid formation, and Marrassini and Luciani in the domestic fowl found the enlarged cells containing a varying number of globules. These we have seen in the enlarged cells of the rat hypophysis, and believe the large vacuoles which appear so strikingly in the basophiles to be filled with an accumulation of the same substance. It is a colloid-like secretion, we infer, because of its similarity in appearance to the homogeneous colloid substance which appears in the lumen of the pouch of Rathke at the same time.

Zacherl's results ('12) on the white rat show the same early changes which we have described, although his interpretation is somewhat different. He looks upon the cells which enlarge as a new cell type 'castration cells' and does not attempt to trace their development.

Thus, in the various animals examined, even allowing for the several techniques employed, it would appear that the act of castration does not produce the same reaction in the hypophysis. It is generally believed, however, that the balance of the endocrine glands may be different in different animals, and the results of this form of experiment (removal of one of them) lend support to this view.

RELATION BETWEEN INTERSTITIAL CELLS AND HYPOPHYSIS

Since Bouin and Ancel ('03 and later) brought forward strong evidence for the hypothesis that the interstitial cells of the testis represent a gland of internal secretion, it has become the general belief that it is primarily the loss of these cells which gives rise to the castration effects. This is supported by the results of experiments in which the vasa deferentia are tied. This leads to cessation of spermatogenesis, but not destruction of the interstitial cells, and in these animals there are no castration effects. From the point of view of these observations we must regard the loss of the secretion of the interstitial cells by castration, as affecting the balance of the endocrine organs, and in the rat it seems to be chiefly the hypophysis which is stimulated.

SUMMARY

1. Castration in the male albino rat produces definite histological changes in the structure of the ventral glandular portion of the hypophysis (pars anterior). These include changes in the glandular cells, increase in size of blood channels and marked production of colloid.

2. The cells of this portion of the hypophysis are, in the classification here used, acidophiles, basophiles and reserve cells. After castration the basophiles increase in size and number. The largest ones begin to become vacuolated at two months after operation. During succeeding months the number of vacuolated cells increases; the largest ones become ring-shaped, with cytoplasm and nucleus at the periphery, and the central part made up of the colloid-containing vacuole. The acidophiles are not much affected at first, except for a slight diminution in average diameter. Gradually some of them show diminished number of granules and lessened stainability. At seven months after castration the number of distinctively stained acidophiles is decidedly reduced (fig. 5). Some of the reserve cells in the first months after castration appear to become basophile cells. In the later months some of the acidophiles appear to gradually dedifferentiate into reserve cells.

3. The basophiles, after certain fixations, show a large cyto-plasmic body (macula), which may be the reticular apparatus of Golgi, or centrosphere, or both combined. The acidophiles show a similar structure, much smaller in size.

4. The experiments afford strong evidence for the view that there are at least two actively functionating types of cells in this part of the hypophysis and that both of these are derived in histogenesis from a less differentiated form (reserve cells). Some of the latter persist during mature life, and serve as the source of supply for the other two types.

In conclusion, I wish to thank Professor Piersol for advice and criticism, and Professor Donaldson, of The Wistar Institute, for constant interest in the study. I also wish to acknowledge the kind assistance of Mr. E. F. Faber, instructor in art in the School of Medicine, in the preparation of the drawings.

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a, acidophile cells   *c*, reserve cells
b, basophile cells   *m*, macula
b′, basophile cells with vacuoles   *n*, nucleus

Ventral glandular portion of hypophyses of male albino rats, normal and experimented. All drawings made from the region (x) indicated in figure 1, p. 444. Figures 2 and 3 were made from preparations fixed in neutral formol-Zenker and stained with Mallory's aniline blue-orange G. Figures 4 and 5 were made from preparations fixed in neutral formol-potassium bichromate, and stained with Mallory's aniline blue-orange G. Outlines were drawn with the aid of a camera lucida, using Spencer apochromat lens, 1.5 mm. and Spencer compensating ocular, No. 5. The drawings were made at a magnification of 700 diameters and reduced in reproduction to 600 diameters.

2. Normal hypophysis of male albino rat, two months old.
3. Hypophysis of rat of same age as in figure 2, one month after castration.
4. Hypophysis of rat, three months old, two months after castration.
5. Hypophysis of rat, eight months old, seven months after castration.

THE RECOVERY FROM DEPRESSION IN THE PURKINJE CELL AND THE DECLINE TO SENILITY OF DEPRESSION: WITH THE INCIDENTAL HISTOGENESIS OF ABNORMAL PIGMENTATION

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CONTENTS

This study completes the immediate and the remote cycle resulting from the depression of function, of which the retrogressive phase has been previously presented ('13).

DATA OF EXPERIMENTS

All depressant agents produce a common reaction. The choice was therefore governed by convenience. The aim was to produce the maximum of depression which experience had taught was compatible with safe recovery. The animals were known to be young.

Ether

For these experiments, I am indebted to Mr. E. E. Butler ('16), who kindly performed them for this study of recovery in connection with his work on immediate depression.

Experiment anesthesia 13. Dog, female, 4 kg., aged 2 years. Six periods of administration, averaging over 4 hours and $\frac{1}{2}$ pound of ether each. Three administrations from 1 to 3 days apart, then a rest of 10 days, followed by the remaining three. Duration of exeriment 24 days. Recovery period after last administration 6 days.

Experiment anesthesia 14. Dog, male, 9.58 kg., aged about 3 years. Nine periods of administration, averaging $4\frac{1}{2}$ hours and one pound of ether each. Five administrations 1 to 3 days apart, a rest of 18 days, then the remaining four. Duration of experiment 45 days. Recovery period after last·administration 13 days.

Experiment anesthesia 16. Dog, female, 8 kg., aged 3 years. Five periods of administration in 41 days. Followed after one week by a series of morphine injections carried over 67 days to the number of 30. Dose was raised from 0.04 to 0.2 gram at the end. Duration of experiment 170 days. Recovery period after ether 128 days, after morphine 52 days.

Morphine

Experiment morphine 4. Rabbit, 1.5 kg. Administered hypodermatically over a period of 71 days, somewhat irregularly but usually every other day. Dosage raised from the initial 0.007 to 0.133 gram per kg. at end. Duration of recovery after last dose 42 days.

Experiment morphine 6. Rabbit, 1.7 kg. Administered hypodermatically every day for 47 days. Dosage raised from 0.020 to 0.035 gram per kilogram. The same dosage though lowered toward the end killed a companion rabbit (Morphine 5) when the administration was stopped. Duration of recovery 28 days.

Heat

Experiments heat 11 and 12. Two rabbits carried simultaneously in a hot air oven. There were eight exposures, on successive days, ranging from 1 hour to 2 hours and 45 minutes, at a temperature around 45°C. The exhaustion each time was moderate, never destroying an immediate interest in food. For profundity of reaction there is no agent so effective as heat. Experiment 11 was allowed to live 21 days and Experiment 12 six months.

Axone section

Much information is owed to the study of the cutting of Purkinje cell axones made by W. D. Davis in this laboratory (unpublished thesis for the Master's Degree, '14). The degenerative changes have been identified anatomically as depression phenomena, and physiologically are to be correlated with the loss of functional tonus and of active functional stimuli. The changes

are not due to the injury, the most prominent secondary factor, for mechanical stimuli are known never to produce depression, but only its opposite, excitation. There were nine experiments on dogs, followed from 2 to 27 days. At the latter time recovery, in such cells as may recover, namely, not completely isolated by the section, is for the most part well advanced. In isolated cells, death appears to take place before direct or collateral connections are reëstablished.

Microscopic technic

The fresh material was fixed in saturated corrosive sublimate plus 2 per cent formaldehyde, in alcohol, in formalin, and in vom Rath's fluid. Sections were stained by erythrosin-toluidin blue after mercury and alcohol, Grenacher's borax-carmine and iron hematoxylin after mercury, neutral red (Johnston, '16) after alcohol and formalin, and Delafield's hematoxylin and eosin after vom Rath.

THE RECOVERY WITHOUT OR WITH TEMPORARY DISORGANIZATION

Just as functional activity is the quantitative opposite of depression, so the process of recovery from depression is one of contrast to the process of recovery from activity. Without reference to its contrasted state, no interpretation as a process could be made, and certain changes indeed would probably appear meaningless or not worthy of regard. The morphological contrast between activity and depression may first be summarized. It must be remembered that on account of the initial shifts of size and chromatin in activity, this is not an inclusive comparison.

Functional activity	Functional Depression
Cytoplasm	
Size: Progressive increase of volume	Absolutely smaller stage for stage.
Structure: Edematous	Compact, granular: albuminous and glycogen deposit.
Chromatic content: Dechromatization only as end result.	Dechromatization of any stage, resting as well as active.

Nucleus

Size: Relatively small to volume plasma.	Relatively large to volume plasma.
Structure: Vesicular, edematous.	Dense, packed.
Chromatin: Progressively diminishes (absorbed by plasma at expense of nucleus).	Hyperchromatism stage for stage (increase at expense of plasma).
A decrease relative to the cytoplasmic content.	Absolute increase over activity, relative increase to its cytoplasm.
Karyosome: Karyorrhexis and karyolysis with plasmic resorption. Karyolysis predominates.	Karyorrhexis and karyolysis without plasmic resorption. Karyorrhexis predominates.
Nucleolar substance: Progressive diminution.	Increase relative to activity stage for stage.

Terminal relations

Upset of the nucleus-plasma relation in favor of plasma.	Upset of nucleus-plasma relation in favor of nucleus.

Functional activity ends in nuclear exhaustion. The residual chromatin and the nucleolar substance are used up. Its recovery therefore involves the necessarily slow resynthesis of these substances. But as fast as the synthesis occurs the cytoplasm exacts its complementary share of chromatic substance, resorbs it. It makes no difference to the essentials of the process whether this resorbed chromatic substance, the Nissl substance, be identical with nuclear chromatin or merely an allied derivative (Dolley, '13 a, p. 532, '13 b, p. 106). The restoration of Nissl substance, which progresses slowly, and the loss of water—shrinkage to normal size—are the initial features of recovery. On the contrary, the most conspicuous and prolonged feature is the lagging of the nucleus, both in size and intranuclear chromatin. Usually not until the cytoplasmic content is complete does the residual chromatin cover the nucleolus to form a karyosome (Dolley, '11).

It will be clearer to examine the state of a depressed cell which falls short of degenerative change before stating the morphological indications of recovery. The nucleus-plasma interchange is blocked. The breakdown of the reciprocal relation is due to the cytoplasm. It fails to furnish proper materials to the nucleus, the lack of synthesis being demonstrated by the

deposition of raw food material.   It fails to resorb the chromatic substance from the nucleus, hence there ensues a progressive diminution of that substance as it is used up without adequate restorative effort, which leads to absolute dechromatization. Being of such genesis, depression can effect the resting as well as the active cell.

The nuclear picture is of a cell which for its functional stage shows an increase in measured volume and objective substances. To account for this there is in the first place the chromatin, because some of what is synthesized of preformed material is not resorbed.   Second, the nucleolar substance at the least holds its own because further synthesis in which it is consumed has stopped for lack of the cytoplasmic compounds.   There is evidence, in addition, that the nucleolar substance is actually increased and that the nucleus synthesizes this itself (Dolley, '14, p. 489, in agreement with Verworn, '91).   The nucleus stands, therefore, well-prepared; it is the cytoplasm which fails to reciprocate.

The plasma picture is one devoid of basic staining substance, murky, reddish and granular from the albuminous deposit.   It is not only relatively small to its nucleus but absolutely small in comparison with its normal stage on account of the loss of chromatic substance and failure of continuous primary synthesis.

The earliest and most striking feature of recovery is the return of the extranuclear chromatic (Nissl) substance.   This is intelligible in the light of the nucleus-plasma relation and the state of the nucleus.   As soon as the plasma begins to recover its resorptive and its synthetic powers, it draws on the accumulated nuclear chromatic substance, and also finds ready abundant nucleolar substance for the immediate new synthesis of chromatin.   This fact of early return of chromatin when referred to the state of the nucleus corroborates fully for the depression cycle the R. Hertwig ('02) theory of chromatin formation.   It is the diametric opposite of recovery from function, which is slow because it involves primarily the synthesis of nucleolar substance.

For example, in Experiment Anesthetic Recovery 13, one-half of the six periods of prolonged etherization were separated from

the other half by ten days. Either of these three exposures would have produced a profound depression in all cells with some degenerative effects according to the results of Butler ('16) from this laboratory. Yet six days of recovery from a reduplication thereof was sufficient for a complete return, in fact if anything a return in excess, in all cells which were not seriously affected organically. More significant, cells which by their atrophy showed the more serious effects of depression exhibited usually a complete renewal of extranuclear chromatic substance. These observations apply universally so far as chromatic recovery is possible.

Considering the karyosome, after exhaustive activity it is frequently the very last organelle to be restored. Some depression is almost invariably associated with karyorrhexis and karyolysis of the karysome. Yet in the above animal, recovery of the karyosome was almost universal, even in the severely affected cells. The total result of the rapid chromatic recovery with the clearing up of the plasma would be to give a fictitious idea of its completeness were relative sizes to be disregarded.

Recovery of plasma volume is obviously the slowest phase. It is best determinable in those cells in which the depression went to a structural disorganization involving the nucleus. In them, though the chromatic restoration approximates the proper one, both plasma and nucleus are shrunken, frequently irregular, but the plasma is plainly relatively smaller. Measurements, which were applicable to depression itself, functional stage by stage, are out of the question to demonstrate this because of the widely varying degrees of atrophy and other involvement within the stages. But working from the pronounced manifestations, instances of a nucleus with a disproportionately small plasma for every stage of function which became severely depressed are plain after chromatic recovery to one experienced in relative cells sizes. It is probable, however, that the inconsiderable shrinkage of a physiological depression in the strictest sense is rapidly recuperated from, although beyond observation.

These changes of relative and absolute cell atrophy are the first and may be the mildest indication of a permanent structural

disorganization. They are cited here for the light they shed on the regular course of complete recovery.

The contrast between the recovery from exhaustive functional activity and that from depression may accordingly be summarized as follows:

Recovery from activity	*Recovery from depression*
Cytoplasm	
Size: A shrinkage plus renewal of substance; usually the first feature of complete recovery.	A growth, renewal of substance only; the last feature of complete recovery.
Chromatic content: Relative to depression slowly. Second feature or associated with size.	The first feature of complete recovery—a nuclear phenomenon.
Nucleus	
Size: A growth of materials; slow to recover.	The excess materials are reduced by renewed cytoplasmic resorption and further synthesis.
Karyosome: The last feature of recovery.	An early feature, probably along with cytoplasmic restoration of chromatic substance.
Chromatin independent of karyosome: Where it normally appears a relative deficiency until the end of the process.	Reduction of chromatin in excess.
Nucleolar substance: Slow, a synthetic growth.	Recovery proceeds on an unaffected or increased absolute amount.
Time relations	
Measured in weeks and months—the nucleus in disadvantage.	Measured in days as regards chromatic substance—the nucleus in advantage.

THE RECOVERY WITH PERMANENT DISORGANIZATION (SENILITY OF DEPRESSION)

A permanent cellular disorganization rests clearly on the anlage of acute depression. The antecedent of the permanent state in this experimental work is the degenerative or better necrobiotic climax of severe depression. The term degenerative is only used to apply to the continuation of the same physiological process, though to some degree finally a modified process, not in the sense of a fixed pathological condition. From the principles in-

volved, it seems safe to deduce that a mild chronic depression, never reaching such an extreme degree, would end the same way.

The earlier conclusion that karyorrhexis is not flatly degenerative (Dolley, '13) is confirmed by the early recovery of the amphinucleolus.

The changes which unless interrupted proceed to cell death are: a shrunken, homogeneous, eosinophilic and hyaline-like plasma. Corresponding to this is a likewise shrunken hyperchromatic nucleus, if in the Hodge stage of normal shrunken nucleus, still more shrunken. So far it would maintain the relative excess of nuclear materials. If more advanced, solution of the nuclear membrane follows, and the nucleus is no longer organically isolated from the cytoplasm, though the presence of chromatic substance proves the existence of nuclear material. The last stage is the loss of this chromatic factor, and the cell is reduced to a hyaline-like remnant. From any one of these varied states, then, more or less recovery is possible.

The decrease of chromatic substance

It must be remembered further that tinctorial methods do not demonstrate even at the last point the complete disappearance of nuclear material in the shape of nucleolar substance, an acid reaction in common with the plasma. To preclude the possibility of some recovery, the anucleated appearance must stand for an absolute reality. This consideration possibly explains some states of least possible restoration. For example, formless remnants of cells appear in all recoveries without any perceptible localized nucleus, yet containing scattered basichromatic granules. The tinctorial reaction of these granules, if paler, suggests a basis of nucleolar substance, that is, their usual composition. This in one way presents no difficulty for the suggestion has been made elsewhere that the extranuclear chromatic synthesis may happen within the plasma (Dolley, '13). The potentiality of further recovery, however, involves the same problem of nuclear relocalization that exists in less atrophic cells with loss of the nuclear membrane, and will be discussed under the latter phenomenon.

It follows, therefore, from the preceding discussion that the permanent involvement of the chromatic substance is a relative, not an absolute one. The production of chromatic substances, however minimal, ceases only with loss of the nuclear substance, and hence with cell death. This belongs to the quantitative principle which governs the functional reaction.

The minimal of chromatic production, as it exhibits itself after about two months of recovery after prolonged depression (ether and morphine), is reduced to the vanishing point. Only in some of the depressed hyperchromatic stages in terms of the normal functional cycle does any extranuclear chromatic substance appear. Mainly only cells of such type show intranuclear chromatin, and field after field is characterized by no chromatic substance whatever. It is obvious that the cells are working on such a quantitatively lower levee of production that all is consumed as fast as made. On the other hand, there is the presumption with more time of some greater restoration proportionate to the degree of permanent involvement.

Cell death and cell atrophy

Of permanent changes cell death is the absolute one. The disappearance and irreparable damage of cells are to be traced through acute into chronic states. Though reached by opposite processes, functional activity and depression of function have finally this end in common, just as immediate recuperable extremes of either are characterized by a common functional incapacity—exhaustion or a complete metabolic standstill.

Cell atrophy, grading as it does to cell disappearance, is a permanent change, though a relative one. Its initial phase in the plasma has already been discussed. Set against acute depression, it is clear that atrophy of the nucleus, if it occurs—and depression goes far without it—results from actual nuclear disorganization. Yet, though this might appear to necessitate the ending of the characteristic nucleus-plasma relation of depression in favor of the nucleus, that is not the case. In imperfect recoveries, the nucleus, though atrophic, undoubtedly holds the relative ad-

vantage, though on a lower level. It is true that the volumetric estimation of it must be a matter of judgment, not of measurement, but it is sufficiently demonstrated aside from that by the relative excess of nucleolar substance, and by plasmosomes, in addition to the nucleolar basis of the karyosome, even without the excess of chromatin which may occur. This is in conspicuous contrast with senility of activity, for any atrophic cells of corresponding grade would almost surely lack either element of the amphinucleolus (Dolley, '11). Incidentally, the packing of the nucleus with nucleolar substance is the marked feature of chronic depression next to lack of chromatic substance in the plasma.

Thus in the cell atrophy, which in some way represents disorganization, there persists the index of the original process which is physiological. So far at least, in respect to function, the essential permanent changes of depression maintain the quantitative principle which has subtended all other functional states. Only the absolute level and the relative level of plasma to nucleus are lowered, it may be to the vanishing point.

Reorganization of the nuclear membrane and its failure

The next phenomenon is the hold over into chronic depression of an undemonstrable nuclear membrane. This is best seen after one to four weeks of recovery. Cells appear, without much atrophy, and with some restoration of chromatic substance in the plasma, though far from the standard amount, whose nuclear materials are plainly localized in their lighter eosinophilic character (nucleolar substance), but merge without demarcating line into the plasma. On the other hand, from the fact that after more prolonged recovery such appearances are lacking in similar cells, the nucleus is able to reorganize a perceptible protective covering.

The nuclear membrane is generally conceded to be a distinct, individualized structure, difficultly soluble and resistant to ordinary reagents (Heidenhain, '07, p. 132), but there is no question of the phenomenon of its solution in depression. It has been noted in all studies of the morphology of severe depression (R. Hertwig, '04 and Howard, '08, for Actinosphaerium; Calkins, '04 and

Popoff, '07, for Paramaecium; Howard and Schultz, '11, for tumor cells; Dolley, '13).

If one could apply to the nerve cell the work of Albrecht ('02) which substitutes the conclusion of a 'fluid partition wall, neither mixing with the cytoplasm nor with the nucleus, for the usual conception of an individualized, contingently porous, not fluid, or even doubled nuclear membrane' (Gurwitsch, '04, p. 20), the process of its being laid down anew would be hypothetically more easily conceived. Further, it is stated by Gurwitsch, who cites Albrecht's work at length, that this different partition layer, comparable to the ectoplasm of the amoeba, must be able both to differentiate itself from the nuclear substance and to mix with it again.

Does this loss of nuclear integrity become permanent in chronic states? Here belong the formless knobs of atrophic anucleated cells already mentioned, with isolated chromatic granules. Certainly the anucleated state is found after months of recovery, but such a state may be only a phase of a continuous retrogression to death, not relatively more permanent. On a final permanent inability to relocalize a nucleus, though nuclear materials are present, I am not able yet to speak positively, as it is a unique phenomenon. Objectively and theoretically, I am inclined to believe it possible and compatible with function for some little time. Search through the ordinary sources for information on this point has been fruitless. This is rather to be expected, for it falls without the domain of normal cytology, and further for the nerve cell in which the Nissl substance is not yet conceded generally to be of nuclear origin, the presence of that substance without a formed nucleus would not suggest the possibilities.

The potentiality of recovery from depression as compared with that from organic exhaustion

Depression, both acute and chronic, would appear to have a greater potentiality of recovery than organic exhaustion, even to the senile state. The more rapid restoration of the chromatic substance has already been discussed. In this, as in other

features, the problem relates to the less involvement of the nucleus relative to the plasma in depression than in activity. This nuclear relationship, even in advanced necrobiosis or in permanent disorganization, brings about a condition quantitatively analogous at least to that existing in the developmental stage of the nerve cell. The cell emerges from the divisional phase and enters on its function with a large nucleus but a minimal plasma. Functional growth of both elements occurs, but vastly greater in volume and content in the plasma. The nucleus must preserve a similar influence in depression, and one would predicate, therefore, the possibility of considerable plasmatic functional growth with any existent nucleus, while such a nucleus itself is also capable of further functional growth. What its limits are from a minimal point must remain yet undetermined. The consideration does, however, explain the marked recovery after some time over the known effects of acute profound depression and would lead one to expect still greater recovery, say after years, if the depressant agent had ceased to act. On the other hand, after some point of retrogression, particularly with a continuously acting depressant, perfect recovery becomes impossible just as in organic senility.

Only such an irreparable damage can be declared a senility of depression. The factors of permanence may be summed up as follows, and at the same time compared with senility of activity.

Senility of activity	*Senility of depression*
Structural end result	
Disappearance of cells. (Exhaustion.)	Disappearance of cells. (Functional or disuse atrophy.)
Cell atrophy. (Nucleus relatively small to plasma.)	Cell atrophy. (Nucleus relatively large in volume and content.)
Chromatic substance	
Nuclear chromatin: Absolute decrease from normal; decrease relative to chromidial apparatus.	Absolute diminishment in less degree: proportion to plasma greater.
Karyosome: Disappears earlier.	Tends to persist very late.
Chromidial apparatus (Nissl substance): Absolute decrease; proportion to nuclear chromatin greater than senile depression.	Absolute decrease in greater degree: relative decrease to nuclear chromatin.
No change tinctorial reaction.	Definite change possible.

Nucleolar substance

Absolute decrease from normal. Relative decrease to senile depression.	Absolute decrease in less degree; proportion to senile activity greater.
No pigment ever occurs. (Purkinje cell.)	Pigment deposit the final result.

THE HISTOGENESIS OF ABNORMAL PIGMENTATION

In connection with pigment formation, a peculiar phenomenon of modification or alteration of the chromatin is first to be noted. There was described in the discussion of acute depression (Dolley, '13 b, p. 90) the analogue of this change. Appearing best in hyperchromatic cells—from the excess of chromatin which would show it, usually confined to the nucleus, though perhaps extending in places to the plasma—the deep blue color became a light grayish or steel blue. It was found with toluidin blue only but invariably in depression. The correctness of the interpretation as a phase of acute depression is confirmed by the following observations after prolonged recovery.

The nucleus, or somewhat more commonly here, the nucleus and the plasma about the nucleus lose their structural markings in a yellow or yellowish brown diffused or washed look with toluidin blue after mercury or alcohol. If the plasma is involved, the nuclear membrane is indistinct or lost and the nuclear site generally visible only as a lighter place. The presence of actual chromatic granules may mottle the appearance of the plasma with darker specks or streaks. Some granules may even stain blue in the mildest plasmatic form, while in the extreme the whole cells become homogeneous, yellow, and somewhat glistening, with no trace of nucleus. As in the acute analogue, the condition in its extreme predominates in relatively hyperchromatic cells in terms of their functional stage (stages 3 to 5). It is, however, apparent in all functional stages, it may be only as a browning of the now achromatic amphinucleolus. Iron hematoxylin reproduces the alteration as a diffused rusty brown of the same distribution. As to its extent, the severer and more prolonged Experiments Anesthetic 16 and Morphine 4 showed

the alteration in most pronounced degree and most frequently. In the ether-morphine dog, there was a peculiar phenomenon. Only by prolonged overstaining could any chromatic substance be demonstrated even in the nucleus. After several days these diffuse blue nuclei were found changed to the typical brown of the rabbit.

The color at once suggests an associated pigmentary deposit. Accordingly, a fine amorphous light brownish pigment was identified in many cells after all fixations in stained and unstained sections in both cytoplasm and nucleus. However, the gross amount of changed material is far from being entirely formed pigment, and represents a transformation of the chromatic substance only demonstrable by staining. It is also to be noted that pigment occurs in the cells of the granular layer and extracellularly.

The possibility of extraneous matter simulating the pigment. which is very troublesome where the pigment is scant and scattered, was controlled as fully as was feasible. Unstained sections were also mounted in xylol and in water, and the mercury material subjected to a second prolonged iodin treatment in sections.

There is no doubt that the pigment is derived from nuclear substances. The observations above of the primary location within the nucleus predicate that. On account of the uncertainty which may still exist as to the nature of the so-called Nissl substance, the genesis within the nucleus is fortunate with respect to the genesis of the extranuclear deposit. For under the conception of the extranuclear chromatic substance as chromidial apparatus, extranuclear functioning nuclear material in the sense of Goldschmidt ('04), which all the evidence obtained by me corroborates for the nerve cell, one deals with a common origin of pigment in both plasma and nucleus. The demonstration may be made more conclusive by citing the evidence for the nerve cell in terms of that from other sources.

R. Hertwig ('04) described the transformation of chromidia into pigment in Actinosphaerium eichhorni in the state of depression. By the term chromidia, he designated excessive chromatin which had been extruded from the nucleus to reduce

the hyperchromatism of depression. It is a phenomenon then of reorganization necessary for the restoration of the normal nucleus-plasma balance. Howard ('08) verified Hertwig's findings on the same animal under the same conditions, and contributed the further observation of pigment transformation within the hypertrophied and hyperchromatic nuclei themselves.

The parallelism with the nerve cell is definite in a common state—depression, acting on a common source—nuclear materials, with a common result—pigment. However, before going farther the reader must, to avoid a misunderstanding, remember the distinction between chromidia and chromidial apparatus: extruded nuclear material of no functional value, against functioning extranuclear material, a specialized organelle of actively functioning cells. Chromidia as such have probably no place in the nerve cell. The resorption of nuclear materials by the plasma is a normal process in that cell devoted to the upbuilding of the chromidial apparatus, and hence even in profound depression any interchange of nuclear materials follows that process. The point of resemblance in this respect to the cells mentioned is rather that in profound depression on account of the blocking of interchange the material within the nucleus, while potentially functional, is actually in a useless position, and in this restriction it corresponds to the chromidia forming nucleus in possibilities. Hence it is that pigmentation in the Purkinje cell is more essentially an intranuclear phenomenon. However, the fate of cytoplasmic nuclear materials, though not chromidia in process, appears modified by the disorganized state of the plasma, and this permits their transformation into pigment which will be discussed. Further it may be that the change in tinctorial reaction leading to pigmentation described around the nucleus is significant of the plasmatic pigment being itself in part a resorption product.

Further observations in the same category as the preceding on the genesis of pigment from nuclear materials are as follows· Rössle ('04) has traced, in the cells of melano-sarcoma, the extrusion of nucleolar substance and its transformation into pigment in the cytoplasm. Howard and Schultz ('11) have also noted the occasional transformation of chromidia into pigment in

tumors derived from nonpigmented cells.   Again in their work
the process emanated from a state of depression.

For cells characterized by chromidial apparatus, the obser-
vations of Schultz ('12) on the formation of pigment in the dermal
chromatophore are especially illuminating and conclusive. Schultz
regards the chromatophore as a "highly differentiated cell whose
normal function is the formation of pigment; the process is the
result of specialized physiological activity."   The process begins
in the undifferentiated mesoblastic cell by the extrusion of chro-
matin leading to the formation of a functional chromidial net.
Later the chromatin in the cytoplasm becomes changed into a
material which has the staining reactions of nucleolar substance.
Further change leads to the transformation of this material into
pigment.   However, Schultz believes that probably specialized
plasmatic agents, chemical or catalytic, take part in this trans-
formation in the specialized chromotophore, because none or a
very small proportion of the extruded chromatin is transformed
into pigment in other cells.

The parallel between the process in the nerve cell and in the
dermal chromatophore is sufficiently obvious, especially within
the nucleus of the former.   The evidence for the nerve cell corrob-
orates Hertwig's idea ('02), which Schultz likewise follows in
his deductions, that the function of the nucleolar substance is the
synthesis of chromatin by uniting to itself substances derived
from the cytoplasm.   When therefore the chromatin within the
nucleolus or preferably karyosome of the nerve cell is used up or
breaks down, as occurs respectively in exhaustion and in depression
(karyolysis and karyorrhexis) the basic stain gives way to the
acid stain of the nucleolar substance—the true nucleolar remnant
of that amphinucleolus.   Consequently, the browning of this
nucleolus, which was noted in the description of chronic depression,
follows exactly the sequence of the chromatophore.   The chro-
matin breaks down to nucleolar substance and this is trans-
formed into pigment.   More simply, the direct transformation
of the nucleolar substance outside of the karyosome is shown
by its replacement by pigment granules.

To what extent the full process takes place in the cytoplasm I am unable to say for this type of nerve cell. The pigment might come through the full sequence or through the nucleolar substance alone, which must be extruded in line with other cells under special conditions and which perhaps in this case is unemployed in synthetic reactions from the cytoplasmic breakdown. The usual description of chromidial extrusions of whatever nature is that of Schultz's chromatophore,—the breaking down of the chromatin leaves a basis of nucleolar substance. Most probably that is true for the constitution of the chromidial apparatus of the nerve cell as the tinctorial reaction in one micron sections positively suggests, but it possesses this peculiarity that in its normal reaction the chromatic granules disintegrate completely. That is, as the cell approaches functional and chromatic exhaustion, and consequently a paleness and a watery dilution which would permit the observation of a residue of nucleolar substance for the individual granules, if it remained, it · is not apparent by ordinary methods. This is only to be expected from the nature of its specialization, and as a matter of fact accords with the absence of pigment from its normal function which will be discussed. So the problem is of no moment under normal conditions.

In depression, however, it very probably happens that a breaking down of the chromatic granule with a residue of nucleolar substance occurs in the disorganized plasma, as it does prematurely in the nucleus. The browning of isolated chromatic granules which is to be seen is suggestive of the full sequence. Unfortunately, the possibility of origin from nucleolar substance alone is complicated by the coincident occurrence of other eosinophilic material, as for example, the albuminous stuff, and by a diffuse red staining of the whole mass. In the light of the facts stated, however, it is fair to assume that part of the acid reaction is due to nucleolar substance, and to trace the origin of cytoplasmic pigment from this source as well as from chromatic granules themselves and possibly from nuclear extrusion. Its scantiness, however, indicates how slightly the normal fate of extranuclear materials is disturbed.

While I have thus attempted to give in detail the presumptive sequence of the development of cytoplasmic pigment, it must not cloud by its theoretical element the primary objective fact that cytoplasmic pigment is associated in its origin with obvious nuclear materials in the inclusive sense.

The beginning of a generalization for pigmentation of the nervous system may be made. Broadly, it necesarily falls into two groups, the normal and the abnormal. Of normal pigment in the sense of a constant morphological structure, the substantia nigra, the locus coeruleus, and certain other cells constitute examples.

A discussion of these is beyond the scope of this paper, but at least a suggestion may be ventured. Nerve cells as a class possess universally a chromidial apparatus ordinarily called the Nissl substance. The relation of such an organelle to pigment formation becomes clear in a cell where pigment formation is the specialized function as in the dermal chromatophore elucidated by Schultz. Is not pigment formation when it is a normal structure to be correlated with some such differentiation in the nerve cell? This would include all normal pigment as phases of differentiation. Of course, the reference here and elsewhere is to autochthonous pigmentation, not to accidental pigmentation, such as the hematogenous.

Excluding all normal pigmentations, the preliminary generalization for the abnormal autochthonous group seems reasonably clear. It is a phenomenon of chronic depression, or depressant senility. It is not a phenomenon of normal functional activity, so far as the Purkinje cell represents the typical reaction of a chromidial apparatus.

Normal function and depression together include the possibilities of reactions of irritability, in other words, the whole range of reactions of the specialized nerve cell. Pigment has never come to notice in the ordinary routine in normally functioning Purkinje cells, nor particularly in naturally senile cells where it has been the object of special search. This corresponds to the statement of Marinesco ('09, p. 288) for the cerebellum. It would not be expected to be, for such a type cell even to senility

passes out its nuclear materials and they are consumed. There is no block of reciprocal relations between plasma and nucleus, with its possibilities from unused stuff in physiological depression, even without chemical influence directly transforming the chromatin in experimental and toxic depression.

In conclusion, metabolic pigment is definitely derived from nuclear material in all somatic cells which have been studied from this point of view. With this widespread evidence for a specific origin which applies to one type of nerve cell, it must apply to all nerve cells. This disposes of the histogenesis for both the normal and abnormal. Abnormal autochthonous pigmentation in all nerve cells reacting like the Purkinje cell must come by way of depression. Activity in such cells is excluded, the only other possibility. For the normal pigmentation, in all other cell types for which it is likely that the further differentiation of the chromidial apparatus which all nerve cells possess takes the direction of pigment formation, whether a specific and fixed characteristic or possibly a functional by-product, the effect of abnormal conditions remains also to be determined. Cytologically, this covers the possibilities. If the generalization goes too far in its inclusiveness, at least the apology is that it is a prediction which enables one intelligently to investigate and experiment.

For the elucidation of abnormal states, while the pigmentation of depression is a degeneration in the Purkinje cell in the sense of a perversion of its normal activities, it results by a process, and one which finds its homologue in normal situations.

THE DUALITY OF THE SENESCENT PROCESS IN THE NERVE CELL
(SENILITY OF FUNCTION AND SENILITY OF DEPRESSION)

Previous work (Hodge, '94, Dolley, '11, '14, Kurtz, '15) on the nerve cell has shown that the inevitable result of its natural functional metabolic activities is old age. The present work determines a senility of contrasted sort in structure and in genesis and the dual nature of senility for a highly differentiated cell is thus established. There were two possibilities and only

two, for again the nerve cell specialized toward irritability has two processes of reaction, activity and depression.

The attempt here will be to cover a few essential points of what in scope belongs to a monograph. Much that has appeared discordant in observation and deduction is now clearly harmonized in respect to the nerve cell.

There is first the view of Minot ('90, '95, '08) as opposed to the view of R. Hertwig ('03, '08), apparently diametrically opposed, as Conklin ('12, '13) remarks. On the one side, there is the natural senility, the 'final fatigue,' so correctly recognized by Hodge, the (relative) increase of plasma of Minot, structurally the eventually absolute upset of the nucleus-plasma relation in favor of the plasma, and metabolically the senility of final organic exhaustion. On the other side, there is the 'physiological degeneration' of Hertwig as a senescent state, the permanent upset of the nucleus-plasma relation in favor of the nucleus, and metabolically the blocking and hence progressively lowered level of reciprocal interchange between plasma and nucleus. Thus in the cell specialized for function, increase of plasma and increase of nucleus each has its disproportionate display relative to the other, and the views of Minot and Hertwig both apply in respect to the phase which each encountered and investigated. Conklin, however, is entirely correct that changes in the nucleus-plasma relation are not the cause of senescence: they are indexes of the two possibilities of lowered metabolism which will be considered. Instead of the cause, such changes in size relations are an effect.

In discussion of the metabolic relations of the two distinct kinds of senility, it will first be shown that they accord with the generalization of Conklin and Child in respect to senility as a general state. Conklin says, "Longevity is determined by the duration of the excess of an anabolism over katabolism" ('13, p. 191); "Slow interchange (between the chromatin and the protoplasm) is the condition of slow metabolism, and of senescence" ('12, p. 62). Child ('11) concludes that senescence and rejuvenescence are caused by a decrease or an increase in the fundamental metabolic reactions. A decrease in the power of constructive metabolism, as Conklin terms it, is obviously the metabolic

factor in both natural senility and senility of depression in the nerve cell, as follows.

In natural senility, the progressive atrophy of plasma and nucleus, with prior disappearance of the nucleus, the progressive deficiency of chromatin elaboration due to nuclear exhaustion, associated with deficiency of nucleolar substance, and the progressive failure of the power of recuperation or regrowth, all predicate the ascendency of katabolism, the decrease of constructive metabolism. Natural senility is one of organic exhaustion, and in this respect it fulfills the state of the metabolism conditioned by these investigators.

In depressant senility, the decrease or failure of constructive metabolism is the outstanding exhibition of the cellular process. Again there is progressive atrophy from lack of synthetic up-building, in this case primarily a cytoplasmic factor, a progressive deficiency of the chromatin supply, due now not only to lack of primary plasmic building substances, but to failure of reciprocal nucleus-plasma relations, and a loss of recuperative power for the same reasons. Senility of depression likewise fulfills the condition of lowered metabolism.

As to the rate of metabolism which Child emphazises, there seems no reason to doubt that both kinds of senility would involve a decrease in rate. The reciprocal interchange between plasma and nucleus is disturbed or interrupted, in one mainly on the part of the plasma (depression), in the other mainly on the part of the nucleus (exhaustion).

This relation of senility to metabolism has been purposely written in very general terms. It could be written, perhaps more convincingly but beyond the scope of this paper, in detailed terms of the central theme to which all lines of work have converged,—a common metabolic basis for all nervous reactions of a cell type from which there is only a quantitative divergence. Thus both natural senility and senility of depression can be shown to be only quantitatively different, each in its way, in respect to metabolism of the normal cell. For, in some explanation, the nucleus-plasma relation has been interpreted to be a reciprocal metabolic relation between plasma and nucleus. The specific end result

of this relation is the elaboration of chromatin, finally displayed as the chromidial apparatus, the immediate source of functional energy. The nerve cell is devoted solely to function, and its whole series of anabolic processes is directed to the above specific end. That cell having been shown to have no function other than irritability, one needs go no farther than a basis for that property. Hence it follows that the constructive metabolism of the nerve cell is its function in respect to the potential energy involved in any functional act—that energy is stored as chromidial apparatus.

But the various shifts in the nucleus-plasma relation which subserve functional states, changes in size and in size relations, are merely the outward token of the inward chemistry of chromatin anabolism, objectively to be interpreted as phases of the reciprocal relation. So far then, structure depends on function. Further, this relation of structure to function unquestionably goes deeper. For in a study of the development of the nerve cell, unfortunately yet unpublished save in abstract ('16), the evidence from volumetric relations at least indicates that non-divisional growth back to a very early period of development is a functional growth. So far as this goes, the development of structure for the nerve cell depends thus further on function. The statement of Child ('15, p. 44) therefore unequivocally applies to this special case: (The nerve cell) "constructs itself *by* function." The development of the nerve cell (growth in mass), the maintenance of its *status quo* in maturity and its possible functional hypertrophy depend thus on a common property of function. Moreover, just as immediate function is quantitative in respect to the normal metabolic level, so the volumetric evidence indicates that the lasting effect of function is quantitative. For example, the functional hypertrophy is an absolute increase of materials in the same proportion.

It follows immediately from this conception with reference to senility that in a cell so highly specialized for function that all other properties are lost, whatever may be true of less specialized cells, there is no necessity of distinguishing between structural and functional causes of senescence, as Conklin ('13, '14) does.

though not to their mutual exclusion. The metabolism of the nerve cell is its function on the constructive side, and its function determines its structure. This is no critical objection, as will appear, but the establishment of the place of one specialized type of cell in respect to a generalization for the senescence of all cells. The primary conditions of senescence in the nerve cell are solely functional, whether excitation to an ultimate exhaustion or depression to the minimum, and structural changes are secondary and dependent thereupon.

In terms of the preceding discussion, natural senility becomes a quantitative process. Heretofore, in discussing this senility, the reservation of a qualitative side has been made to explain the loss of recuperative power. In ignorance of the relation between growth and function, and hence with a tacit admission of some inherent growth impulse in deference to the common idea that was inevitable. But with the newer light on growth and function involving the same substances in a functional growth, the basis of this kind of senility becomes quantitative, that is, after the usury of a life-time, certain primary materials are consumed, decline of growth occurs and senile activity takes place on a different quantitative level. Thus the senile deviation of the nucleus-plasma relation from the norm represents a difference in the quantitative reciprocal interchange of the same metabolic materials. The quantitative principle which holds all through life does not change for a natural end result of that life.

That senility of depression is quantitative in respect to the same metabolic materials as in the norm is more self-evident. Depression physiologically (Verworn, '96) and cytologically (Dolley, '14) is the quantitative opposite of activity, a lower level instead of a higher level of metabolism. Senility of depression is only a further reducement of reciprocal interchange which becomes permanent, as was emphasized in the objective description. It is the quantitative opposite of natural senility (see schema under recovery with permanent disorganization). The upset in favor of the nucleus opposes the upset in favor of the plasma, but senile function in both is subserved by the same materials. Hence either type of senile cell not only functions in

manner as before, but either type can grow again, though as in the norm, only under the functional stimulus.

A final statement concerns the relation of the senescence of the nerve cell to its differentiation. The differentiation of the nerve cell is such that it does not appear in continuous progressive relationship with senescence, though this statement does not contraindicate the fact that its potentiality of senescence depends upon its peculiar *status quo* of differentiation. For the quantitative nature of the dynamic reactions of that cell predicates that differentiation is determined at the moment the quantitative principle of function becomes operative. In the Purkinje cell of the dog that moment is as close to birth as ten days and probably earlier, and it is at least sure that the differentiation remains a constant factor through adult life and senescence. Thus within that quantitative principle, the nerve cell is insusceptible to dedifferentiation (compare Child, '15, p. 281). It regresses, of course, but this is the functional atrophy, and a quantitative regression; it may again go forward in renewed functional growth, but there is no rejuvenation in the manner of certain other cells. All of this connects up with established knowledge. Let it be understood that this is not written in antagonism to the principle of dedifferentiation, without which the pathologist would face an insuperable barrier to intelligent interpretations.

Child ('15, p. 288) makes a place for such a degree of specialization, as follows:

Atrophy in the higher animals differs from reduction in the lower forms in that, while decrease in size occurs, there is little or no dedifferentiation. The cell has apparently become so highly differentiated that it has lost the capacity for synthesizing a substratum adequate in quantity or constitution to carry on metabolism. Consequently the losses from degradation and breakdown of the existing substratum are not compensated by the synthesis of new substratal substance, and sooner or later the fundamental mechanism of the cell is destroyed and degeneration and death occur. The atrophy of old age in organs of such fundamental importance as the nervous system indicates that there is some truth in the statement, so often made, that the later stages of senescence are a 'wearing out' of the physiological mechanism or some essential part of it. Apparently the nerve cells or some of them do wear out because they are no longer able to synthesize the substratum necessary for their continued function.

Having thus attempted to make clear that the nerve cell's differentiation is not an active structural factor in senescence, I can suggest how that fact does not antagonize the generalization of Conklin and Child that accumulation of the products of metabolism and of differentiation is the basis of senescence. The reason from the point of view of differentiation why the nerve cell remains unimpaired through long years of life, rejuvenescent rather than senescent, is that the specific products of its differentiation are entirely consumed in the functional reaction and the by-products eliminated. The high metabolic rate which Child ('15, p. 281) offers to explain its stability is the objective cytological fact. It is continuously forced to the renewal of the superficial substances, such as the chromatin and nucleolar substance, which are the final products of its differentiation. There is no absolute rest for the nerve cell, even without actual function, for it is necessarily in some degree of metabolic 'tone' as long as it exists. Hence the condition of rapid interchange between plasma and nucleus (Conklin), of rapid metabolism (Child), the condition of youth, is sufficiently fulfilled, unless the inner exhaustion goes too deeply or unless external conditions depress it.

There is no rejuvenescence in actual fact, for there is no dedifferentiation, and a recovery from a profound depression or an immediate exhaustion involves in either case the same substance as preexisted. Since the factor of metabolism which conditions rejuvenescence is so nearly a constant, provided the function which conditions it in the nerve cell is properly balanced, the stability of its protoplasm holds, the *status quo* remains essentially youthful, and it is not placed in need of the actuality of rejuvenescence, which is for it impossible. The return to the normal after fatiguing or exhausting nervous activity or after depression is simply a recovery and not a rejuvenescence. Of course, the elimination of the waste products of metabolism is possibly to be conceded as a daily rejuvenescence in the most superficial sense, but it is not only probably an infinitesimal factor under normal conditions, in respect to that, but also the more important phase here is the recovery, regrowth of materials which these

waste products have impeded, as well as the restoration of materials lost by usury. While Child ('15, p. 297) argues for the possibility of some rejuvenescence of the nervous system, basing it on the subjective effects of vacation or change of activity, the benefits of these are the benefits of rest and recovery of the whole or of one part at the expense of other parts. In respect to placing the nerve cell, the real support of his conception of senescence and rejuvenescence, with which I interpret myself in complete agreement, appears to rest upon the preceding fundamental properties of the nerve cell's differentiation.

Finally, speculations on a potential immortality from the actualities of dedifferentiation and rejuvenescence are naturally consequent for undifferentiated cells and cells of a certain order of differentiation, and interesting in not only an abstract but a concrete scientific sense. But when the implication is attached, as it has been of late years in nonscientific writings, of a potential immortality for the highly organized soma, it becomes an absurdity. For any organism possessing a true nervous system, it would be a brainless immortality. Natural senescence and natural death are inescapable for this one order—as well as other orders—of differentiated cells. For, though the conditions above which lower its metabolism may be escaped for a much more prolonged period than the traditional span of three score and ten for the human organism, they are eventually inevitable in such an organism. There is no use to speculate on its absolute potential viability by itself, for it has no isolated existence. "Senescence is a retardation resulting from continued dynamic activity under certain conditions in a system" (Child, '15, p. 465).

CONCLUSIONS

The process of recovery from organic depression reduces its quantitative upset of the nucleus-plasma relation in favor of the nucleus in essentially the opposite way to recovery from exhaustive functional activity, which is a quantitative upset in favor of the plasma. The quantitative principle of the functional reaction is thus maintained, and all phases of function are now demonstrated to rest upon a quantitative divergence only.

The objective fact is correlated with the quantitative principle that some relative recovery is possible for every grade of depression necrobiosis until nuclear death is absolute. Depression has a comparatively greater potentiality of recovery than has exhaustion, which is to be referred to the relative advantage maintained by the nucleus in both acute and chronic states.

A depression which goes too far in degree or time results in a permanent disorganization of the cell. Progressive diminution of chromidial apparatus, atrophy of plasma and nucleus, pigmentation, loss of nuclear integrity, and finally, disappearance of cells are indicative of such a change, relative or absolute. Up to cell death, however, the nucleus-plasma relation of depression holds as an index of a continued process, and the capacity of functional metabolism on lowered quantitative levels and of some relative recovery by functional growth persists.

The essentially permanent state of disorganization constitutes an organic senility of depression. This is the quantitative opposite of senility of function, of final organic exhaustion, and the dual nature of the senescent process for the nerve cell in its state of differentiation is thus established.

Both senility of function and senility of depression conform to the lowered functional metabolism which has been conditioned by other investigators for the senile state.

There is no rejuvenescence of the nerve cell, for there is no dedifferentiation within the working of the quantitative principle, but only a recovery, which, whether from depression, from disuse atrophy or exhaustion, involves the regrowth of the same preexisting substances. The reason why the nerve cell remains unimpaired through long years of life is that the specific products of its differentiation are entirely consumed in the functional reaction and the by-products eliminated, while the condition of rapid metabolism, which is the condition of youth, is sufficiently fulfilled, unless the inner exhaustion goes too deeply, or unless external conditions depress it. From its place in the organic system it follows that senility and death of the nerve cell are at some time inevitable.

Pigmentation of the Purkinje cell and of all nerve cells for which it represents the typical reaction of a chromidial apparatus is a phenomenon of chronic depression, not of functional activity, the only other possibility.

The histogenesis of the pigment is from nuclear materials, both within the nucleus and from the chromidial apparatus.

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COMPARATIVE STUDIES ON THE GROWTH OF THE CEREBRAL CORTEX

I. ON THE CHANGES IN THE SIZE AND SHAPE OF THE CEREBRUM DURING THE POSTNATAL GROWTH OF THE BRAIN. ALBINO RAT

NAOKI SUGITA

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TWO FIGURES AND TWO CHARTS

I. INTRODUCTION

The present study is preliminary to an investigation on the postnatal growth of the cerebral cortex in thickness, in the case of the albino rat. For this purpose it was necessary to learn the changes in shape and size of brain—represented by the cerebrum —which occur during postnatal growth and to record these changes in terms of linear measurements.

The growth of the brain, as represented by increase in weight, is tabulated in "The Rat" (Donaldson, '15). As yet however we have no records concerning the shape and dimensions of the brain—or cerebrum—in relation to increasing weight or age. Hatai ('07) has given detailed measurements on the skull of mature albino rats, from which we can infer approximately the shape and size of the adult albino rat brain, but measurements for the younger stages were not included.

As a first step in this study, I had to ascertain the relation existing between the weight of the entire brain and of the cerebrum during growth. For the purpose of a more detailed study, Donaldson has divided the entire brain into four parts, i.e., the cerebrum, the stem, the cerebellum and the olfactory bulbs, and investigated the increase in weight of each of these parts and the changes in the weight relations among them, during postnatal growth (unpublished observations, partly presented in a Harvey Lecture, December, 1916). From a like investigation on my part,

on a small number of cases, the following may be reported. In table 1 are given for ten ages the total brain weights and their ratios, the weights of the parts and the ratios of the weights of parts to their respective initial weights at birth. It is seen from these data that throughout the early period of life (first 150 days), the cerebellum develops most rapidly, the olfactory bulbs show the next highest rate, while the stem develops rather steadily

TABLE 1

Giving for ten ages the total brain weights and their ratios, the weights of the parts and the ratios of the weights of parts to their respective initial weights at birth, albino rat brain

NUMBER OF CASES	AGE IN DAYS	TOTAL BRAIN WEIGHT		CEREBRUM		CEREBELLUM		STEM		OLFACTORY BULBS	
		Observed	Ratio	Observed	Ratio	Observed	Ratio	Observed	Ratio	Observed	Ratio
		grams		*grams*		*grams*		*grams*		*grams*	
4	B	0.236	1.0	0.150	1.0	0.008	1.0	0.073	1.0	0.005	1.0
4	4	0.482	2.0	0.338	2.3	0.023	2.9	0.105	1.4	0.016	3.2
3	5	0.503	2.1	0.349	2.3	0.026	3.3	0.113	1.5	0 015	2 0
2	20	1.153	4.9	0.817	5.4	0.126	15.8	0.171	2.3	0.039	7.8
3	35	1.355	5.7	0.870	5.8	0.197	23.4	0.248	3.4	0.040	8.0
4	50	1.469	6.2	0.974	6.5	0.197	23.4	0.258	3.5	0.040	8.0
3	60	1.531	6.5	0.959	6.4	0.226	28.3	0.278	3.8	0.068	13.6
2	70	1.612	6.8	1.033	6.9	0.215	26.9	0.331	4.5	0.033	6.6[1]
5	90	1.779	7.5	1.121	7.5	0.244	30.5	0.337	4.6	0.077	15.4
4	150	1.933	8.2	1.191	8.0	0.275	34.4	0.373	5.1	0.094	18.8

[1] The olfactory bulbs are very variable in weight. Undeveloped bulbs like the above sometimes occur.

during that period. The cerebrum, however, develops in a ratio equal to that of the entire brain, from which fact we may conclude that the developmental stage of the cerebrum is fairly represented by that of the entire brain.

This preliminary examination was made to ascertain whether or not the developmental weight phases of the cerebrum are represented by those of the total brain weight. It appears that they are thus represented, so that total brain weights may be used when cerebrum weights are not available.

To study the developmental changes in the shape and size of the cerebrum, I selected five diameters of the entire cerebrum,

from the measurements of which its general shape and size can be closely determined.

The materials used in this study were all employed for the further investigation on the cortical development, including a study on the thickness of the cerebral cortex. The present research was begun in October, 1915, and concluded in June, 1916, at The Wistar Institute of Anatomy and Biology.

In connection with this study, which is the first of a series made during my stay in Philadelphia, I desire to express my sincere thanks to Dr. M. J. Greenman, Director of The Wistar Institute, for extending to me the privileges of the Institute and putting its facilities at my disposal—and also to acknowledge my obligations to Prof. Henry H. Donaldson under whose direction these researches have been made.

II. MATERIAL AND TECHNIQUE

The material, consisting of 141 albino rats (106 males, 32 females and 3, sex undetermined)—representing every phase of postnatal growth and having approximately standard body measurements—were all from the rat colony at The Wistar Institute. After dissection the entire brain was severed from the spinal cord by a transverse section at the level of the calamus scriptorius and weighed to a milligram in a weighing bottle. The brain was next put on a glass plate, base down, without any lateral support. Five diameters of the hemispheres were then measured with a sliding calipers to a twentieth of millimeter, according to the method to be later described.

These measured values are, of course, slightly different from the values of the same diameters taken on the hemispheres in situ within the skull, because normally the basal surface of the skull does not lie in a plane, but is slightly arched, and the sides are supported by the temporal walls of the skull. Thus, as measured, the height of the fresh brain is somewhat reduced and the width increased. To measure the brain in situ was however not satisfactory and I thought it better to bring the brain to a position convenient for measurement, in order to get more exact

values. As I measured all the material in the same manner, the data thus obtained are comparable among themselves.

I have arranged the individuals in twenty groups, numbered according to the number of decigrams in the brain weight of each, for example, brains weighing 0.300–0.399 grams form Group III and brains weighing 1.500 to 1.599 grams Group XV, etc. In each group, the individual was designated as a, or b, or c., etc. in the order of the date of the dissection. Each individual carries the same designation when the record for it appears in the other studies in this series. Measurements of the body and brain weights, body and tail lengths, were made by the usual methods employed at The Wistar Institute (see "The Rat," Donaldson, '15).

As the individual records will, for the most part, be given in a study, which follows, on the growth of the cortex, and as all of the records are on file at The Wistar Institute, it has not been thought necessary to print them here. In table 2, the mean values for each brain weight group are entered with a statement of the number of individuals on which the average has been based.

In table 2 are given for comparison the standard body weight and body and tail lengths and the age, corresponding to the observed brain weights, and obtained by the use of formulas devised by Hatai (see "The Rat," Donaldson, '15). Some discrepancies seen in the case of Groups XVI–XX between the observed and calculated values are due to the fact that in grown-up rats the brain weight increases very slowly, while the body measurements are open to wide variations.

III. POSITIONS OF DIAMETERS

The positions of the five diameters, by measurement of which the shape and size of an albino rat cerebrum are to be determined, are as follows (figs. 1 and 2).

1. Width AB (abbreviation *W. B*), the greatest width along the frontal plane (fig. 1).

2. Width CD (abbreviation *W. D*), passing through the middle point O of the fissura sagittalis and parallel to AB (fig. 1).

TABLE 2

The body measurements as observed are here compared with the standard values for the body measurements based on the observed brain weights and computed by the formulas devised by Hatai ("The Rat," Donaldson, '15)

BRAIN WEIGHT GROUP	NUMBER OF CASES	OBSERVED					STANDARD			
		Age in days	Body weight	Body length	Tail length	Brain weight	Tail length	Body length	Body weight	Age in days
			grams	*mm.*	*mm.*	*grams*	*mm.*	*mm.*	*grams*	
I	3	—[1]	3.0	38	14	0.161	—	—	—	—
II	10	B.	5.2	48	18	0.254	19	51	5.2	1—
III	11	3—	6.2	52	20	0.341	24	56	6.0	2+
IV	10	4+	7.7	56	23	0.424	27	58	6.7	4—
V	19	6	9.6	61	27	0.544	31	61	8.0	6—
VI	4	7	9.6	63	28	0.622	34	64	9.0	7—
VII	7	8+	9.6	64	32	0.769	40	69	11.1	8+
VIII	10	9+	12.4	68	33	0.845	42	71	11.9	9+
IX	5	11+	13.9	74	38	0.954	46	75	13.6	11—
X	6	13—	15.5	76	46	1.047	51	80	15.8	15—
XI	6	18	16.1	78	50	1.156	59	87	19.7	20+
XII	6	(27)	25.8	92	69	1.253	68	96	24.9	26
XIII	7		28.1	99	73	1.334	77	105	31.1	32
XIV	5		55.8	125	102	1.449	93	121	44.4	41
XV	7	(50)	74.1	141	129	1.558	111	139	64.1	54
XVI	9		156.8	177	158	1.662	130	158	92.3	65
XVII	7	(100)	157.8	180	157	1.737	144	174	122.0	76
XVIII	5		184.3	188	160	1.832	164	194	175.0	107
XIX	1	2 years	300.2	225	195	1.924	183	215	250.5	205
XX	3	2 years	289.2	—[2]	199	2.037	207	241	388.0	—

[1] Prematurely born.   [2] Rigor mortis.
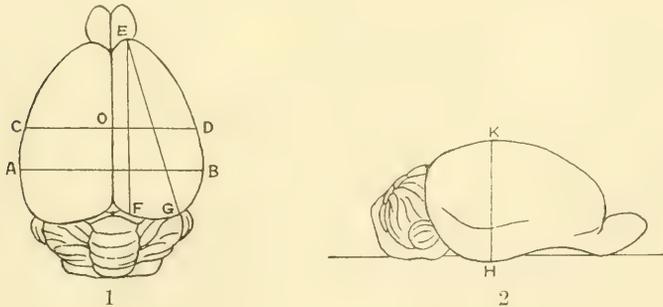
Fig. 1 Dorsal view of the albino rat brain weighing 1.5 grams. Enlarged 1.8 diameters. To show the positions at which the two measurements for the width and the two measurements for the length were taken. AB = Width $W.B$, CD = Width $W.D$, EF = Length $L.F$ and EG = Length $L.G$.

Fig. 2 Lateral view of the albino rat brain weighing 1.5 grams. Enlarged 1.8 diameters. To show the position at which the height was measured. HK = Height Ht.

3. Length EF (abbreviation *L. F.*), passing through the frontal pole at E and running parallel to the mesial surface of the hemisphere (fig. 1).

4. Length EG (abbreviation *L. G*), passing from the frontal pole at E to the occipital pole at G (fig. 1). This measurement gives the greatest length.

5. Height HK (abbreviation *Ht.*), from the stalk of the hypophysis to the dorsal surface and vertical to the plane on which the brain is resting (fig. 2).

These lines here marked on the surface of a cerebrum really indicate the shortest distance between the terminal points, a distance which has been exactly measured with sliding calipers to a twentieth of millimeter.

As a matter of fact, the point G is difficult to fix, while the other points are relatively easily found and fixed. I took as G the extreme point of lateral contact of the cerebrum and the cerebellum (disregarding the paraflocculi). Sometimes this line of contact was disturbed in the removal of the brain, but in such instances I replaced the parts and measured the greatest distance from the frontal tip to a point at the occipital pole which was supposed to be G.

To measure the height, I brought the brain to the edge of the glass plate, inserted one end of the calipers under the basal surface at the stalk of the hypophysis and, holding the calipers vertically to the plate, carefully measured the distance to the dorsal surface of the brain.

In a fresh brain it is of course hard to get exact measurements owing to the softness of the brain substance, but each measurement was repeated more than three times for each diameter and the average value was recorded.

These measurements were intended first, to show the rate of increase in each dimension of the cerebrum during growth and second, to furnish a basis with which to compare the corresponding measurements after fixation for histological study and at various later stages. All these data are necessary in order to determine the coefficients needed to convert the observed values of the

cortical thickness, cortical area, etc., as seen on the slide, into the values for the fresh condition of the material.

Among above diameters, the *L. F* was measured in the plane, from which the sagittal sections were taken for the investigations on the cortex, and the diameter *W. D.* in the plane from which the frontal sections were taken for the same purpose. So the diameter *L. F* measured in the fresh brain is comparable with the correspondng diameter in the sagittal sections and the diameter *W. D* with that in the frontal sections. The diameters *W. B*, *W. D* and *L. G* are controllable in the horizontal sections, which were taken in a plane passing through the frontal pole and approximately parallel to the basal surface of the brain. The measurements *W. B*, *W. D* and *L. F* were utilized as factors for correction-coefficients used to convert the cortical thickness as measured on the sections into the values for fresh condition, – as will be described in another study. The height is not controllable directly in any section prepared by me. The diameters *W. B*, *L. G* and *Ht.* indicate the maximum values in each dimension of the cerebrum and by them it would be possible to outline the shape of a cerebrum of the albino rat, because it is very simple in form.

These five diameters representing three dimensions of the cerebrum are therefore available for a systematic comparison of the changes which occur in the shape and size of the cerebrum during its growth.

IV. MEASUREMENTS PRESENTED IN TABLE AND CHART

Table 3 gives for each brain weight group the data obtained by measurements on fresh brains according to the above mentioned procedure. The individual measurements have been placed on file at The Wistar Institute.

Chart 1 shows graphically the linear measurements given in table 3.

V. DISCUSSION

From the study of table 3 and chart 1, it is seen that the cerebrum of the albino rats does not increase in volume so as to maintain the proportions present at birth, but that the rates of increase differ a little in the several dimensions.

The diameter *W. B.* has an almost fixed rate of increase gaining a little less rapidly than the cube root of the brain weight

TABLE 3

Giving the brain weights for each brain weight group, the cube roots of the brain weights and the linear measurements for width, length and height of the cerebrum.
Albino rat

BRAIN WEIGHT GROUP	NUMBER OF CASES	AVERAGE BRAIN WEIGHT	CUBE ROOT OF THE BRAIN WEIGHT	LINEAR MEASUREMENTS				
				W. B	W. D	L. G	L. F	Ht.
		grams		*mm.*	*mm.*	*mm.*	*mm.*	*mm.*
I	3	0.161	0.545	7.02	6.58	6.18	5.60	4.52
II B.	10	0.254	0.633	8.64	8.05	7.12	6.34	5.39
III	11	0.341	0.699	9.28	8.52	7.84	7.20	5.85
IV	10	0.424	0.750	10.02	9.11	8.50	7.92	6.35
V	19	0.544	0.816	10.78	10.02	9.41	8.69	6.93
VI	4	0.622	0.854	11.24	10.44	10.00	9.39	7.23
VII	7	0.769	0.916	12.05	11.10	11.09	10.59	7.91
VIII	10	0.845	0.945	12.26	11.48	11.43	10.88	8.22
IX	5	0.954	0.984	12.90	12.10	12.12	11.42	8.54
X	6	1.047	1.016	13.05	12.22	12.23	12.03	8.50
XI	6	1.156	1.049	13.50	12.61	12.61	12.35	8.94
XII	6	1.253	1.078	13.79	12.99	13.04	12.50	8.93
XIII	7	1.334	1.101	13.87	13.14	13.45	13.09	9.16
XIV	5	1.449	1.132	14.04	13.42	13.77	13.31	9.25
XV	7	1.558	1.159	14.42	13.76	14.28	13.61	9.50
XVI	9	1.662	1.184	14.44	13.62	14.93	14.10	9.34
XVII	7	1.737	1.202	14.80	14.03	15.29	14.51	9.53
XVIII	5	1.832	1.224	14.88	14.22	15.47	14.63	9.68
XIX	1	1.924	1.243	14.65	14.10	16.00	15.40	9.75
XX	3	2.037	1.267	15.39	14.62	16.65	15.30	10.02
Ratios II–XX		8.02	2.00	1.78	1.81	2.34	2.41	1.86

Chart 1 Giving for each brain weight group, in millimeters, on brain weight in grams, the values for the several diameters. *W.B.* and *W.D.*, width; *L.F.* and *L.G.*, length; *Ht.*, height.

$$\bullet \!-\!\bullet \!-\!\bullet = W.B. \qquad\qquad \circ \!-\!\circ \!-\!\circ = L.G.$$
$$\bullet \text{----} \bullet = W.D. \qquad\qquad \circ \text{----} \circ = L.F.$$
$$\times \text{--} \cdot \text{--} \times \text{--} \cdot \text{--} \times = Ht.$$

(volume) (see also table 4, column C). The approximate value of *W. B* can be estimated by formula (1) as follows:

$$W.\,B.\ (mm.) = C_W \times \sqrt[3]{\text{Brain weight (grams)}} \qquad (1)$$

where C_W will be 13.5 for a brain weighing 0.3–0.5
  13.2 for a brain weighing 0.5–1.0
  12.9 for a brain weighing 1.0–1.3
  12.5 for a brain weighing 1.3–1.6
  12.2 for a brain weighing 1.6–2.1

The value for the measurement *W. D* stands in an almost fixed relation to that for *W. B*. The latter averages through its course 0.6 to 1.0 mm. below the former, the average difference being 0.76 mm. Thus in chart 1 both graphs run nearly parallel to each other.

The measurement $L.\ G$ increases rapidly as compared with the other diameters. The formula (2) which expresses the relation between it and the brain weight is as follows:

$$L.\ G.\ \text{(mm.)} = \text{C}_\text{L} \times \sqrt[3]{\overline{\text{Brain weight (grams)}}} \qquad (2)$$
$$\text{grams}$$

where C_L will be 11.2 for a brain weighing 0.3–0.5
11.5 for a brain weighing 0.5–0.7
12.2 for a brain weighing 0.7–1.6
12.7 for a brain weighing 1.6–2.0
13.1 for a brain weighing 2.0–2.1

The graph for the measurement $L.\ F$ runs in general parallel to that for $L.\ G$, as might be inferred from the relation of the two diameters, but after the brain has reached the weight of 1.3 grams, the difference between them tends to increase owing to the slightly more rapid growth of $L.\ G$. The difference between $L.\ F$ and $L.\ G$ ranges between 0.20 and 1.35 mm. In general, the difference tends to decrease from birth (0.78 mm.) to the brain weighing 1.0 to 1.1 grams, at which stage the difference is least (0.20 to 0.26 mm.), and then increases again (up to 1.35 mm.) as the brain weight advances.

At birth the width ($W.\ B$ and $W.\ D$) surpasses considerably the length ($L.\ G$ and $L.\ F$) and the cerebrum is short and rounded. But very soon the length begins to increase more rapidly than the width and the shape becomes more and more elongated. The measurement $L.\ G$ surpasses $W.\ B$ and the measurement $L.\ F$ surpasses $W.\ D$ at the stage when the brain reaches 1.6 grams in weight.

The width-length index of the brain, here used, is obtained by the formula $\dfrac{W.\ D \times 100}{L.\ F}$. It is at birth about 127, reaches 100 in a brain weighing 1.3 − 1.5 grams and is about 95 in old age.

The height of a brain may be obtained by the following formula (3):

$$Ht.\ \text{(mm.)} = \text{C}_\text{H} \times \sqrt[3]{\overline{\text{Brain weight (grams)}}} \qquad (3)$$
$$\text{grams}$$

where C_H will be 8.6 for a brain weighing 0.3–1.2
8.3 for a brain weighing 1.2–1.6
7.9 for a brain weighing 1.6–2.1

The increase of *Ht.* is somewhat more rapid than the increase in width, but is rather slow as compared with the increase in length, so that in relation to its length the brain flattens somewhat as the age advances.

If the initial values of the diameters in the newborn brain group (Group II) be taken as unity and the corresponding values of the diameters in the other successive groups be compared with these units, the series of ratios in columns D, E and F, table 4, are found. If the shape of the cerebrum remained the same throughout growth, the product of *W. B* × *L. G* × *Ht.* would give the relative volume of the cerebrum at maturity as compared with its volume at birth.

As already stated, the weight of the cerebrum stands in an almost fixed relation to the weight of the entire brain, so the ratio of the total brain weight to its weight at birth would be the same as the ratio for the cerebrum and the cube root of that ratio would indicate the theoretical increase in one (mean) dimension, if the cerebrum did not change in form. These calculated ratios based on the data in table 3 are given in table 4. From among the values given in table 4, the cube root of the brain weight ratio and the ratios of *W. B*, *L. B* and *Ht.* are presented in chart 2 in smoothed graphs.

On examining chart 2, we see that the diameter *W. B* increases in an almost fixed relation to the theoretical curve denoted by $\sqrt[3]{C}$ (representing the cube root of the bran weight ratios), indicating that *W. B* is growing almost in proportion to the increase in total volume. The graph for *L. G* shows the rapid growth of this dimension. The rate of increase is most rapid in brains weighing 0.25 to 0.90 grams, then, in the brains weighing 0.90 to 1.25 grams, the curve runs nearly parallel to $\sqrt[3]{C}$, and after that period the rate becomes more rapid again. Up to a brain weight of 1.1 grams, the increase of *Ht.* is nearly equal to that of $\sqrt[3]{C}$ and after that becomes slower. Generally speaking, in the period in which the brain weighs 0.9 to 1.2 grams, the three dimensions of the cerebrum increase in nearly the same proportions, a fact to which we shall return later when considering the growth in thickness of the cerebral cortex.

If, in table 4, the brain weight ratio be compared with the ratio of $W.\ B \times L.\ G \times Ht.$ for any brain weight group, it appears that the values are nearly equal in the groups II–XII, though some fluctuations under 0.2 may be seen. But in groups beyond XII, the values in the two series deviate from one another; those for the brain weight ratios being consistently larger. This shows

TABLE 4

Giving from birth to maturity the ratios of the cube root of the brain weight, ratios of W. B, of L. G and of Ht., and also the ratios of the products of these three values

BRAIN WEIGHT GROUP	A Brain weight	B Ratio of brain weight	C Cube root of the ratio	D W.B	E L. G.	F Ht.	G Ratio of W. B × L. G × Ht.
					Their ratios to the initial values at birth		
	grams						
II	0.254	1.000	1.000	1.000	1.000	1.000	1.000
III	0.341	1.343	1.103	1.074	1.104	1.085	1.284
IV	0.424	1.669	1.186	1.160	1.194	1.178	1.631
V	0.544	2.142	1.289	1.248	1.322	1.286	2.120
VI	0.622	2.449	1.348	1.301	1.405	1.341	2.451
VII	0.769	3.028	1.447	1.396	1.558	1.467	3.188
VIII	0.845	3.327	1.493	1.419	1.605	1.525	3.474
IX	0.954	3.756	1.554	1.493	1.703	1.584	4.026
X	1.047	4.122	1.603	1.511	1.718	1.577	4.091
XI	1.156	4.551	1.657	1.563	1.771	1.659	4.590
XII	1.253	4.933	1.702	1.596	1.832	1.657	4.843
XIII	1.334	5.252	1.738	1.606	1.889	1.700	5.154
XIV	1.449	5.705	1.787	1.625	1.934	1.716	5.393
XV	1.558	6.134	1.831	1.669	2.005	1.763	5.899
XVI	1.662	6.543	1.870	1.671	2.097	1.733	6.073
XVII	1.737	6.839	1.898	1.713	2.147	1.768	6.504
XVIII	1.832	7.213	1.932	1.722	2.173	1.796	6.720
XIX	1.924	7.575	1.964	1.696	2.247	1.809	6.893
XX	2.037	8.020	2.002	1.781	2.338	1.859	7.744

roughly that in the brains weighing 0.25 to 1.20 grams the volume and weight keep a fixed relation, but that in the brains weighing more than 1.2 grams the total weight increases more rapidly than the cerebral volume, as measured by the diameters which were chosen. This relation probably depends on several causes.

1. As already noted, at the beginning of the present paper, the cerebellum develops more rapidly than the other parts of the

brain, attaining at maturity over thirty-four times its initial weight. This will be one of the reasons why the relative weight increase of the total brain is greater than the relative volume increase of the cerebrum only.

2. The specific gravity of the brain substance is probably increasing as the age advances, especially after the brain has attained
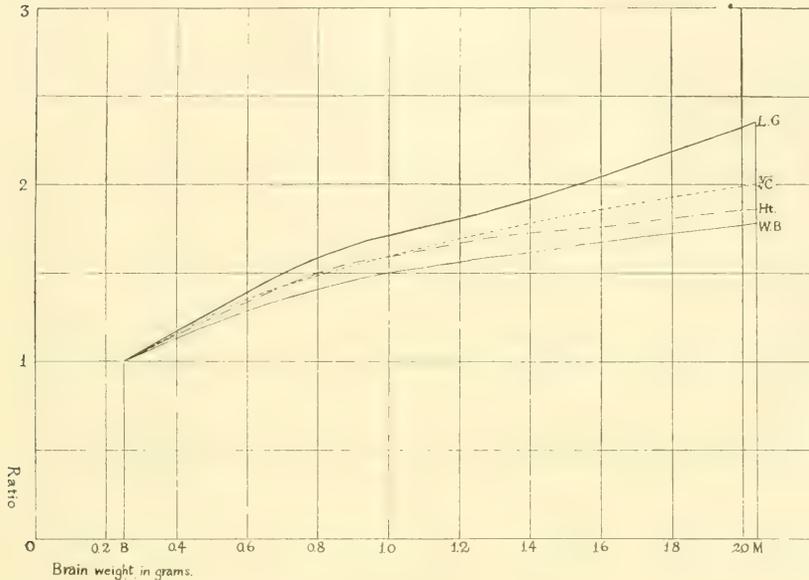
Chart 2 Showing on brain weight by smoothed graphs, the ratios of the width $W.B.$, the length $L.G.$ and the height Ht, as compared with the cube root of the brain weight $\sqrt[3]{C}$.

tained 1.2 grams in weight, for as already determined by Watson ('03) the myelination of the brain begins in the albino rat at the age of fourteen to twenty days and this process, which increases the specific gravity of the brain, continues most energetically during and after the fourth week, when the brain attains 1.2 to 1.3 grams in weight.

3. Finally it is at about this period, when the brain has reached 1.2 grams in weight, that the more rapid growth in the length appears and the change in the proportions between the lengths of

the diameters which is thus brought about tends, as a mere matter of arithmetic, to make the values of their product less than it would have been had their proportions remain the unaltered.

Sex characteristics. When brains of different sexes but of like weight were compared, there was not detected any considerable sex-differences in measurements, except in three cases. These individuals had apparently an elongated form of brain, their lengths being markedly high as compared with the average values for the brains of like weight. These three were all old females, exact age unknown.[1] In other respects, even these brains, showed no peculiar characteristics.

Hatai ('07) concluded from his skull measurements of the mature albino rats that, in every respect other than the nasal bone, the female cranium might be considered as an undersized male cranium, and vice versâ, since the other differences found between the two sexes were too small to be significant. This statement is probably true for the form of the cranial cavity, i.e., the form of the brain, as indicated by my measurements on brains of like weight but different sex. According to Hatai ('07) the skull capacity of the mature albino rat measured and represented by shot weight, is thus; male: 10.896 grams, female: 10.368 grams, which empirically correspond to the brains weighing 1.822 grams (male) and 1.725 grams (female) respectively, suggesting that the specific gravity of the male brain substance is slightly higher than that of the female (male 6.009: female 5.980).

VI. SUMMARY

1. On the fresh brain of the albino rat, five diameters were measured at fixed localities. By these the shape and the size of the cerebrum can be indicated and by the changes in them, the postnatal growth can be studied. As material 141 albino rats at every stage of growth were used.

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[1] According to my observations, the brains from rats which were severely underfed for a long time and whose body weights were reduced considerably, in the younger age, not only weigh less than the brains of standard rats of like age, but have generally an elongated shape as compared with the controls.

2. The greatest width ($W.B$), the greatest length ($L.G$) and the height ($Ht.$) of the cerebrum at birth are respectively 8.6, 7.1 and 5.4 mm. The same measurements at full maturity are respectively 15.4, 16.7 and 10.0 mm. (table 3). The ratio of each measurement in the mature brain to its initial value at birth is, therefore, respectively 1.78, 2.34 and 1.86. Using the product of these three diameters as a measure the ratio of volume of the mature cerebrum to the volume at birth is 7.74.

3. The increase in the ratio of the weight of a cerebrum according to age is quite equal to the increase in the ratio of total weight of the brain, which includes the cerebellum, the stem and the olfactory bulbs, besides the cerebrum. Thus the developmental stage of the cerebrum (in weight) corresponds to the developmental stage of the entire brain (in weight).

4. As the cerebrum increases its volume with age, it does not enlarge proportionally in all dimensions. In general, the length increases most rapidly while the width and height increase more slowly. However, in the period between the tenth and the twentieth day after birth (brain weight 0.95 to 1.2 grams), the cerebrum is enlarging in volume quite uniformly in all its dimensions.

5. In the newborn cerebrum the width is greater than the length (width-length index, '127). After birth, the increase in the longitudinal diameter surpasses that of the transverse diameter and finally at full maturity the cerebrum has a somewhat elongated form (width-length index 95).

6. Sex differences in the size and shape of the cerebrum are not significant, when brains of like weight are compared.

7. A rough estimation indicates that the specific gravity of the brain increases after brain has attained 1.2 grams in weight. This is due probably to myelination.

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COMPARATIVE STUDIES ON THE GROWTH OF THE CEREBRAL CORTEX

II. ON THE INCREASE IN THE THICKNESS OF THE CEREBRAL CORTEX DURING THE POSTNATAL GROWTH OF THE BRAIN. ALBINO RAT

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From The Wistar Institute of Anatomy and Biology

TWELVE FIGURES AND TWELVE CHARTS

I. INTRODUCTION

The object of this paper is to present the data collected upon the increase in the thickness of the cerebral cortex of the albino rat from birth to maturity and so to give an insight into the postnatal growth of one important portion of the cerebrum. A study of this kind may be of value in several ways: It should be of fundamental biological interest, it should be useful for reference in experimental work on the central nervous system and in connection with extensive data on the rat collected by Donaldson ('15), and finally it should shed some light on comparative psychology.

Papers on the thickness of the cerebral cortex of the mature human brain have been published by several anatomists, for example, Schwalbe ('81), Donaldson ('91), Hammarberg ('95), Kaes ('05, '07), Brodmann ('09), etc. His ('04) has also carefully described the developmental changes of the cerebral cortex in the early fetal period of the human brain, including the cortical thickness. Furthermore, there has been only one study on the development of the human cortex after birth, a paper by Kaes ('07) which, however, is unfortunately open to very serious criticism. Brodmann ('09) has made an extensive collection of results obtained by comparative anatomists on the cerebral cortex in several orders of the mammals. It is not possible, however,

to compare the results in these valuable papers, because, the authors are not in accord in respect to technical methods or the localities where the thickness was measured. My intention in the study which follows is to present the results of the exact measurements systematically applied to the thickness of the cerebral cortex during postnatal growth, using ample material and treating this with uniform methods of dissection, fixation, imbedding and staining. The systematic examination of sections thus prepared should make it possible to trace the steps in the growth of the cerebral cortex of the albino rat from birth to maturity in a way which could be controlled by subsequent workers in this field.

Hitherto, there has been no systematic study in this field so that I have not had any previous example to follow. I have therefore used my own judgment as to the localities to be sectioned and the methods of measurement. The reasons for the various methods will be given in the appropriate chapters. In a later part of this series of studies, I shall try to review collectively the data presented by previous authors concerning the cortical thickness of human and animal brains. As these researches have, however, been all directed towards the solution of phylogenetic problems and have been made on the adult individual, they have but little immediate bearing on the present problem.

In connection with this research, and using the same material, I have studied also the developmental changes during growth in the size and shape of the ganglion cells, the differentiation of the cell-layers of the cortex, the distribution of the several kinds of ganglion cells in the cerebral cortex, and further, the changes in the relations between the elements of the central nervous system according to the growth phase of the brain. These results will be presented in papers which are to follow.

This study was started in October, 1915, and completed in June, 1916, under the direction of Prof. H. H. Donaldson at The Wistar Institute of Anatomy and Biology and I am much indebted to Prof. Donaldson for his valuable suggestions.

II. MATERIAL

The animals used in the present study were all from the rat colony of The Wistar Institute. I employed 124 albino rats, 96 males and 28 females, representing every stage of postnatal growth and having approximately standard body weights. After .dissection, the entire brain was separated from the spinal cord by a section at the level of the calamus scriptorius and was weighed in a small weighing bottle. I have classified the individuals in twenty groups, according to the number of decigrams in the brain weight of each, for example, brains weighing 0.300 to 0.399 grams form Group III and brains weighing 1.500 to 1.599 grams, Group XV, etc. In each group, the individual was designated as a, b, c in the order of the date of the dissection. The body and brain weights, body and tail lengths, sex and age of each individual are given in tables 1 and 2. The measurements were made by the usual methods employed at The Wistar Institute.

My reason for classifying the material according to brain weight, is that my own observations as well as those of others have shown that the brain weight increases most regularly according to age, and is much more resistant to outside influences than the body weight, for example. Hence it seemed to me to be not only more convenient, but also more precise to arrange the material according to the brain weight, rather than according to the body weight, or some other physical character.

Tables 1 and 2 show the sex, body and tail lengths, body and brain weights of the albino rats used in this study on the thickness of the cerebral cortex (table 1) in the sagittal and the frontal sections and (table 2) in the horizontal sections respectively, entered in the order of the increasing brain weight.

These animals were collected at random from many different litters and at various seasons. Although individual variations appear when the data are compared with the values in the reference tables for the rat (Donaldson, '15), nevertheless, by grouping these data according to the number of decigrams of brain weight, and obtaining the average values, it is found that these

TABLE 1

Showing the sex, body and tail lengths, body and brain weights of the albino rats used in this study on the thickness of the cerebral cortex in the sagittal and frontal sections, entered according to increasing brain weight

NUMBER	SEX	AGE	BODY WEIGHT[1]	BODY LENGTH	TAIL LENGTH	BRAIN WEIGHT
		days	*grams*	*mm.*	*mm.*	*grams*
I a			2.9	38	14	0.153
c			2.9	34	14	0.154
b			3.2	42	15	0.177
II a	m	B	4.8	47	16	0.213
b	m	B	4.6	45	16	0.221
c	m	B	5.0	48	19	0.261
d	m	B	5.0	51	18	0.271
e	m	1	5.7	50	18	0.288
III a	m	2	5.5	50	17	0.311
b	f	3	5.2	49	18	0.322
g	m	4	7.1	55	23	0.374
c	m	3	6.5	54	20	0.390
i	f	4	5.7	53	22	0.395
IV b	m	4	7.2	56	22	0.400
a	m	4	6.8	54	22	0.402
c	m	5	7.6	56	22	0.420
i	m	4	6.6	57	23	0.443
d	m	5	8.9	59	26	0.459
e	m	5	9.8	60	25	0.466
V i	m	5	8.2	61	25	0.501
a	m	5	8.3	58	25	0.525
b	f	7	8.5	61	26	0.528
c	m	6	12.2	63	30	0.534
d	m	7	10.9	65	27	0.537
e	m	6	8.2	60	25	0.555
f	m	6	8.8	60	30	0.558
g	m	6	8.5	57	25	0.564
h	f	7	10.5	63	29	0.579
VI c	m	7	8.5	62	28	0.610
a	m	7	12.1	63	26	0.617
e	f	8	8.6	61	29	0.690
VII a	m	9	8.4	61	32	0.740
b	m	8	8.9	62	33	0.760

TABLE 1—Continued

NUMBER	SEX	AGE	BODY WEIGHT[1]	BODY LENGTH	TAIL LENGTH	BRAIN WEIGHT
		days	grams	mm.	mm.	grams
VIII a	m	9	12.2	66	30	0.800
h	m	9	8.9	61	32	0.805
b	m	9	10.8	67	32	0.822
c	m	10	12.4	68	36	0.849
k	m	11	11.6	69	39	0.870
d	f	10	13.9	70	30	0.898
IX d	m	11	13.3	73	38	0.959
e	f	12	14.6	79	42	0.960
a	m	11	14.1	75	40	0.972
X a	m	12	15.2	74	45	1.033
b	m	13	15.8	77	43	1.036
·e	m	15	15.5	73	49	1.051
XI a	m	17	13.4	75	51	1.107
b	m.	22	20.4	82	46	1.189
c	m	15	15.8	79	47	1.193
d	m	20	18.7	80	58	1.195
XII c	m	25	20.5	84	54	1.234
a	f	28	35.4	103	88	1.273
XIII a	m	16	20.4	82	60	1.301
g	m		40.9	120	69[2]	1.307
b	m		30.6	102	·85	1.327
c	m		29.8	106	73	1.346
h	f	28	38.5	114	90	1.392
XIV a	m		32.1	108	77	1.412
e	m		77.4	144	130	1.441
b	m	30	38.1	110	86	1.483
XV a	m	50	77.8	142	135	1.530
b	f	35	54.9	127	119	1.542
c	f	60	79.4	140	134	1.552
d	f	41	70.1	139	120	1.573
e	f	60	66.7	138	123	1.574
XVI a	m	105	121.0	167	151	1.642
g	f		163.0	191	173	1.643
c	f		216.8[3]	198	170	1.647
e	m		145.0	178	147	1.690

TABLE 1—Concluded

NUMBER	SEX	AGE	BODY WEIGHT[1]	BODY LENGTH	TAIL LENGTH	BRAIN WEIGHT
		days	grams	mm.	mm.	grams
XVII f	f		234.0	201	174	1.720
a	m	77	119.5	163	150	1.721
b	m	90	123.0	169	141	1.730
c	f	108	125.3	162	150	1.731
XVIII c	m	112	204.7	195	160	1.817
a	m	150	191.0	192	165	1.844
e	m	112	210.0	198	164	1.855
XIX a	m	2 years	300.2	225	195	1.924
XX a	m	2 years	251.5	162[4]	215	2.039
b	m	2 years	321.0	180[4]	187	2.069

[1] Under 10 grams net weight, over 10 grams gross weight given.

[2] Cut.

[3] Pregnant.

[4] Because of rigor mortis, this figure is not reliable..

average values are in fair accord with the reference table values as shown in tables 3 and 4, corresponding respectively to tables 1 and 2.

These latter values have been obtained by the use of the formulas devised by Hatai and given in "The Rat" (Donaldson, '15). A comparison of the observed and calculated values shows that in the case of Groups II–XV in table 3 and Groups II–XIII in table 4, the agreement is close, and as these groups go up to 30 days of age or more they include the period in which there is any important increase in the thickness of the cortex. After 30 days of age the discrepancies which appear between the observed and computed body weights and body and tail lengths are not significant for the present investigation and may therefore be neglected.

TABLE 2

Showing the sex, body and tail lengths, body and brain weights of the albino rats used in this study on the thickness of the cerebral cortex in the horizontal sections, entered according to increasing brain weight

NUMBER	SEX	AGE	BODY WEIGHT	BODY LENGTH	TAIL LENGTH	BRAIN WEIGHT
		days	grams	mm.	mm.	grams
II f	m	1	5.5	50	21	0.288
g	m	2	6.9	53	19	0.296
III d	m	1	6.1	52	22	0.303
f	m	2	5.9	53	20	0.318
e	m	2	6.1	50	22	0.331
IV g	m	3	6.4	53	23	0.415
h	m	3	6.9	54	23	0.421
f	m	5	8.4	60	24	0.423
V j	m	5	9.9	63	29	0.520
l	m	5	9.1	61	27	0.535
k	m	7	11.3	63	28	0.541
n	m	7	10.6	63	28	0.563
m	m	7	11.6	63	30	0.569
VI d	m	7	9.0	62	28	0.613
b	m	7	8.7	63	28	0.650
VII d	m	8	9.9	64	29	0.728
c	m	7	9.3	63	32	0.794
VIII e	m	10	12.1	68	33	0.809
i	m	8	8.3	62	32	0.829
f	m	10	13.2	69	33	0.868
g	f	9	18.9	80	41	0.884
IX b	m	11	13.8	71	39	0.914
c	m	12	13.6	73	40	0.964
X d	f	11	15.1	75	48	1.028
c	m	12	15.6	77	44	1.035
f	m	14	15.2	79	45	1.098
XI e	m	16	16.2	78	50	1.121
XII d	m		20.9	84	51	1.209
b	f		21.3	82	60	1.255
e	m		23.9	92	78	1.257

TABLE 2—Concluded

NUMBER	SEX	AGE	BODY WEIGHT	BODY LENGTH	TAIL LENGTH	BRAIN WEIGHT
		days	grams	mm.	mm.	grams
XIII d	m		21.9	84	62	1.332
f	m	33	25.1	96	77	1.344
e	f		28.3	100	82	1.377
XIV d	f		76.7	135	125	1.448
c	m		54.9	126	93	1.461
XV f	f	50	83.0	152	135	1.533
g	f		86.5	147	140	1.599
XVI b	m	68	130.0	164	150	1.674
h	f		188.7	189	175	1.675
d	f		95.7	151	136	1.680
f	m		252.2	202	188	1.683
XVII e	f		168.5	190	162	1.723
d	m		184.0	195	156	1.738
XVIII b	f		127.5	168	157	1.802
d	m	112	188.4	188	152	1.844
XX c	m	2 years	295.0	203	194	2.004

III. TECHNIQUE

Fixation

For the exact measurement of the thickness of the cerebral cortex, it is desirable to have material which has suffered the least possible change in volume as result of fixation and also to employ a uniform technique. As a preliminary, I went over King's work ('10) on the effects of various fixatives on the adult albino rat brain, applying several kinds of fixing fluids to brains in different stages of growth, and imbedding them in paraffine. These brains were sectioned and the cell pictures studied under the microscope to determine the influence of the several fixatives upon the cells and the surrounding tissues. War conditions prevented the use of celloidin and photoxylin as imbedding media to be compared with paraffine.

TABLE 3

Showing the average age, body weight, body and tail lengths and brain weight grouped by brain weight of the albino rats used in this study for sagittal and frontal sections, accompanied by the calculated standard age, body weight, body and tail lengths corresponding to the given brain weights

GROUP	OBSERVED					CALCULATED STANDARDS[3]			
	Age	Body weight	ody length	Tail length	Brain weight	Tail length	Body length	Body weight	Age
	days	grams	mm.	mm.	grams	mm.	mm.	grams	days
I		3.0	38	14	0.161	—	—	—	—
II	B+	5.0	48	17	0.251	17	49	5.0	B+
III	3+	6.0	52	20	0.358	25	56	6.1	2+
IV	5−	7.8	57	23	0.432	27	58	6.8	4−
V	6+	9.3	61	27	0.542	31	62	8.0	6−
VI	7+	9.7	62	28	0.639	35	65	9.2	7−
VII	9−	8.7	62	33	0.750	39	69	10.9	8+
VIII	10−	11.6	67	33	0.841	42	71	11.8	9
IX	11+	14.0	76	40	0.964	47	76	13.3	11−
X	13+	15.5	75	46	1.040	51	80	15.6	14+
XI	19−	17.1	79	51	1.171	60	89	20.4	21+
XII	27−	28.0	94	71	1.253	68	96	25.0	26+
XIII	—	32.0	105	77	1.335	78	105	31.3	32−
XIV	—	49.2	121	98	1.445	93	121	44.0	41
XV	49+	69.8	137	126	1.554	110	138	63.0	52−
XVI	—	143.0[1]	179[1]	157[1]	1.656	129	157	91.2	64+
XVII	—	150.5	174	154	1.726	142	171	117.6	75−
XVIII	125	201.9	195	163	1.839	165	196	179.6	110+
XIX	2 years	300.2	225	195	1.924	183	215	250.5	205
XX	2 years	286.3	[2]	201	2.054	212	246	417.5	—

[1] Pregnant one omitted.

[2] Rigor mortis.

[3] All calculated by formulas for sexes combined.

As a result, Bouin's fluid (picric acid solution in water at room temperature, saturated, 75 vol., formalin 25 vol. and glacial acetic acid 5 vol.) was selected as most suitable for the present work. When the rat brain is fixed in this fluid for 24 hours, the shape and the weight of the total brain suffer but little, if any, change, as the result of the fixation. The other fluids commonly used for fixation either swell or shrink the brain very noticeably (King, '10). On comparing the sections obtained from brains fixed in other fluids with those fixed in Bouin's fluid,

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TABLE 4

Showing the average age, body weight, body and tail lengths and brain weight grouped by brain weight of the albino rats used in this study for horizontal sections, accompanied by the calculated standard age, body weight, body and tail lengths corresponding to the given brain weights

GROUP	OBSERVED					CALCULATED STANDARDS			
	Age	Body weight	Body length	Tail length	Brain weight	Tail length	Body length	Body weight	Age
	days	grams	mm.	mm.	grams	mm.	mm.	grams	days
II	2−	6.2	52	20	0.292	22	54	5.5	1+
III	2−	6.0	52	21	0.317	23	55	5.7	2−
IV	4−	7.2	56	23	0.419	27	58	6.6	3+
V	6+	10.5	63	28	0.546	31	62	8.1	5+
VI	7	8.9	63	28	0.631	35	64	9.1	7−
VII	8−	9.6	64	31	0.761	40	69	· 11.0	8+
VIII	9+	13.2	70	35	0.848	42	71	11.9	9+
IX	12−	13.7	72	40	0.939	46	75	13.3	10−
X	12+	15.3	77	46	1.054	52	80	16.0	15−
XI	16	16.2	78	50	1.121	56	85	18.3	18+
XII	−	22.0	86	63	1.240	67	95	24.1	25+
XIII	−	25.1	93	74	1.351	80	107	32.7	33
XIV	−	65.8	131	109	1.455	94	122	45.3	42−
XV	−	84.8	150	138	1.566	111	139	64.0	53+
XVI	−	166.7	177	162	1.678	133	162	99.0	68−
XVII	−	176.3	193	159	1.730	143	172	119.0	75+
XVIII	−	158.0	178	155	1.823	162	192	170.0	102+
XX	2 years	295.0	203	194	2.004	200	233	340.0	−

I was convinced that, for the present study, the latter should be exclusively used (cf. also Allen, '16).

From an examination of the cell bodies and nuclei, I have concluded that they were not much modified by either swelling or shrinkage. The sections fixed with Bouin's fluid are, however, less well stained with Nissl's soap-methylene-blue-solution, toluidin blue or thionine than the sections fixed with formalin or alcohol. But, if carbol-thionine solution (1 gram thionine powder dissolved in 99 cc. of 0.5 per cent aqueous solution of carbolic acid) be used as the stain, this defect is remedied, and in the rat brain at least, they stain distinctly.

As a result of these preliminary tests the sections used for this study were all prepared by the methods just described and in doing this, the procedure outlined in the next section was followed.

The method of procedure

The rat was chloroformed and its sex, body and tail lengths and body weight recorded. After complete evisceration, the brain was exposed and the pia mater carefully removed from the surface of the brain. Then the entire brain was severed from the cord by a transverse section at the level of the calamus scriptorius and taken out, care being taken to preserve the paraflocculi and the olfactory bulbs. The brain was put on a glass plate, basal surface down and without lateral support. The five diameters of the hemispheres were next measured—according to the method described in the first paper of this series (Sugita, '17)—this procedure being desirable not only for the present study but also for a study on the changes in the size and shape of the brain according to age. The total brain was then put into a closed weighing bottle and weighed to the tenth of milligram. The brain was next transferred to Bouin's fluid for 24 hours at the room temperature, the basal surface of the brain being in even contact with the bottom of the vessel. After fixation, the brain was washed with running water for 20 minutes and then put into 20 cc. of 80 per cent alcohol for 24 hours and into 20 cc. of 90 per cent alcohol for 24 hours successively, for dehydration and further fixation. On the occasion of each change of fluid the diameters of the hemispheres were measured and the total weight taken in order to record the modifications produced.

With india ink and a fine brush lines were circumscribed on the surface of the brain in order to designate the planes from which sections should be taken after imbedding. The positions of these lines will be described in the following chapter. Slices about 2 mm. in thickness and including the level from which the sections were to be taken, were made, and placed in 10 cc. of absolute alcohol for 6 hours, then in xylol for one and a half hours at the room temperature, transferred into the xylol-parafine mixture for one and a half hours in the oven at 37°C., and finally imbedded in paraffine for two hours at about 56°C. I have used the paraffine with the melting point at 54°C. in winter

time and that at 56°C. in summer time, for the convenience of
making sections.

A series of sections 10 micra thick was cut exactly from the
designated level, adjusting the microtome carefully to this end,
and these sections were affixed on the glass slide by the albumin-
glycerine method.   After the sections had dried in the oven over-
night at 37°C., they were washed in the xylol bath, in order to
dissolve away the paraffine from the sections, and then again
washed with absolute alcohol to remove the residual xylol.
After having passed through the several grades of alcohol, they
were brought to the water bath, and kept there until the yellow
tone of the sections due to the picric acid in Bouin's fluid totally
faded away.   Now the sections were placed in the carbol-thionine
solution above described and remained there for two hours at
room temperature.   After being washed slightly with running
water, and passed through 70 per cent, 80 per cent, 90 per cent,
95 per cent and absolute alcohols successively, each for a short
time, in which they were not only dehydrated but at the same
time decolorized, they were cleared in xylol or carbol-xylol and,
at last, mounted in the neutral Canada balsam.

Examination of sections

The sections were each projected on a sheet of paper by the
Leitz-Edinger projection-apparatus, at a magnification of twenty
diameters exactly.   The outline of the image was then accurately
traced on the sheet, and, perpendicular to the brain surface,
lines were drawn parallel to the radiations of the cortex at sev-
eral selected localities, at which the thickness of the cortex was to
be measured.   These localities are shown on the figures which
will be described in the following chapter.   The length of the
line between the external margin and the lowest cell-layer of the
cortex, where it is in contact with the white matter, was measured
with the sliding calipers accurately to a tenth of a millimeter,
and one twentieth of this value was recorded as the actual thick-
ness of the cortex on the slide at this locality.

Two or more measurements of each locality on a given section were always made and, where these differed, the mean value was recorded. The same locality measured on the different sections from one and the same series, taken from a given slice, showed for the most part some differences in thickness, though never large differences. The main reasons for these differences appear to be the following:

1. As 6 to 12 sections were taken in succession from a given slice, at the level of the mark of india ink, these sections should not be identical as to thickness of the cortex, owing to the fact that the surface of the hemisphere is, of course, not strictly vertical to the plane of the section. Changes in cortical thickness due to this cause are, in general, very slight.

2. Although the outer boundary of the cortex is very distinct, the borderline between the cortex and the white substance is not equally sharp and this may prove a source of slight error in determining the thickness of the cortex. Direct tests indicate a variability of less than one-half of 1 per cent plus or minus, so that this error may be neglected.

3. In the procedure of attaching sections to slide by means of albumin-glycerine, the sections were first warmed over a small flame, in order to unfold and flatten them. During this manipulation, slight differences of extension sometimes occur in consequence of the dissimilarity of the temperature used. If the temperature be a little higher than the melting point of the paraffine, sections extend at first much, and, when they are cooled, they may shrink rather suddenly, thereby reducing the size of sections below that found before heating. If the temperature be raised only just to the melting point, or a little below it, the size of the sections on slide will be quite unmodified, but it is not always easy to control the temperature precisely, and significant alterations in the size of the sections due to this influence do occur. With a view to adjusting these differences, a method of correcting the direct observations was devised and the manner in which the correction was applied will be given in a later chapter.

IV. THE SECTIONS

Sagittal sections

After complete fixation of the brain, the sagittal sections were obtained by slicing the right hemisphere in a plane parallel to the mesial surface. The cut passes through the frontal point (*S*), as shown in figure 1 diagrammatically, and touches tangentially the lateral boundary of the infundibulum, while passing sagittally through the olfactory bulb. The plane of this cut is marked with india ink on the brain, by a circumscribing
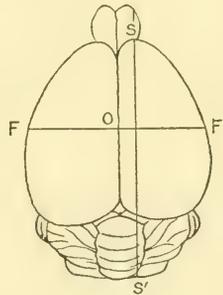
Fig. 1   Diagram of the entire brain of the albino rat seen from above, showing the levels from which the sagittal and frontal sections were taken. *SS'*, the sagittal section; *FF'*, the frontal section; *O*, middle point of the sagittal fissure.

line *SS'* around the parietal and basal surface of the right hemisphere, along which the sagittal section is to be taken, and a slice of the hemisphere, about 2 mm. in thickness, containing the delineated plane in the middle, is cut out and imbedded in paraffine as previously described. From this slice and exactly in the plane determined by the circumscribing line, 6 to 12 sections were cut in series, stained and mounted. From the left hemisphere of the same brain, frontal sections were also taken.

Figure 2 is a somewhat diagrammatic picture of the sagittal section from the albino rat brain at about thirty days in age, and is intended to show the cell-lamination of the cortex and the localities at which the thickness of the cortex was measured. Cytoarchitecturally the cortex of the sagittal section is divided

into several areas characterized by the difference of cell-lamination.

The cerebral cortex of the albino rat has five cell layers (fig. 3) if a typical locality be taken. The most external layer is the lamina zonalis (I), which has a few scattered glia-cells. Under this, there is the lamina pyramidalis (III) consisting of typical, deeply-staining, pyramidal cells lying closely together,
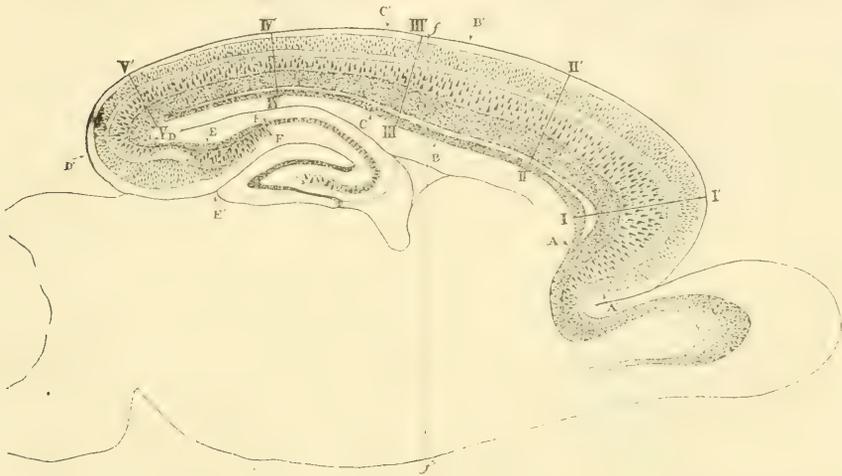
Fig. 2   Diagram of the sagittal section, from the albino rat brain weighing 1.5 grams, at about 30 days in age, showing the cell-lamination of the cortex and the localities at which the thickness of the cortex was measured.   *ff'* is the level from which the frontal section was to be taken.   Lines *AA'*, *BB'*, *CC'*, *DD'*, *EE'* and *FF'* indicate the borders of the areas showing different types of cell-lamination.

which corresponds to the third layer of Brodmann ('09).   In the rodent brain, the lamina granularis externa (II), or the second layer of Brodmann, is always indistinct, and it is almost impossible to distinguish it from the lam. pyr. (III).   Beneath the lam. pyr., the lamina granular is interna (IV) is situated, composed of crowded, deeply-staining, small granules, somewhat resembling glia-cells.   Below this layer, there is the lamina ganglionaris (V), which has dispersed, large-sized, deeply-staining

pyramids.   Next to the lam. gang., there is the lamina multi-
formis (VI) with polymorphous cells.

In the sagittal section (fig. 2) the cortical area lying between
AA' and BB',—AA' marking the knee where the gray of the
olfactory bulb passes over to the frontal cerebral cortex and
BB' marking almost the middle of the parietal cortex covering
the lateral ventricle,—is distinctly provided with all the five
cell layers (fig. 3).   The lam. zon., the lam. pyr., and the lam.
gang. are all typically constructed.   In the lam. gran. int., espe-

I. Lamina zonalis.

III. Lamina pyramidalis.

IV. Lamina granularis interna.

V. Lamina ganglionaris.

Ectal sublayer ⎫
               ⎬ Lamina multiformis.
Ental sublayer ⎭
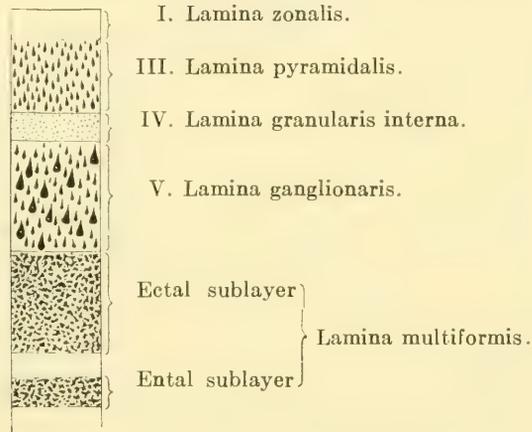
Fig. 3   Diagram of the typical cerebral cortex of the albino rat, for illustration
of figures 2, 4 and 6.

cially in material fixed in formol, the tissue surrounding the
granules may stain deeply, giving by low-power magnification
the appearance of a distinctly stained band, but this appearance
is not so evident in the material fixed in Bouin's fluid.   The lam.
gran. int. may be distinguished from the lam. pyr. by the fact
that in the former the cells are small and crowded densely.
There is a narrow band poor in cells, between the lam. gran. int.
and the succeeding lam. gang.   The lam. mult., in the albino
rat, is distinctly separated into two sublayers by a narrow, light
band very poor in cells.   The broader (ectal) sublayer which
lies immediately below the lam. gang. is rich in cells, about equal

in size to the cells of the lam. pyr., but slightly less stained. The narrower (ental) sublayer which lies under the band poor in cells forms the boundary to the white substance, and consists of polymorphous cells, somewhat larger in size than the small pyramids and tinted a little more deeply. In the area $AA'-BB'$, the lam. mult. is the thickest layer and it occupies more than one-third of the total thickness of the cortex. The lam. gang. is the next in thickness, almost equal to the sum of the thickness of the laminae pyr. et gran. int. The lam. gran. int. is the thinnest.

The small area between BB' and CC'' has almost the same cell lamination as the area $AA'-BB'$, but the cortex of this area becomes thinner towards the occipital pole. Especially the lam. gang. loses in thickness remarkably. The characteristic of this area is the appearance in the lam. gang. of the giant pyramids, representing the largest nerve cells in the cerebral cortex of the rat.

The area $CC''-DD'$,—DD' marking the occipital pole of the cortex—covers as a cap the cornu Ammonis. In it, the total thickness of the cerebral cortex diminishes on the average to a half of that in the area $AA'-BB'$. In this area, the lam. pyr. and the lam. gran. int. do not show much difference as compared with the foregoing areas, the cells of the both layers staining deeply. The lam. gang., however, becomes very narrow in this area, and, in addition to this, the size of pyramids is much reduced. The lam. mult. loses greatly in thickness, but the character of the cells remains unchanged. Near the occipital pole (DD') the light band which divides the lam. mult. into two sublayers disappears.

The area $DD'-EE'$ shows a quite characteristic cell-lamination, for example, the lam. zon. thickens distinctly while the laminae pyr. et. gran. int. thicken suddenly at the part, where the cortex is turned over the occipital pole and the surface of the hemisphere makes contact with the dorsal surface of the corpora quadrigemina. Immediately beneath the lam. gran. int. comes the lam. mult., the lam. gang. almost vanishing for a while. The lam. mult. exhibits, in this area, one layer instead of two.

The area EE'-FF' represents the subiculum cornu Ammonis.
The cortex consists of a thick lam. zon., the lam. gang. which
has again resumed its former breadth, and the very thin lam.
mult. At FF' the pyramids of the lam. gang. come more closely
together, and the breadth of the layer diminishes remarkably,
the cells arranging themselves in about three or four rows, as
they pass over to the specific ganglion cell layer of the cornu
Ammonis.

Figure 2 shows also the positions of the localities at which the
thickness of the cerebral cortex was carefully measured. These
localities have been so selected as to be fairly representative of
the entire cortex as regards thickness.

Locality I. On the line of I-I', drawn through the frontal tip
and running parallel to the radiation of the ganglion cells, the
diameter I-I' is measured, between the outer limit of the lam.
zon. and the inner border of the lam. mult. As a matter of fact,
the cell-radiation in this locality is not exactly in a straight line,
but somewhat bent, owing to the rapid curvature of the cortex
and its great thickness. Notwithstanding this, I have measured
the thickness of the cortex by a straight line, as indicated above,
The thickness at I-I' is the greatest found in this study.

Locality V. The thickness V-V' is measured at a point some-
what cephalad to the occipital pole, on the line directed along the
cell-radiation. I thought it would be more advisable to measure
the occipital cortex not exactly at the tip of the occipital lobe,
but somewhat cephalad, thus making the direction of the line
V-V' not very divergent from the direction of the line IV-IV',
if not parallel to it. The locality chosen (V) seemed suitable
and has been used throughout. The thickness at V-V' repre-
sents the thinnest part of the cortex.

Locality III is midway between localities I and V and falls
nearly in the middle of the area BB'-CC', the area in which the
largest pyramidal cells appear. The locality is marked by the
line III-III', drawn perpendicular to the cortex and tangent to
the ganglion-cell band at the frontal tip of the cornu Ammonis.

Locality II. The line at II-II' runs parallel with the radiat-
ing cells and lies midway between the localities I and III. The

line II–II' was drawn so that the distance between the middle-points of the lines I–I' and II–II' is equal to the distance between the middle-points of the lines II–II' and II–III', when the distances are measured along the cell band of the lam. gang. Locality II represents the central part of the area AA'–BB'.

Locality IV. The line IV–IV' is drawn midway between positions of the localities III and V, determined in the same manner as the position of the line II–II'. This locality represents the central part of the area CC'–DD'.

The lines I–I' and II–II' represent the thickness of the cortex in the area AA'–BB', the lines IV–IV' and V–V' the thickness of the cortex in the area CC'–DD', and through the increments in the lengths of these lines according to growth the development of the frontal and occipitals parts of the cortex of the rat brain as they appear in this plane may be determined.

For convenience of comparison in the final statement I have averaged the values of the above named five measurements and this is designated as the "average thickness of the cerebral cortex in the sagittal section."

Frontal sections

In figure 2 the line ff' marks the part of the brain from which the frontal sections were taken (FF' fig. 1). The frontal sections were obtained by cutting the hemisphere in a plane passing through approximately the middle point of the mesial surface, the corpus callosum, the commissura anterior and the chiasma opticum (fig. 1). For the frontal sections the left hemisphere of the same individual, from which the sagittal sections of the right hemisphere had been taken, was used. The technical procedure was similar to that used for the sagittal sections.

Figure 4 is the general diagram of the frontal section, from the albino rat brain, about thirty days of age, and illustrates the cell-lamination and the positions of the cortical localities which were measured. The cortex of the frontal section shows several areas characterized by the structure of the cell layers.

At the bottom of the sagittal fissure appears a small area, where

only a few cells, probably of the lam. mult., are to be seen (indusium).   Next to this, comes the area GG'-HH', GG' marking the mesial tip of the cortex, and HH' the knee of the cortical curvature.   This area has all the layers at HH', but all except the lam. mult. disappear at GG'.
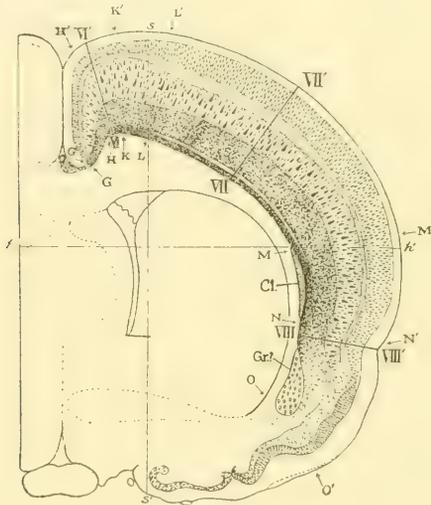
Fig. 4   Diagram of the frontal section, from the albino rat brain weighing 1.5 grams, at about 30 days in age, showing the cell-lamination of the cortex and the localities at which the thickness of the cortex was measured. ss', plane in which the sagittal section was taken; hh', level from which the horizontal section was taken.   Lines GG', HH', KK', LL', MM', NN' and OO' indicate the borders of the areas showing different types of cell-lamination. N' fissura rhinalis.   Between M and N the claustrum—(Cl)—is seen. $Gr.?$ marks the unidentified cell group between NN' and OO'.

In the area HH'-KK', the radiation of the cells is perpendicular to the curved surface of the cortex, and the lam. mult. is formed of a single layer.   This area is here noted, because the locality VI is in the middle of it and the line VI-VI' passes through the tip of the curvature.

The area KK'-LL' corresponds to the part, from which the sagittal sections were cut, and shows distinctly the five layers. In this area the lam. mult. is divided by a pale band into two

sublayers.   The cells of the lam. pyr., the lam. gran. int., the lam. gang. and the ental sublayer of the lam. mult. are all stained deeply, but the cells of the ectal sublayer of the lam. mult. are stained somewhat paler.   The lam. gang. is here relatively broad, occupying about one-third of the total thickness of the cortex while the laminae pyr. et gran. int. are relatively thin.

In the area $LL'-MM'$, representing the greater part of the cortex in the frontal section, the laminae pyr. et gran. int. are on the whole much thicker than in the areas just described, and the granules especially are densely crowded.   In the material fixed in formol, as remarked already, the intercellular tissue of the lam. gran. int. takes the stain so well, as to show apparently a deeply stained band by a low magnification.   The lam. gang. becomes thin as the laminae pyr. et gran. int. increase, and finally becomes thinner than the sum of both these layers.   The lam. mult. is divided into two sublayers, as was seen in the sagittal section, and its relative thickness does not vary greatly.

At the fissura rhinalis, denoted by N', the lam. zon. thickens markedly, while the total thickness of the other layers diminishes.   In the area $MM'-NN'$, the claustrum is seen at the bottom of the cortex, covered by the lam. mult.   Just ventrad to the line MM', the two sublayers of the latter fuse into a single layer and no special layer exists between this and the claustrum (Cl).

Between NN' and OO', the polymorphous cells of the lam. mult. are scattered and dispersed and, beneath the lam. mult., a cell group is seen, composed of large-sized, deeply-staining cells, which are hardly distinguished from the cells of the ental sublayer of the lam. mult.   The pyramids of the lam. pyr. are here more crowded together and make a wavy band.   Beneath this in a single layer, scantily scattered, the small cells of the lam. gran. int. and the large pyramids of the lam. gang., are seen.   There is a pale broad band between this layer and the above-mentioned cell group ($Gr.?$).

Median to OO', there is the tractus olfactorius ectal to the lam. pyr., which latter has been thrown into waves.

Figure 4 shows also the three localities, at which the thickness of the cortex has been measured.

Locality VI.   The line $VI-VI'$ starts from the tip of the dorso-mesial curve of the pallium perpendicular to the surface and runs parallel to the cell radiation.   This line represents the cortical thickness in the area $HH'-KK'$, where the laminae pyr. et gran. int. are so thin that the sum of the both layers does not amount to one-seventh of the total thickness of the entire cortex.   The lam. gang. is somewhat thicker than the lam. mult. and the latter becomes a single layer just at this point.

Locality VII.   The line $VII-VII'$ has been drawn at the middle of the area $LL'-MM'$.   In this area the laminae pyr. et gran. int. have increased in thickness, so as to amount to about one-third of the total thickness of the cortex, while the lam. gang. has undergone a corresponding diminution.

Locality VIII.   The line $VIII-VIII'$ is measured at the bottom of the fissura rhinalis.

The thickness of the cortex in the frontal section is greatest at $VII-VII'$, and least at $VIII-VIII'$, the level of the fissura rhinalis, while the thickness at $VI-VI'$ is intermediate.

For convenience of comparison I have averaged the values of the three thicknesses and named this the "average thickness of the cerebral cortex in the frontal section."

Horizontal sections

In figure 4 the line hh' indicates the level from which the horizontal sections were taken (HH' fig. 5).   The horizontal sections were made by cutting through the entire brain in a plane approximately parallel to the basal surface of the brain and passing through the frontal poles of both hemispheres and the points at which the occipital poles and the paraflocculi touch (fig. 5).   In the young rat brain, this section is tangent to the dorsal surface of the bulbus olfactorius.   In adults, however, it cuts somewhat obliquely through the bulbs, because, owing to the rapid development of the cerebellum in the early days of life, the paraflocculi, used as the marking points, extend dorsad, causing an upward
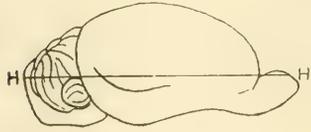
Fig. 5 Diagram of the entire brain of the albino rat, seen from the side and showing the level from which the horizontal sections were taken. *HH'*, horizontal section.
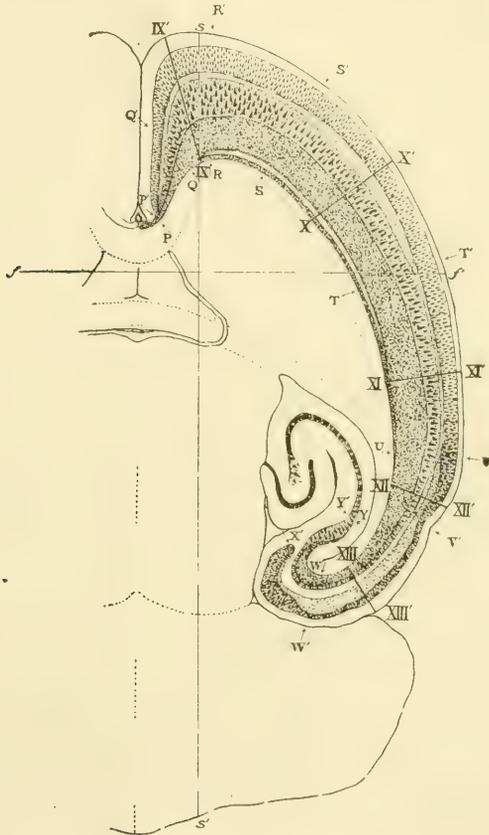
Fig. 6 Diagram of the horizontal section, from the albino rat brain weighing 1.5 grams, indicating the position of the localities measured on the section and the cortical cell-lamination. *ff'* and *ss'* show respectively the levels from which the frontal and the sagittal sections were taken.   The lines *PP'*, *QQ'*, *RR'*, *SS'*, *TT'*, *UU'*, *WW'*, *X'* and *YY'* indicate the borders of areas which show differences of cell-lamination and *V'* indicates the rhinal fissure.

shift of the occipital end of the plane of section.   The technique used in obtaining the horizontal sections was similar to that employed for the sagittal and frontal sections.

Figure 6 gives a schematic view of a horizontal section in this plane, from the albino rat at about thirty days of age.   It shows diagrammatically the cell structure of the cortex and the positions of the localities subjected to measurement.   The cortex of the horizontal section is divided into areas as follows.

The small area median to PP', lying at the bottom of the sagittal fissure between the two hemispheres, corresponds to the similar area median to GG' in the frontal section (fig. 4), which consists only of the cells belonging to the lam. mult. (indusium).   As we pass towards QQ', in the area $PP'-QQ'$, all the cortical layers appear and increase in thickness.   The area $QQ'-RR'$ contains the knee of the frontal cortex and the line IX-IX' runs through the very tip of the frontal pole.

The area $RR'-SS'$ is similar in cell-lamination to the area $AA'-BB'$ in the sagittal section (fig. 2) and the area KK'-LL' (fig. 4), and needs no explanation.   At RR' the two sublayers of the lam. mult. as seen towards SS', fuse into one.

In the area $SS'-TT'$, as in the area LL'-MM' (fig. 4), the lam. gran. int. increases markedly in thickness and cell-density, the lam. gang. at the same time becoming thinner.

In the area $TT'-UU'$, the total thickness of the cortex decreases.   Every layer takes part in this thinning and the cell arrangement in every layer loses little by little its regularity. Speaking broadly, the area $TT'-UU'$ has a structure different from that of $SS'-TT'$, in that the lam. gran. int. becomes so thin that it is difficult to recognize it, even in material fixed in formol.   Though the lam. mult. in this area does not show the pale band separating it into two sublayers, yet we can distinguish the two sublayers, the ectal or subganglional sublayer consisting of inflated cells and the ental, narrow band consisting of several rows of the somewhat larger, polymorphous cells, more deeply stained.

The area $UU'-WW'$ has a modified structure.   The fissura

rhinalis, denoted by V', lies midway in this area. Between the fissura rhinalis and the cornu Ammonis, there is a light band free from cells in the place of the lam. gran. int., although, near the fissura rhinalis, this layer has a small number of more or less scattered cells. The lam. pyr. shows distinctly two sublayers, the ectal or subzonal one consisting of a band of the large-sized, deeply-stained, crowded pyramids, and the other ental sublayer of a band of somewhat lightly stained, smaller-sized, inflated cells, which are less crowded than in the subzonal sublayer. This ental sublayer occupies about two-thirds of the thickness of the entire lam. pyr. The lam. gang. is scarcely to be distinguished and the lam. mult. is here formed by a single layer and is also thin. I would like to call attention here to the fact that though the large-sized, deeply-stained pyramids lying subzonally in the area $UU'-WW'$ are usually held to be derived from the cells of the lam. pyr. by change of form, I am much inclined to attribute these cells to another source, but the discussion of this question must be reserved for another paper.

The area $WW'-X'$ shows a cell-lamination, which is very peculiar in that the subzonal sublayer of the lam. pyr. suddenly increases in thickness, the pyramids become crowded, and the ental, epigranular sublayer becomes so thin as to be almost indistinguishable. The light cell-free band corresponding to the lam. gran. int. widens here.

At X' the lam. pyr. ceases abruptly. In the area $X'-YY'$, the cell-free parts of the cortex becomes much thickened, the laminae pyr. et gran. int. disappear entirely and the remainder of the lam. gang. undergoes a sudden thickening and becomes characterized by large-sized pyramids well dispersed. The lam. mult. also disappears at YY', giving place to the pyramids of the lam. gang., which continues into the cell band of the cornu Ammonis beyond YY'. The area $X'-YY'$ is the same part of the brain as that represented in the area $EE'-FF'$ of figure 2, namely the subiculum cornu Ammonis.

Figure 6 shows also the positions of the five localities at which the thickness of the cortex was measured on the horizontal section.

Locality IX. The line *IX–IX'* has been measured through the
tip of the frontal pole along the line of the cell radiation. This
measurement corresponds nearly to that at *I–I'* in the sagittal
section but lies a little nearer the median plane. Here the lam.
pyr. occupies about one-fifth of the total thickness, the lam. gran.
int. is thin, while the lam. gang. as well as the lam. mult. are
thick, the latter being represented here by one layer.

Locality X. The line *X–X'* has been measured at the middle
of the area *SS'–TT'*. The line *X–X'* was determined by joining
the point midway between the frontal border of the cornu Am-
monis and the tip of the cortex at *PP'*, with the lateral cortex
by a line perpendicular to its ectal surface. In this part, just
as in the corresponding area *LL'–MM'* in figure 4, the lam. gran.
int. increases its thickness considerably.

Locality XI. This has been measured, like locality III, at
the level of the forepart of the cornu Ammonis where the cere-
bral cortex is becoming gradually thinner.

Locality XIII. Near the occipital pole of the hemisphere,
this line *XIII–XIII'* has been measured at the latero-caudal point
where the cerebellum and the occipital lobe come into contact.
This locality is situated between *V'* and *WW'* and here the light
cell-free band, corresponding to the position of the lam. gran.
int., is clearly marked.

Locality XII. This locality has been measured about mid-
way between localities XI and XIII, near the fissura rhinalis
(*V'*). This corresponds roughly to the locality VIII in the frontal
section. For convenience of comparison I averaged the five
measurements here described and this value is named the "aver-
age thickness of the cerebral cortex in the horizontal section."

The measurements at localities IX–X–XI–XII–XIII in the
horizontal section, like those at localities I–II–III–IV–V in the
sagittal section, both reveal a gradual decrease in the thickness
of the cortex from the frontal to the occipital pole. By the
thickness at *IX* and *X* the development of the frontal cortex,
by the thickness at *XI* the development of the temporal cortex,
and by the thickness at *XII* and *XIII* the development of the
occipital cortex can be judged.

V. NOTES ON THE CORTEX OF THE NEWBORN ALBINO RAT BRAIN

In the foregoing chapter I have given a general description of the lamination of the mature cerebral cortex of the albino rat. But, in the case of the younger animals, especially at birth, the appearance of the sections is, of course, not the same, since the cortex is in an earlier stage of development. Figure 7 shows a general picture of the sagittal section of the newborn rat brain
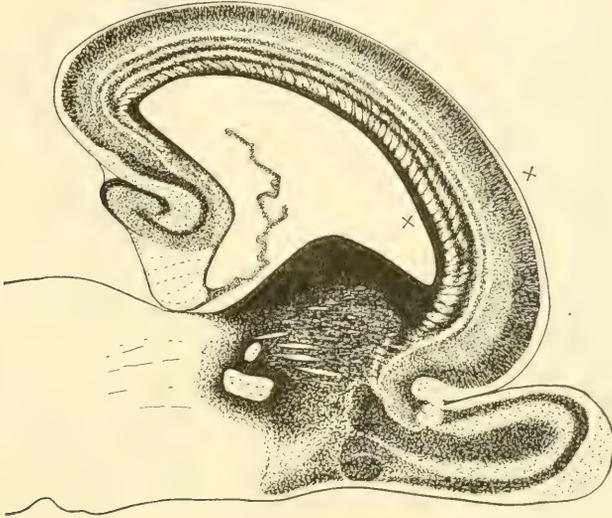
Fig. 7 Diagram showing the cortical cell-lamination of the newborn albino rat brain, which weighs 0.2 gram. Crosses ($\times$–$\times$) show the location from which figure 8 was taken.

(based on Rat. No. II a, brain weight 0.2132 grams), and figure 8 shows an enlarged, diagrammatic picture of the cell-lamination from the part marked with two crosses in figure 7, corresponding to locality II (fig. 2). In comparison with figure 9, which shows the corresponding part of the cerebral cortex in the sagittal section of the mature rat brain, locality II (fig. 2), the newborn cortex presents an appearance of more complexity, especially in the regions adjoining to the ventricular walls. The cells which
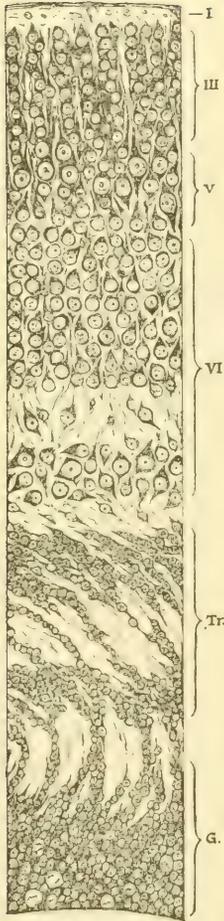
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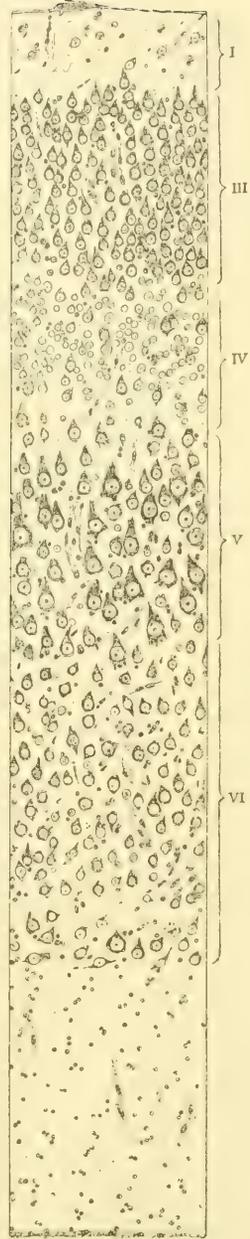
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are becoming ganglion cells have originated from the germinal cells lying in the ventricular wall and have migrated from there to their final position in the cortex. In the rat brain, cell migration is yet in progress at birth, giving the sections a peculiar aspect. The complication of the cortical lamination in the newborn rat is due to the existence of one or two transitional layers (so-called 'Uebergangsschichten', fig. 8, *Tr.*) between the germinal cells (fig. 8, *G*) in the ventricular wall and the cortex proper. These transitional layers are no more to be seen in the brain weighing more than 0.5 grams (age about 5 days).

On examining figures 7 and 8 it is seen that the five cortical layers are not yet clearly distinguished. The lam. zon. (fig. 8, *I*) is fairly thick and the borderline between this layer and the underlying lam. pyr. (III) does not run smoothly as in the adult, but shows a fine zigzag, suggesting that the invasion of the zonal region by the pyramids is still going on. The pyramids are not yet in a mature condition, their protoplasm is scanty and homogeneously stained and their form somewhat spindle-shaped. The nuclei stain less deeply than in the adult brain and the chromatin is not completely visible. The lam. gran. int. cannot be distinguished. The position of the lam. gang. (V) is already sparsely occupied by large-sized pyramids rich in protoplasm, but mixed up with them is a large number of small-sized pyramids, some of which are growing to be ganglion cells and some, probably, on their way to the lam. pyr. The lam. mult. (VI) is divided into two sublayers by a band, poor in cells, as is seen in the adult (fig. 9), but at this phase the number of the cells in the ental sublayer is very much greater and they are larger and better stained than the cells in the ectal sublayer. Their orientation is irregu-

Fig. 8 Diagram of cell-lamination of the prematurely-born albino rat brain weighing 0.2 gram, schematically enlarged from the designated part (×–×) of figure 7. *I*, lamina zonalis; *III*, lamina pyramidalis; *V*, lamina ganglionaris; *VI*, lamina multiformis; *Tr.*, transitional layers; *G*, germinal layer or matrix at the ventricular wall. Magnification × 100.

Fig. 9 Diagram of cell lamination of the adult albino rat brain weighing 1.8 grams, schematically enlarged from the locality II, figure 2, and corresponding to that selected for figure 8. *I*, lamina zonalis; *II*, lamina pyramidalis; *III*, lamina granularis interna; *V*, lamina ganglionaris; *VI*, lamina multiformis. Magnification × 65.

lar, some having the apical process vertical, some oblique and
some in an inverted direction (Hatai '02). This arrangement
indicates possibly that this sublayer serves as a secondary sta-
tion for the migrating neuroblasts, where immature cells in part
mature and orient themselves, though most of them must have
finished their rotation while passing through the transitional
layers. But we cannot regard this sublayer as purely temporary,
for it remains all through life persisting as a special thin layer
containing large, polymorphous, deeply-staining cells, that is
the ental sublayer of the lam. mult. However, in this earlier
phase it contains transitional elements, since the number of
cells is greater here in younger than in the older brains, so
that in the newborn we see even seven or more rows of cells in
this sublayer, while in the adult only three or four rows appear.
The ectal sublayer of the lam. mult. has already in it polymor-
phous cells destined to become ganglion cells. These are round,
somewhat larger than the pyramids in the lam. pyr., their apical
processes all directed ectad. In this layer a relatively large
number of small, round cells, probably future glia cells, are also
to be seen, while we do not see as yet cells of this type in the
more ectal layers.

In the newborn brain, there are one or in some earlier born, two
transitional layers between the lam. mult. and the ventricular
wall. At the earlier stage before birth there are always two such
layers (fig. 8, *Tr.*), afterwards but one, the ental layer having
disappeared. At birth, the wall of the ventricles consists of
germinal and indifferent cells, among which are many mitotic
figures. The thick layer of these crowded cells is the starting
place of the newly divided cells on their migration, during which
they rotate through 180° as they pass through the temporary
layers to their final station in the cortex. During the earlier
stages and when mitosis is most active (Allen '12), the neuro-
blasts are densely crowded both in the transitional layers and at
the cortex. The cells, crowded in the ventricular wall are small
in size, have deeply-staining nuclei and scanty protoplasm.
These cells poor in protoplasm form chains which fuse into a

loose network, but always show the general direction of the current of migration.

The (one or two) transitional layers lie between the ental sublayer of the lam. mult. and the ventricular wall, separated by pale bands which in turn are bridged by slender chains of cells. These transitional layers are probably the loci where the indifferent cells or neuroblasts perform their rotation. The cells of the transitional layers have small nuclei and more or less rich protoplasm and their arrangement suggests every stage of rotation. In brains a few days before birth (brain weight, 0.15 to 0.17 gram) this layer is clearly divided into two layers by a light band, as seen in figure 8, but in older brains the ental layer diminishes, or may even disappear leaving a single layer. The relatively broad layer poor in cells lying between this layer and the ventricular wall is prettily striped by the chains of the migrating cells which run radially at wide intervals. The light band lying between the transitional layers and the ental sublayer of the lam. mult. is narrow. In contrast to the small cells in the transitional layers, the cells of the lam. mult. are large and better stained.

In the newborn brain these transitional layers can be seen always lying between the ventricular wall and the cortical layers proper, so that in the sagittal sections they extend from the frontal tip where the cerebral cortex goes into the olfactory bulb, to the beginning of the cornu Ammonis. In frontal and horizontal sections, those transitional layers are also seen where the ventricular wall is close to the cerebral cortex. Where the cortex overlies interbrain structures, the transitional cell layers are not distinct.

These transitional layers disappear three or four days after birth and are not to be seen any more in brains of over 0.5 gram, in which the indifferent cells or neuroblasts radiate in loose chains from the ventricular wall directly to the ental sublayer of the lam. mult., without forming distinct transitional layers. The cells making the chains are less crowded and majority of them small-sized as seen in the younger brains.

During the first week after birth, when the increase of the cortex in thickness is most energetic, cortical cell-lamination is almost completed. Thus, at the fourth day after birth a light band appears between the lam. pyr. and the lam. gang., which suggests that the differentiation of the latter is now at an end. The ganglion cells are then in five or more rows. At birth, the ental sublayer of the lam. mult. has seven or more rows of multiform cells, but these decrease with advancing age and by the eighth day they have been reduced to four rows of cells or less. I will reserve the details of these changes till I take up in a later paper the development of each type of ganglion cell according to age.

According to His ('04) the transitional layers of the cerebral cortex may be recognized in the human embryo at 4 months, and, according to Mellus ('12) they are still visible in the brain of an eight months foetus and also of a newborn (stillborn) child. Anyhow, the fact that they are not so distinctly visible in a newborn human child as in a newborn rat, and that they persist till after birth in the latter brain, shows, as already stated by Donaldson ('08), that the albino rat is born with a brain somewhat less mature than that of the human child.

The transitional layers have not been subjected to measurement, because they do not belong to the cerebral cortex proper, and in making the measurements care has been taken to exclude the transitional layers in the very young brains.

VI. THE CELL-LAMINATION OF THE CORTEX OF THE ALBINO RAT

In previous chapters (figs. 2, 4, and 6), I have described the laminar structure of the cortex of the albino rat, as far as the cortex is presented to view in my sections. A description of the cortical lamination of the entire hemisphere is not included in the plan of this study, but, as there has been no description previously published on the cortical lamination of the albino rat, it has seemed worth while to refer here to some papers on the cortical cell-lamination in the Norway rat, from which the Albino has been derived, and in some other mammals more or less closely related and to compare the present observations with those previously made.

The first author to study the cell-lamination of the cerebral cortex of the rodents was Bevan Lewis ('81). He took the rabbit and the Norway rat together as the representatives of the rodents, but gave the details for the rabbit brain only, recording for it the thickness of the cortex and of size of the cortical cells. Bevan Lewis found cell-lamination in the cortex of the rabbit similar to that in the rat, so I will here cite his types of the cell-lamination for the rabbit only.

He divided the entire cortex of the hemisphere into eight areas distinguished by the laminar structure.

1. Type of upper limbic arc.
2. Modified upper limbic type.
3. Outer olfactory type.
4. Inner olfactory type. ⎫ Comprised within the limits of the lower and
5. Modified olfactory type. ⎰ anterior limbic arcs.
6. Extra-limbic type.
7. Type of cornu Ammonis.
8. Type of olfactory bulb.

Figure 10 reproduces the figures given by Lewis to show the distribution of these types of cortical structure. So far as these areas occur in my sections, a comparison of the lamination of the cortex of the albino rat with that of the rabbit shows the two cortices to be similar. It, however, seems hardly necessary to record the details of the comparison on this occasion, although such a detailed comparison has been made by me. According to Lewis the parietal cortex of the rabbit has a thickness of 2.8 mm.[1] while at the corresponding locality (Locality VII shown in figure 4) the cortex of the rat brain is 2.2 mm. thick (corrected value). From this, it would appear that the rabbit has a thicker cortex, but systematic investigations would be required to really determine this point.

Recently Fortuyn ('14) has studied thoroughly the laminar structure of the cortex in several rodents. He examined nine

[1] Measured on the section which was cut by the freezing microtome from the fresh material and then hardened by osmic acid, stained by aniline black and mounted in Canada balsam. According to his statement, we obtain, by this method, the natural depth of the cortex, no shrinking occurring if the preparations have been carefully made (Lewis, '78).

species and among these was Mus decumanus (Pall), or Mus norvegicus (Erx.), that is, the Norway rat, the wild form from which the Albino has been derived. He has divided the entire hemispherical surface into twenty-seven areas according to the characteristics of the laminar structure and these are
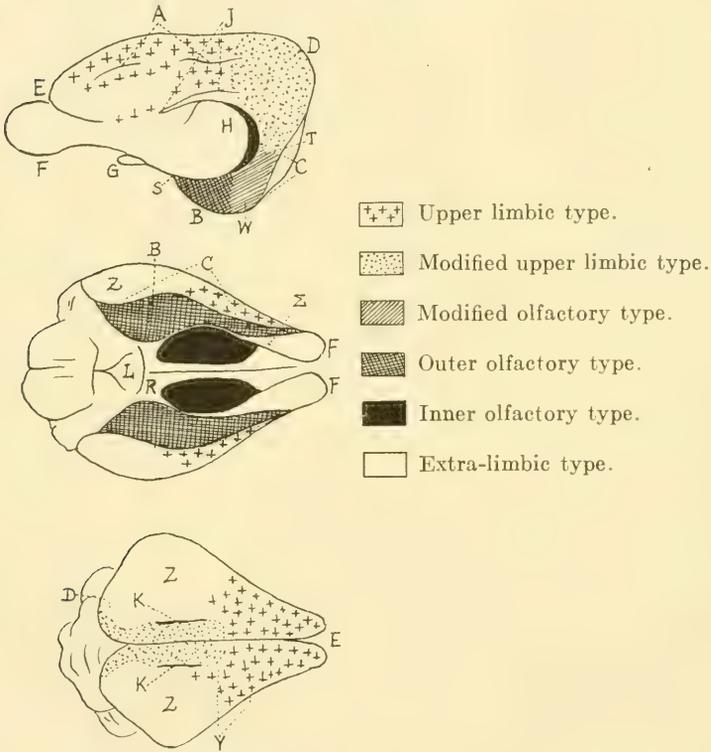
Fig. 10 Cortical areas of rodent's hemisphere—rabbit—reproduced from the original by Bevan Lewis.

shown in figure 11 copied from his paper. In figure 11, I have designated with *FF*, *SS* and *HH* the levels at which my sections were taken; *FF*, *SS* and *HH* being the abbreviations respectively of the frontal, the sagittal and the horizontal sections.

The following table is from Fortuyn's description of the cortical cell-lamination of the Norway rat brain. Under the letters desig-
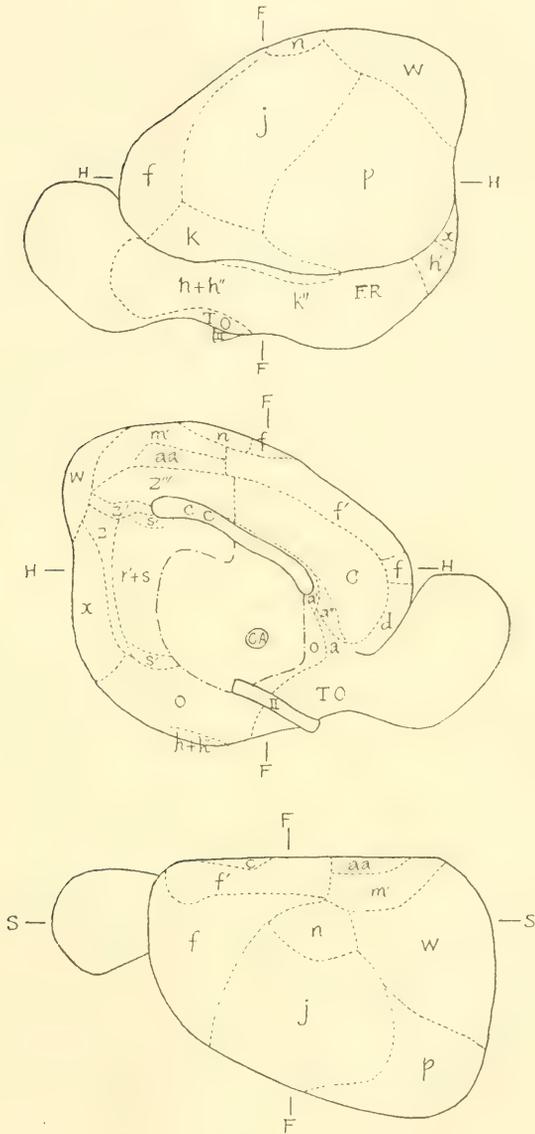
Fig. 11 Cortical areas of Mus decumanus (Pall), slightly modified from the original by Dr. Fortuyn; the characteristics of each area designated on the map are described in text. *FF*, *SS*, and *HH* show respectively the levels from which the sagittal, frontal and the horizontal sections were taken for my present studies.

nating the areas shown in the maps, the layers are given by Roman
numbers, that is; I. means the lamina zonalis, II. means the
lamina granularis externa, which is not distinguishable in the
rat brain, III. means the lamina pyramidalis, IV. means the
lamina granularis interna, V. means the lamina ganglionaris and
VI. means the lamina multiformis. In several areas, some of
these Roman numbers are absent, implying that in these areas
the corresponding layers are not distinguishable or are lacking.
Mathematical symbols, as = or +, show the relations of the
thickness of each layer. Pyr. means pyramidal cells. If the cell
form is not specially given, it is assumed to be polymorphous.
The general characteristics of the lamination of the area are in-
dicated in brackets and some characteristics of the individual
layers are indicated by a word or two after the Roman number.
The absence of remark indicates the ordinary arrangement of a
layer.

Area a.
    I. ......
    V. typical Pyr.
Area a'. (compact structure).
    I. ......
    III. = narrow; Pyr. crowded.
    IV. ......
    V. Pyr.
    VI. = V.
Area a''.
    I. ......
    V. = $\frac{3}{2} \times$ VI; Pyr.
    VI. ......
Area c.
    I. ......
    III. = narrow.
    IV. = narrower than III.
    V. = III + IV; Pyr.
    VI. = $\frac{1}{2} \times$ V.
Area d. (radiating and agranular).
    I. ......
    III. Pyr.
    V. Pyr.
    VI. ......
Area f.
    I. ......
    III. = narrow; typical Pyr.

    IV. = very narrow.
    V. = broader than III + IV;
       many Pyr.
    VI. divided into two sublayers
       by a light band.
Area f'. (radiating; otherwise similar
      to area f).
    I. ......
    III. ......
    IV. ......
    V. elongated Pyr.
    VI. not divided into two sub-
      layers.
Area g. (bottom of the fissura rhi-
      nalis at frontal part; not
      shown in figure 11).
    I. ......
    III. Pyr. resembling granules.
    IV. not numerous.
    V. Pyr.
    VI. ......
Area h + h''.
    I. ......
    III. Pyr. crowded together.
    IV. few cells scattered.
    VI. ......

Area h'.
    I. . . . . . .
  IIIA. = narrow; large Pyr.
  IIIB. = broad; small Pyr.
   IV. = narrow; poor in cells.
    V. = narrow; few Pyr., indistinct.
   VI. many cells.
Area j.
    I. . . . . . .
   III. = $2 \times$ IV; Pyr.
   IV. clouded.
    V. Pyr.
   VI. Similar to VI of area f, divided into two sublayers.
Area k.
    I. . . . . . .
   III. Pyr.
   IV. = narrower than III; less granules than in area j.
    V. = III + IV; small typical Pyr.
   VI. = narrow.
  Claustrum.
Area k''.
    I. . . . . . .
   III. = narrower than III in area k.
   IV. very few cells, light band.
    V. = narrower than V in area k.
   VI. = narrower than VI in area k.
  Claustrum.
Area l. (compactly constructed, situated at bottom of the fissura rhinalis near h'; not shown in figure 11).
    I. . . . . . .
   IV. . . . . . .
    V. = narrow.
   VI. = narrow.
Area m'. (radiating).
    I. . . . . . .
   III. = rather narrow; Pyr.
   IV. = narrow; poor in cells.
    V. = broad; Pyr; rather poor in cells.
   VI. very small cells.

Area n.
    I. . . . . . .
   III. . . . . . .
   IV. granular density great.
    V. many largest Pyr.
   VI. . . . . . .
Area o. (free from cortex).
Area p.
    I. . . . . . .
   III. . . . . . .
   IV. = narrow; few cells.
    V. Pyr.
   VI. nor divided by a light band, but close to the white substance a narrow layer of spindle-shaped cells.
Area r. (fascia dentata).
    I. . . . . . .
   IV.= narrow; cells crowded together, beneath IV some scattered cells.
Area s. (cornu Ammonis).
    I. . . . . . .
    V. Pyr. dispersed.
Area w.
    I. . . . . . .
   III. typical Pyr.
   IV. rich in cells, distinct.
    V. small typical Pyr.
   VI. . . . . . .
Area x.
    I. . . . . . .
  IIIA. Pyr.
  IIIB. = $\frac{3}{2} \times$ IIIA; Pyr.
   IV. no cells, light band.
   VI. . . . . . .
Area z. (agranular, light bands between III and V, and also between V and VI).
    I. . . . . . .
   III. typical Pyr.
    V. Pyr.
   VI. . . . . . .
Area z''.
    I. . . . . . .
   IV. . . . . . .
    V. = IV; Pyr.
   VI. = narrow.

Area z'''.
    I. ......
    III. = narrow; Pyr. crowded.
    IV. = 2 × III: cells closer
        crowded near III.
    V. = 2 × (III + IV); cells dispersed.
    VI. = V.

Area aa.
    I. ......
    III. = $\frac{1}{2}$ × III of area z'''.
    IV. = $\frac{1}{2}$ × IV of area z'''.
    V. = broad; Pyr.
    VI. ......

The areas appearing in my sections are collated below, with the corresponding areas from Fortuyn's map. The laminar structure was found generally similar to that described by Fortuyn.

Sagittal sections (fig. 2)   *Fortuyn's map*

Area AA'–BB'...f and f'
Area BB' CC' ....j or n
Area CC'–DD' ....w
Area DD'–EE'....................not precisely described, probably o?
Area EE'–FF'...s'?

Frontal sections (fig. 4)

Area GG'–HH'..c
Area HH'–KK'..f'
Area KK' LL'...f
Area LL'–MM' ....j
Area MM'–OO'...k
Area OO'...h + h''

Horizontal sections (fig. 6)

Area PP'–QQ'...c
Area QQ'–RR'..f'
Area RR'–SS'...f
Area SS'–TT'...j
Area TT'–UU' ....p
Area UU'–VV' ....w
Area VV'–WW'...h'
Area WW'–XX'....................not precisely described, probably o?
Area XX'–YY'...s'?

Thus we see that the description by Fortuyn of the cortex of the Norway rat is in the main applicable to the albino rat. This is, of course, what we should expect. However, there appear to be some slight differences shown by the areas VV'–WW', WW'–

X', X'–YY', DD'–EE' and DD'–FF' in my sections. All of these areas lie near to the cornu Ammonis and show a structure somewhat different from that described by Fortuyn. But, fortunately, none of these areas include localities where measurements of the cortex were made, so that the questions thus raised may be reserved for discussion in another paper.

In addition to the authors just cited, Brodmann ('09), in his valuable work on the cortical localisation of function in mammals, has examined the localisation in the cerebral cortex of the rabbit (Lepus cuniculus) and of the spermophilus citellus, as the representatives of the rodents. Though his description of the rabbit cortex does not extend to the details of the structure of the cell layers and for this reason, his 'Hirnkarte' can not be precisely compared with my sections in respect to the laminar structure, yet I think his diagrams do not deviate much from Fortuyn's map. For the sake of completeness, therefore, I give a table collating his regional terms with the areas mapped by Fortuyn and also with the corresponding areas shown in my own sections.

Brodmann's term. (anatomico-physiological)	Fortuyn	My sections
Regio praecentralis	f, f'	Areas AA'–BB', HH'–KK', KK'–LL', QQ'–RR'.
Regio parietalis	j, n	Areas BB'–CC', LL'–MM', SS'–TT'.
Regio occipitalis	w	Areas CC'–DD', UU'–VV'.
Regio insularis	k	Area MM'–OO'.
Regio temporalis	p, x	Area TT'–UU'.
Regio cingularis	c	Areas GG'–HH', PP'–QQ'.
Regio retrosplenialis	z, z'', z'''	No section.
Regio hippocampica	r', s	
Regio olfactoria	h + h''	Area OO'.

VII. THE THICKNESS OF THE CEREBRAL CORTEX ACCORDING TO BRAIN WEIGHT—TABLES AND CHARTS

A. Direct measurements on the slide

In the first instance, the thickness of the cortex was measured at localities I–XIII inclusive, on the sections as prepared and recorded without any corrections.

The results are condensed in table 5, where the average thickness for the sections in each plane is given and also the general average for the three sections is combined, the arrangement of the data being according to brain weight groups.

Chart 1 repeats in graphic form the data in table 5.

TABLE 5

Showing the general average thickness of the cerebral cortex of the albino rat according to brain weight groups, also the average thickness in the sagittal, the frontal and the horizontal sections

BRAIN WEIGHT GROUP	SAGITTAL SECTION			FRONTAL SECTION	HORIZONTAL SECTION			GENERAL AVERAGE	
	Number of cases	Brain weight	Thickness	Thickness	Number of cases	Brain weight	Thickness	Brain weight	Thickness
		grams	mm.	mm.		grams	mm.	grams	mm.
I	3	0.161	0.47	0.51					
II	5	0.251	0.58	0.65	2	0.292	0.70	0.265	0.64
III	5	0.358	0.83	0.90	3	0.317	0.74	0.344	0.85
IV	6	0.432	0.93	1.00	3	0.419	0.90	0.428	0.94
V	9	0.542	1.06	1.18	5	0.546	1.05	0.543	1.10
VI	3	0.639	1.16	1.30	2	0.631	1.14	0.636	1.20
VII	2	0.750	1.30	1.43	2	0.761	1.26	0.754	1.33
VIII	6	0.841	1.32	1.47	4	0.848	1.23	0.843	1.34
IX	3	0.964	1.36	1.53	2	0.939	1.35	0.956	1.41
X	3	1.040	1.31	1.51	3	1.054	1.37	1.045	1.40
XI	4	1.171	1.40	1.53	1	1.121	1.49	1.154	1.47
XII	2	1.253	1.43	1.55	3	1.240	1.44	1.249	1.47
XIII	5	1.335	1.39	1.52	3	1.351	1.39	1.340	1.43
XIV	3	1.445	1.40	1.51	2	1.455	1.48	1.448	1.47
XV	5	1.554	1.44	1.50	2	1.566	1.48	1.558	1.47
XVI	4	1.656	1.42	1.46	4	1.678	1.51	1.663	1.46
XVII	4	1.726	1.47	1.51	2	1.730	1.42	1.727	1.47
XVIII	3	1.839	1.52	1.52	2	1.823	1.54	1.833	1.53
XIX	1	1.924	1.44	1.50					
XX	2	2.054	1.53	1.47	1	2.004	1.69	2.037	1.56

The methods used in making the measurements have been described already. In applying these methods, from 2 to 12 measurements were made in each locality of each brain and the average of these recorded as the observed value. These data for each locality, in each brain of a brain weight group, were then averaged and the value obtained taken as that for the

group. The average measurements for each locality in each plane (section) were then again averaged to give the average thickness of the cortex in the sagittal, frontal and horizontal sections of each brain weight group. In chart 1, these are the values used for the ordinates, the average brain weight of the group being entered on the abscissa.
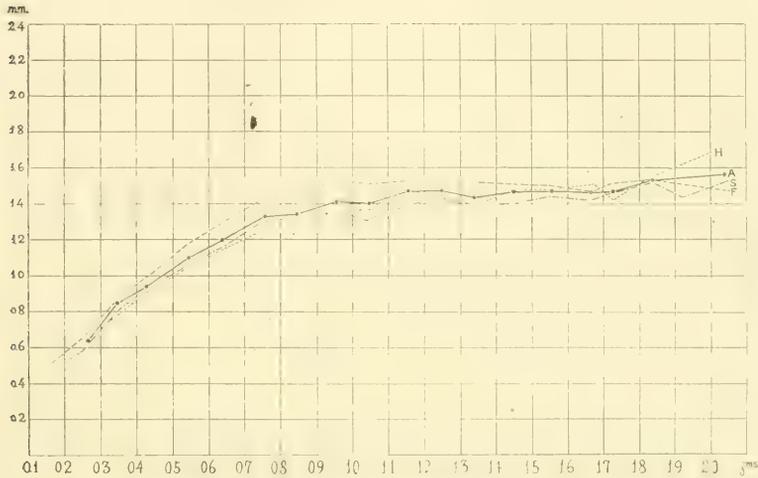
Chart 1 Giving the thickness of the cortex on slide (not corrected) in sagittal, frontal and horizontal sections and the general average thickness, according to brain weight. Based on table 5. •—•—•—S Average thickness of the cortex in sagittal section, measured on slide. – – – – –F Average thickness of the cortex in frontal section, measured on slide. - - - - - -H Average thickness of the cortex in horizontal section, measured on slide. •———•A General average thickness of the cortex of three kinds of sections, measured on slide.

All these data have been tabulated in detail and are on file at The Wistar Institute together with the sections used. The full tables have not been printed here because it is evident that the observations are open to a correction. The sections are from brains that have been subjected to an elaborate technique, while the brain weights are from the fresh specimens, and what we should like to know is the thickness of the cortex in the fresh brain. This can be obtained only by applying a correction to the observed values.

B. Measurements corrected for the effects of technique

The figures given in the foregoing table 5 and chart 1 were based on the direct measurement of sections prepared by the uniform technique as explained in a former chapter. But, since the shape and volume of the brain suffer some passive changes during preparation for study in this way, the values obtained by measurement on the slide do not represent those for the cortex in the fresh condition. We might think of these modified measurements as comparable among themselves, but, as will appear later, even that is not the case, since the change shown by a brain is related to its age (or size).

Thus, during fixation in the Bouin's fluid, the younger brains are little influenced in size, but, the more the age advances, the more the fluid causes shrinkage during fixation in all dimensions, especially in the sagittal direction. During fixation and dehydration, while the brain is passing through the several grades of alcohol, the older brain has more substance extracted by alcohol than younger. As a matter of routine I took the total weight of brain, just before it was transferred from 90 per cent into the absolute alcohol. In younger brains the weight is reduced to ca. 80 per cent of the fresh weight, while older brains, for example, that of a rat 150 days old or more, are reduced in weight to 66 per cent of the fresh weight.[2] Accordingly, of course, the size of the total brain suffers more shrinkage, as the age advances, during the process of dehydration.

It follows from this that the younger brain should have a relatively thicker and the older brain a relatively thinner cortex on the slide, as a result of the foregoing treatment. It is a question whether the white and the gray substance respond in exactly the same manner, but for the moment we assume that they do. Measurements indicate, however, that the shrinkage of fibers along their length is larger than that along their transverse direction, but the difference is so small that it may be neglected.

[2] Correction was not made for the weight lost by the replacement of water by alcohol. Details on this point will appear in a later part of this series of studies.

In two brains of the same age and treated by the same method, the ratio between any diameter measured on the fresh brain and that measured after imbedding in paraffine is almost constant, although heavier brains suffer slightly more shrinkage. But the ratio between a given diameter on slide and the same diameter in paraffine block has proved less constant, probably because, as mentioned earlier, on extending or unfolding the sections on slides the results are modified by slight differences in the temperature applied or in the duration of heating. I could not avoid this irregularity, though I endeavored to do so. These minor effects of the technique might be ignored in case of purely histological or pathological investigations, which aim only to detect changes in the formal aspects of the elements of tissue and do not regard the minute changes in size caused by the technique; but in the present study which requires painstaking exactness at every point, some effort must be made to correct for these changes.

At the beginning of this study, it was appreciated that such changes would occur, and the necessary preliminary observations were made (Sugita, '17). On the fresh rat brain I measured in each case the following diameters to 0.05 mm. by placing the brain on the glass-plate, basal surface down. Figure 12 gives the position of these diameters.

1. Width AB, (*W.B*,) the greatest width along the frontal plane.

2. Width CD, (*W.D*), passing through the middle point of the fissura sagittalis and parallel to AB. This corresponds to the plane in which the frontal sections were taken.

3. Length EF, (*L.F*), passing through the frontal pole and running parallel to the mesial surface of the hemisphere. This corresponds to the plane of the sagittal sections.

4. Length EG, (*L.G*), passing from the frontal pole at E to the occipital pole at G. This measurement gives the greatest length.

5. Height HK, (*Ht.*), (fig. 12 *b*) from the stalk of the hypophysis to the dorsal surface and vertical to the basal surface of the brain.

The results of the measurement are given in chart 2. This chart shows the curve of each measured diameter in millimeters given on the ordinates, the brain weight being entered on the abscissa. Generally considered, at birth, the width $W.B$ surpasses the length $L.G$, but after the third week, the length increases more rapidly and finally surpasses the width at the end of the seventh week, when the hemispheres appear somewhat elongated and ovoid.

Among these diameters, $L.F$ is in the plane from which the sagittal sections were taken and $W.D$ in the plane from which the
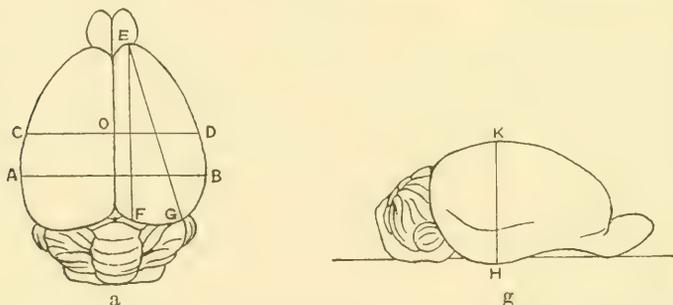
Fig. 12 *a*. Dorsal view of the albino rat brain weighing 1.5 grams, enlarged 1.8 diameters. To show the positions at which the two measurements for the width and the two measurements for the length were taken. AB = Width $W.B$, CD = Width $W.D$, EF = Length $L.F$ and EG = Length $L.G$.

b. Lateral view of the albino rat brain weighing 1.5 grams. Enlarged 1.8 diameters. To show the position at which the height was measured. HK = Height Ht.

frontal sections were taken. So I have used these values as those from which to obtain a coefficient for correction.

Assuming that, by fixation and when unfolded by heat on slides, the section shrinks or extends uniformly, and, that the white and the gray substances suffer approximately the same shrinkage or extension as the result of the treatment, I have selected the following correction-coefficients for use in this series.

In the sagittal section, the thickness of the cortex measured on the slide being represented by T_s and the thickness of the fresh cortex by T_F. Then

$$T_F = T_s \times \frac{L.F \ \text{(fresh)}}{L.F \ \text{(on slide)}}, \tag{1}$$
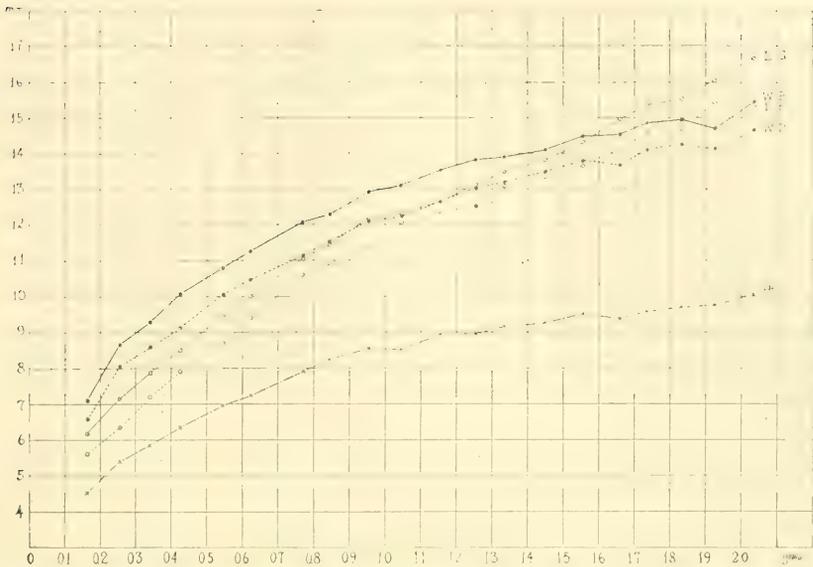
Chart 2 Giving for each brain weight group, in millimeters, on brain weight in grams, the values for the several diameters. $W.B.$ and $W.D.$, width; $L.F.$ and $L.G.$, length; $Ht.$, height. •——• $= W.B.$ •– – –• $= W.D.$ ○ ○ $= L.G.$ ○ – – –○ $= L.F.$ ×–·–·–× $= Ht.$

where EF is the longitudinal diameter in the plane of the sagittal section (fig. 12). The thickness of the cortex at localities I–V was corrected by the use of this coefficient.

For the frontal section the corresponding formula is

$$T_F = T_S \times \frac{W.D \ \text{(fresh)}}{W.D \ \text{(on slide)}}, \tag{2}$$

where CD is the width in the plane of the frontal section (fig. 12). The thickness of the cortex at localities VI, VII, and VIII was corrected by the use of this coefficient. Of course, the actual measurement on the slide was in this section that of one hemisphere only. The observed value obtained was therefore doubled for use in the formula.

After considering several possibilities, I decided to use for horizontal sections, the value for the maximum width of the brain, AB, in order to obtain the necessary formula. Thus,

$$T_\text{F} = T_\text{s} \times \frac{W.B \text{ (fresh)}}{W.B \text{ (on slide)}}, \tag{3}$$

where AB is the greatest width of the brain (fig. 12). The thickness of the cortex at localities IX-XIII was corrected by the use of this coefficient.

VIII. CORRECTED DATA PRESENTED IN TABLES AND CHARTS

Tables 6, 7 and 8 show the corrected values of the cortical thickness at the thirteen localities in the three kinds of sections examined. The data for the coefficients employed in correcting the individual entries are given separately together with the coefficients for each brain weight group. The method of applying the coefficients has already been described. The average thickness of the cortex for each brain was obtained from the corrected individual measurements. Charts 3 to 8 are based on the foregoing tables, charts 3, 5 and 7 showing the individual measurements and charts 4, 6 and 8 the average values of each locality for each brain weight group, in sagittal, frontal and horizontal sections respectively.

In tables 6, 7 and 8, the endeavor has been made to introduce all the details necessary for the interpretation and control of the results. In table 6, for example, the entry III a, age 2 days (see also table 1), is for a rat having a brain weight of 0.3105 grams. For the correction of the observed cortical thickness in the sagittal section, the coefficient was found by formula (1) page 554. This correction coefficient was applied to the measurements, as made on the slide, for each of the localities I to V at which the thickness of the cortex in the sagittal section had been determined. For each locality the corrected measurement in millimeters is given in the table and at the end of the line the average of the five corrected values appears.

Similarly by using the correction coefficient determined by formula (2) correction has been made in a like manner for the direct measurements at the three localities VI–VIII in the frontal section, table 7, taken from the other hemisphere of the brain, one hemisphere of which had been used for the sagittal section.

TABLE 6

Showing the corrected values of the cortical thickness in the sagittal section for each individual and for each brain weight group. The data for the coefficients are indicated separately for each brain and the coefficient is given explicitly in the average for each group. Group averages in italic

BRAIN WEIGHT GROUP	BRAIN WEIGHT	COEFFICIENT		THICKNESS OF THE CORTEX (SAGITTAL SECTION)					
		Diam. L.F on fresh brain	Diam. L.F on slide	Loc. I	Loc. II	Loc. III	Loc. IV	Loc. V	Average
	grams	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*
I a	0.153	5.50	4.97	0.59	0.54	0.49	0.41	0.35	0.48
c	0.154	5.60	4.80	0.73	0.64	0.55	0.44	0.35	0.54
b	0.177	5.70	5.13	0.71	0.67	0.55	0.45	0.39	0.55
	0.161	*1.13*		*0.68*	*0.62*	*0.53*	*0.43*	*0.36*	*0.52*
II a	0.213	5.80	5.13	0.77	0.73	0.60	0.51	0.46	0.61
b	0.221	6.00	5.43	0.71	0.66	0.56	0.48	0.36	0.55
c	0.261	6.60	5.52	0.92	0.86	0.80	0.73	0.72	0.81
d	0.271	6.75	5.80	0.93	0.82	0.70	0.67	0.45	0.69
e	0.288	6.70	6.11	0.91	0.76	0.68	0.52	0.41	0.66
(Birth)	*0.251*	*1.14*		*0.85*	*0.77*	*0.67*	*0.56*	*0.48*	*0.67*
III a	0.311	7.35	6.55	1.01	0.99	0.89	0.74	0.56	0.84
b	0.322	7.20	6.26	1.22	1.04	0.86	0.68	0.50	0.86
g	0.374	7.40	7.65	1.18	1.01	0.89	0.77	0.57	0.88
e	0.390	7.50	6.75	1.24	1.03	0.88	0.73	0.63	0.90
i	0.395	7.95	7.20	1.41	1.08	1.01	0.81	0.65	0.99
(2 days)	*0.358*	*1.09*		*1.21*	*1.03*	*0.91*	*0.75*	*0.58*	*0.90*
IV b	0.400	7.70	6.65	1.30	1.14	1.01	0.74	0.65	0.97
a	0.402	7.75	7.65	1.24	1.05	0.92	0.71	0.57	0.90
c	0.420	7.95	7.40	1.27	1.14	0.92	0.73	0.58	0.93
i	0.443	8.30	8.00	1.48	1.22	1.10	0.82	0.64	1.05
d	0.459	8.05	7.60	1.34	1.14	1.00	0.77	0.61	0.97
e	0.466	8.40	8.30	1.39	1.23	1.10	0.94	0.79	1.09
(4 days)	*0.432*	*1.06*		*1.34*	*1.15*	*1.01*	*0.79*	*0.64*	*0.99*
V i	0.501	8.35	7.90	1.49	1.31	1.13	0.91	0.72	1.11
a	0.525	8.55	8.30	1.48	1.24	0.99	0.76	0.64	1.02
b	0.528	8.50	8.05	1.57	1.31	1.13	0.89	0.67	1.11
c	0.534	8.65	7.70	1.48	1.24	1.10	0.92	0.74	1.10
d	0.537	8.30	7.70	1.50	1.30	1.16	0.89	0.73	1.12
e	0.555	9.25	8.50	1.60	1.49	1.39	1.05	0.74	1.25
f	0.558	9.20	8.60	1.66	1.36	1.20	0.94	0.75	1.18
g	0.564	8.85	8.50	1.51	1.30	1.14	0.96	0.74	1.13
h	0.579	9.10	8.25	1.64	1.40	1.24	0.96	0.73	1.19
(6 days)	*0.542*	*1.07*		*1.55*	*1.33*	*1.16*	*0.92*	*0.72*	*1.14*

TABLE 6—Continued

BRAIN WEIGHT GROUP	BRAIN WEIGHT	COEFFICIENT		THICKNESS OF THE CORTEX (SAGITTAL SECTION)					
		Diam. L.F on fresh brain	Diam. L.F on slide	Loc. I	Loc. II	Loc. III	Loc. IV	Loc. V	Average
	grams	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.
VI c	0.610	9.35	8.25	1.79	1.42	1.37	1.03	0.79	1.28
a	0.617	9.25	8.10	1.75	1.40	1.16	0.99	0.82	1.22
e	0.690	9.60	9.00	1.94	1.54	1.37	1.05	0.93	1.37
(7 days)	0.639	1.11		1.83	1.45	1.30	1.02	0.85	1.29
VII a	0.740	10.50	9.80	1.96	1.74	1.54	1.09	0.88	1.44
b	0.760	10.65	9.50	1.97	1.52	1.48	1.12	0.93	1.40
(8 days)	0.750	1.10		1.97	1.63	1.51	1.11	0.91	1.43
VIII a	0.800	10.50	9.25	1.90	1.57	1.46	1.21	0.91	1.41
h	0.805	10.90	9.20	2.13	1.70	1.58	1.17	0.85	1.49
b	0.822	10.45	9.80	1.94	1.60	1.49	1.17	0.92	1.42
c	0.849	10.50	9.70	2.05	1.70	1.56	1.22	0.95	1.50
k	0.870	10.95	9.70	2.18	1.72	1.62	1.22	0.99	1.55
d	0.898	11.45	10.15	2.08	1.67	1.59	1.26	0.98	1.52
(9 days)	0.841	1.12		2.05	1.66	1.55	1.21	0.93	1.48
IX d	0.959	11.60	10.50	2.13	1.69	1.59	1.29	0.97	1.53
e	0.960	11.40	9.85	2.18	1.74	1.63	1.31	1.06	1.58
a	0.972	11.30	9.80	2.01	1.73	1.59	1.22	1.05	1.52
(10 days)	0.964	1.14		2.11	1.72	1.60	1.27	1.03	1.55
X a	1.033	11.90	9.60	2.35	1.68	1.61	1.28	0.95	1.57
b	1.036	11.85	9.85	2.25	1.78	1.62	1.31	1.01	1.59
e	1.051	12.05	10.05	2.23	1.69	1.60	1.32	1.09	1.59
(15 days)	1.040	1.21		2.28	1.72	1.61	1.30	1.02	1.59
XI a	1.107	12.00	10.00	2.40	1.78	1.66	1.45	1.13	1.68
b	1.189	12.50	10.10	2.32	1.98	1.82	1.40	1.12	1.73
c	1.193	12.65	10.35	2.27	1.88	1.78	1.48	1.24	1.73
d	1.195	12.60	10.15	2.44	1.96	1.70	1.35	1.13	1.72
(20 days)	1.171	1.23		2.36	1.90	1.74	1.42	1.16	1.72
XII c	1.234	12.30	10.40	2.44	1.88	1.70	1.42	1.22	1.73
a	1.273	12.45	9.80	2.58	1.92	1.72	1.31	1.26	1.76
	1.253	1.23		2.51	1.90	1.71	1.37	1.24	1.75
XIII a	1.301	13.00	11.10	2.48	1.84	1.69	1.36	1.08	1.69
g	1.307	12.95	10.10	2.54	1.83	1.64	1.40	1.15	1.71
b	1.327	13.20	10.10	2.51	1.88	1.76	1.36	1.19	1.74
c	1.346	13.00	10.10	2.55	1.85	1.74	1.34	1.16	1.73
h	1.392	13.45	11.40	2.58	1.91	1.77	1.39	1.12	1.75
	1.335	1.24		2.53	1.86	1.72	1.37	1.14	1.72

TABLE 6—Concluded

BRAIN WEIGHT GROUP	BRAIN WEIGHT	COEFFICIENT		THICKNESS OF THE CORTEX (SAGITTAL SECTION)					
		Diam. *L.F* on fresh brain	Diam. *L.F* on slide	Loc. I	Loc. II	Loc. III	Loc. IV	Loc. V	Average
	grams	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*
XIV a	1.412	13.40	10.70	2.50	1.88	1.71	1.40	1.08	1.71
e	1.441	13.25	10.50	2.64	1.77	1.55	1.27	1.09	1.66
b	1.483	13.30	11.60	2.43	1.89	1.70	1.40	1.23	1.73
	1.445	*1.22*		*2.52*	*1.85*	*1.65*	*1.36*	*1.13*	*1.70*
XV a	1.530	13.70	11.30	2.56	1.83	1.74	1.34	1.13	1.72
b	1.542	13.50	11.40	2.53	1.79	1.61	1.30	1.18	1.68
c	1.552	13.70	10.70	2.56	1.92	1.69	1.40	1.23	1.76
d	1.573	13.70	11.20	2.65	1.98	1.76	1.39	1.21	1.80
e	1.574	13.75	11.20	2.70	1.94	1.78	1.44	1.30	1.83
	1.554	*1.22*		*2.60*	*1.89*	*1.72*	*1.37*	*1.21*	*1.76*
XVI a	1.642	14.10	11.30	2.78	2.05	1.80	1.38	1.19	1.84
g	1.643	14.65	11.50	2.72	1.79	1.68	1.28	1.11	1.72
c	1.647	13.75	11.40	2.78	1.84	1.68	1.35	1.16	1.76
e	1.690	13.65	10.90	2.62	1.94	1.76	1.30	1.09	1.74
	1.656	*1.25*		*2.72*	*1.91*	*1.73*	*1.33*	*1.14*	*1.77*
XVII f	1.720	14.90	11.80	2.84	1.81	1.72	1.34	1.16	1.77
a	1.721	13.90	11.40	2.67	1.91	1.78	1.38	1.19	1.79
b	1.730	13.85	11.50	2.67	2.07	1.87	1.46	1.28	1.86
c	1.731	14.30	11.70	2.71	1.98	1.67	1.30	1.15	1.76
	1.726	*1.23*		*2.72*	*1.94*	*1.74*	*1.37*	*1.19*	*1.79*
XVIII c	1.817	15.20	12.10	3.06	1.91	1.77	1.36	1.24	1.87
a	1.844	14.00	11.90	2.77	2.09	1.94	1.44	1.21	1.89
e	1.855	15.05	12.10	2.82	1.90	1.72	1.46	1.24	1.83
	1.839	*1.23*		*2.88*	*1.97*	*1.81*	*1.42*	*1.23*	*1.86*
XIX a	1.924	15.40	12.30	2.89	1.85	1.71	1.35	1.20	1.80
	1.924	*1.25*		*2.89*	*1.85*	*1.71*	*1.35*	*1.20*	*1.80*
XX a	2.039	15.10	12.80	2.82	1.86	1 72	1.36	1.22	1.80
b	2.069	15.55	13.30	2.80	1.99	1.72	1.33	1.16	1.80
	2.054	*1.19*		*2.81*	*1.93*	*1.72*	*1.35*	*1.19*	*1.80*

TABLE 7

Showing the corrected values of the cortical thickness in the frontal section for each individual and for each brain weight group. The data for the correction-coefficients are indicated separately for each brain and the coefficient is given explicitly in the average for each group. Group averages in italic.

BRAIN WEIGHT GROUP	BRAIN WEIGHT	COEFFICIENT		THICKNESS OF THE CORTEX (FRONTAL SECTION)			
		Diam. W.D on fresh brain	Diam. W.D on slide	Loc. VI	Loc. VII	Loc. VIII	Average
	grams	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*
I a	0.153	6.45	5.92	0.51	0.56	0.49	0.52
c	0.154	6.35	5.60	0.53	0.58	0.49	0.53
b	0.177	6.95	6.26	0.68	0.70	0.54	0.64
	0.161	*1.11*		*0.57*	*0.61*	*0.51*	*0.56*
II a	0.213	8.40	6.95	0.88	0.99	0.65	0.84
b	0.221	7.95	6.50	0.67	0.62	0.54	0.61
c	0.261	7.80	7.48	0.72	0.81	0.66	0.73
d	0.271	7.75	6.40	0.82	0.91	0.71	0.81
e	0.288	8.55	6.60	0.96	0.97	0.76	0.90
(Birth)	*0.251*	*1.19*		*0.81*	*0.86*	*0.66*	*0.78*
III a	0.311	8.50	7.65	0.84	0.95	0.84	0.88
b	0.322	8.70	6.80	1.14	1.14	0.93	1.07
g	0.374	8.95	8.45	1.07	1.15	0.92	1.05
c	0.390	8.85	7.40	1.11	1.11	0.93	1.05
i	0.395	9.10	8.60	1.09	1.14	0.99	1.07
(2 days)	*0.358*	*1.13*		*1.05*	*1.10*	*0.92*	*1.02*
IV b	0.400	9.00	8.50	0.97	1.09	0.88	0.98
a	0.402	9.10	7.90	1.17	1.27	0.97	1.14
c	0.420	9.00	8.15	1.07	1.16	0.91	1.05
i	0.443	9.15	8.40	1.12	1.26	0.95	1.11
d	0.459	9.50	7.85	1.25	1.38	0.98	1.20
e	0.466	9.30	9.25	1.18	1.29	1.07	1.18
(4 days)	*0.432*	*1.10*		*1.13*	*1.24*	*0.96*	*1.11*
V i	0.501	9.80	9.20	1.26	1.38	1.01	1.22
a	0.525	9.65	9.15	1.28	1.39	1.04	1.24
b	0.528	9.90	8.60	1.36	1.52	1.14	1.34
c	0.534	10.30	8.05	1.44	1.56	1.15	1.38
d	0.537	10.00	8.80	1.34	1.47	1.12	1.31
e	0.555	9.90	9.20	1.40	1.52	1.13	1.35
f	0.558	10.00	8.55	1.42	1.54	1.15	1.37
g	0.564	10.10	9.15	1.32	1.51	1.20	1.34
h	0.579	10.10	9.50	1.42	1.58	1.15	1.38
(6 days)	*0.542*	*1.12*		*1.36*	*1.50*	*1.12*	*1.33*

TABLE 7—Continued

BRAIN WEIGHT GROUP	BRAIN' WEIGHT	COEFFICIENT		THICKNESS OF THE CORTEX (FRONTAL SECTION)			
		Diam. *W.D* on fresh brain	Diam. *W.D* on slide	Loc. VI	Loc. VII	Loc. VIII	Average
	grams	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*
VI c	0.610	10.15	8.50	1.64	1.73	1.31	1.56
a	0.617	10.55	8.30	1.62	1.72	1.19	1.51
e	0.690	10.60	9.40	1.67	1.72	1.34	1.58
(7 days)	*0.639*	*1.19*		*1.64*	*1.72*	*1.28*	*1.55*
VII a	0.740	11.00	9.20	1.92	1.92	1.47	1.77
b	0.760	11.20	9.00	1.82	1.85	1.43	1.70
(8 days)	*0.750*	*1.22*		*1.87*	*1.89*	*1.45*	*1.74*
VII a	0.800	11.15	8.40	2.08	2.05	1.39	1.84
h	0.805	10.60	8.30	2.04	2.05	1.44	1.84
b	0.822	11.85	10.20	1.92	1.95	1.59	1.82
c	0.849	11.40	9.90	1.81	1.92	1.48	1.74
k	0.870	11.45	9.40	2.00	2.12	1.52	1.88
d	0.898	11.75	9.90	1.84	2.02	1.60	1.82
(9 days)	*0.841*	*1.23*		*1.95*	*2.02*	*1.50*	*1.82*
IX d	0.959	11.80	9.70	1.98	2.03	1.44	1.82
e	0.960	12.15	10.00	1.92	2.07	1.60	1.86
a	0.972	11.95	9.80	2.03	2.19	1.48	1.90
(10 days)	*0.964*	*1.22*		*1.98*	*2.10*	*1.51*	*1.86*
X a	1.033	12.40	10.30	1.94	2.03	1.56	1.84
b	1.036	12.40	9.70	1.91	2.06	1.56	1.84
e	1.051	12.10	10.20	2.01	2.04	1.50	1.85
(15 days)	*1.040*	*1.22*		*1.95*	*2.04*	*1.54*	*1.84*
XI a	1.107	12.90	10.20	2.25	2.03	1.55	1.94
b	1.189	13.15	10.70	1.98	2.02	1.67	1.89
c	1.193	12.70	10.50	1.97	2.06	1.57	1.87
d	1.195	12.50	9.80	2.10	2.15	1.56	1.94
(20 days)	*1.171*	*1.24*		*2.08*	*2.07*	*1.59*	*1.91*
XII c	1.234	12.95	11.00	1.97	1.99	1:60	1.85
a	1.273	12.90	10.00	2.14	2.19	1.56	1.96
	1.253	*1.23*		*2.06*	*2.09*	*1.58*	*1.91*
XIII a	1.301	13.20	10.60	2.11	2.14	1.61	1.95
g	1.307	12.70	10.20	1.97	2.17	1.53	1.89
b	1.327	13.35	9.60	1.98	2.17	1.67	1.94
c	1.346	13.15	9.70	2.08	2.33	1.62	2.01
h	1.392	13.10	11.20	1.98	2.09	1.68	1.92
	1.335	*1.28*		*2.02*	*2.18*	*1.62*	*1.94*

TABLE 7—Concluded

BRAIN WEIGHT GROUP	BRAIN WEIGHT	COEFFICIENT		THICKNESS OF THE CORTEX (FRONTAL SECTION)			
		Diam. $W.D$ on fresh brain	Diam. $W.D$ on slide	Loc. VI	Loc. VII	Loc. VIII	Average
	gms.	mm.	mm.	mm.	mm.	mm.	mm.
XIV a	1.412	13.65	10.30	2.17	2.33	1.63	2.04
c	1.441	13.10	9.50	2.07	2.30	1.53	1.97
b	1.483	13.80	10.80	2.03	2.25	1.65	1.98
	1.445	1.32		2.09	2.29	1.60	1.99
XV a	1.530	13.80	10.40	1.98	2.24	1.50	1.91
b	1.542	13.70	10.40	2.12	2.27	1.67	2.02
c	1.552	13.50	10.30	1.84	2.20	1.57	1.87
d	1.573	13.90	10.60	2.08	2.33	1.65	2.02
e	1.574	13.70	10.80	2.16	2.20	1.66	2.01
	1.554	1.31		2.04	2.25	1.61	1.97
XVI a	1.642	13.80	11.20	1.92	2.31	1.68	1.97
g	1.643	13.40	9.50	1.79	2.17	1.68	1.88
c	1.647	14.00	11.00	2.02	2.22	1.71	1.98
e	1.690	13.45	9.60	1.82	2.25	1.64	1.90
	1.656	1.32		1.89	2.24	1.68	1.94
XVII f	1.720	13.50	10.00	1.88	2.23	1.53	1.88
a	1.721	14.00	11.00	1.83	2.23	1.60	1.89
b	1.730	14.70	12.20	2.02	2.25	1.62	1.96
c	1.731	14.40	11.80	1.88	2.17	1.56	1.87
	1.726	1.26		1.90	2.22	1.58	1.90
XVIII c	1.817	14.00	10.40	1.91	2.15	1.54	1.87
a	1.844	15.00	12.40	2.15	2.36	1.62	2.04
e	1.855	14.30	10.60	1.96	2.37	1.63	1.99
	1.839	1.30		2.01	2.29	1.60	1.97
XIX a	1.924	14.10	11.60	1.78	2.17	1.54	1.83
	1.924	1.22		1.78	2.17	1.54	1.83
XX a	2.039	14.80	12.60	1.67	2.03	1.55	1.75
b	2.069	14.60	12.40	1.60	1.95	1.53	1.70
	2.054	1.18		1.64	1.99	1.54	1.72

TABLE 8

Showing the corrected values of the cortical thickness in the horizontal section for each individual and also for each brain weight group. The data for the correction-coefficients are indicated separately for each brain and the coefficient is given explicitly in the average for each group. Group averages in italic

BRAIN WEIGHT GROUP	BRAIN WEIGHT	COEFFICIENT		THICKNESS OF THE CORTEX (HORIZONTAL SECTION)					
		Diam. W.B on fresh brain	Diam. W.B on slide	Loc. IX	Loc. X	Loc. XI	Loc. XII	Loc. XIII	Average
	gms.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.
I									
II f	0.288	8.60	7.30	1.05	0.89	0.78	0.65	0.48	0.77
g	0.296	8.70	8.40	1.08	0.92	0.74	0.64	0.47	0.77
(1 day)	*0.292*	*1.10*		*1.07*	*0.91*	*0.76*	*0.65*	*0.48*	*0.77*
III d	0.303	8.65	7.75	1.16	1.02	0.84	0.71	0.49	0.84
f	0.316	9.20	7.30	1.26	1.05	0.92	0.81	0.62	0.93
e	0.331	9.20	7.20	1.31	1.07	0.92	0.77	0.57	0.93
(2 days)	*0.317*	*1.21*		*1.24*	*1.05*	*0.89*	*0.76*	*0.56*	*0.90*
IV g	0.415	9.60	7.60	1.58	1.25	1.02	0.87	0.76	1.10
h	0.421	10.00	7.30	1.72	1.41	1.08	1.00	0.86	1.21
f	0.423	10.00	7.90	1.71	1.42	1.19	1.04	0.66	1.20
(3 days)	*0.419*	*1.30*		*1.67*	*1.36*	*1.10*	*0.97*	*0.76*	*1.17*
V j	0.520	10.65	9.35	1.88	1.58	1.36	1.11	0.77	1.34
l	0.535	10.45	8.40	1.78	1.42	1.28	1.11	0.72	1.26
k	0.541	11.00	8.60	1.79	1.38	1.19	1.12	0.78	1.25
n	0.563	11.30	9.40	1.89	1.52	1.27	1.13	0.81	1.32
m	0.569	11.20	9.20	1.68	1.33	1.17	1.08	0.78	1.21
(5 days)	*0.546*	*1.22*		*1.80*	*1.45*	*1.25*	*1.11*	*0.77*	*1.28*
VI d	0.613	11.00	8.60	1.96	1.63	1.40	1.15	0.72	1.37
b	0.650	11.50	9.50	2.07	1.61	1.38	1.27	0.94	1.45
(7 days)	*0.631*	*1.24*		*2.02*	*1.62*	*1.39*	*1.21*	*0.83*	*1.41*
VII d	0.728	12.20	9.70	2.19	1.71.	1.48	1.34	1.11	1.57
c	0.794	12.15	9.55	2.36	1.85	1.62	1.35	0.97	1.63
(8 days)	*0.761*	*1.26*		*2.28*	*1.78*	*1.55*	*1.35*	*1.04*	*1.60*
VIII e	0.809	12.40	9.90	2.32	1.64	1.44	1.35	1.03	1.56
i	0.829	12.10	8.30	2.64	1.86	1.66	1.55	1.06	1.75
f	0.868	12.50	9.40	2.34	1.93	1.66	1.46	1.10	1.70
g	0.884	12.50	8.70	2.57	1.72	1.80	1.51	1.12	1.74
(9 days)	*0.848*	*1.36*		*2.47*	*1.79*	*1.64*	*1.47*	*1.08*	*1.69*
IX b	0.914	13.00	9.85	2.48	1.83	1.66	1.63	1.03	1.71
c	0.964	12.90	9.85	2.63	1.99	1.73	1.60	1.16	1.82
(10 days)	*0.939*	*1.32*		*2.56*	*1.91*	*1.70*	*1.57*	*1.10*	*1.77*

TABLE 8—Concluded

BRAIN WEIGHT GROUP	BRAIN WEIGHT	COEFFICIENT		THICKNESS OF THE CORTEX (HORIZONTAL SECTION)					
		Diam. W.B on fresh brain	Diam. W.B on slide	Loc. IX	Loc. X	Loc. XI	Loc. XII	Loc. XIII	Average
	gms.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.
X d	1.028	13.00	10.00	2.56	1.81	1.79	1.64	1.13	1.79
c	1.035	13.10	8.80	2.89	2.16	1.79	1.67	1.19	1.94
f	1.098	12.80	9.80	2.79	1.92	1.83	1.64	1.11	1.86
(15 days)	1.054	1.36		2.75	1.96	1.80	1.65	1.14	1.86
·XI e	1.121	13.10	10.40	2.88	1.92	1.83	1.66	1.11	1.88
(19 days)	1.121	1.26		2.88	1.92	1.83	1.66	1.11	1.88
XII d	1.209	13.95	10.20	2.82	2.13	1.91	1.78	1.26	1.98
b	1.255	13.75	9.90	2.97	2.22	1.88	1.71	1.23	2.00
e	1.257	14.05	10.50	2.76	2.03	1.83	1.70	1.19	1.90
	1.240	1.36		2.85	2.13	1.87	1.73	1.23	1.96
XIII d	1.332	14.05	10.00	2.67	2.01	1.80	1.58	1.15	1.84
f	1.344	13.90	10.80	2.65	1.98	1.84	1.59	1.26	1.86
e	1.377	14.00	10.30	2.65	2.11	1.92	1.67	1.21	1.91
	1.351	1.35		2.66	2.03	1.85	1.61	1.21	1.87
XIV d	1.448	14.00	10.75	3.06	2.03	1.86	1.61	1.17	1.95
c	1.461	13.95	10.60	2.60	2.12	1.93	1.66	1.29	1.92
	1.455	1.31		2.83	2.08	1.90	1.64	1.23	1.94
XV f	1.533	14.25	11.20	2.73	2.04	1.82	1.62	1.16	1.87
g	1.599	14.40	11.30	2.56	2.03	1.90	1.71	1.26	1.89
	1.566	1.27		2.65	2.04	1.86	1.67	1.21	1.89
XVI b	1.674	14.75	11.40	2.50	1.87	1.82	1.68	1.19	1.81
h	1.675	14.25	10.40	3.30	2.10	2.11	1.68	1.25	2.09
d	1.680	14.30	10.90	2.80	2.03	2.07	1.74	1.26	1.98
f	1.683	14.10	10.50	3.27	2.20	2.08	1.68	1.34	2.12
	1.678	1.33		2.97	2.05	2.02	1.70	1.26	2.00
XVII e	1.723	14.40	10.80	3.45	1.79	1.88	1.55	1.19	1.97
d	1.738	14.45	10.50	2.90	1.88	1.88	1.58	1.18	1.88
	1.730	1.35		3.18	1.84	1.88	1.57	1.19	1.93
XVIII b	1.802	14.50	11.20	2.65	2.13	1.96	1.77	1.19	1.94
d	1.844	14.70	11.40	3.08	2.05	2.03	1.70	1.29	2.03
	1.823	1.29		2.87	2.09	2.00	1.74	1.24	1.99
XIX									
XX c	2.004	15.10	11.60	3.30	2.26	2.26	1.83	1.38	2.21
	2.004	1.30		3.30	2.26	2.26	1.83	1.38	2.21
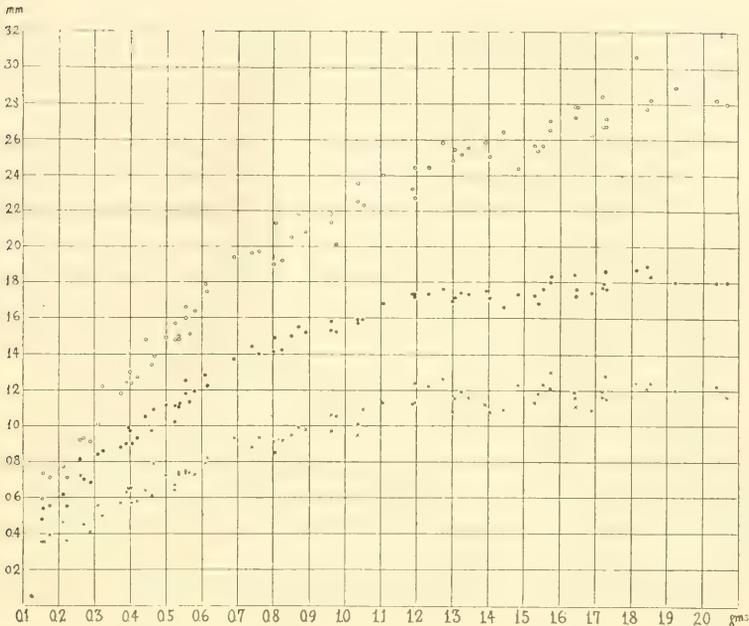
Chart 3 Giving the corrected thickness of the cortex of the albino rat in sagittal section. Individual entries for the cortical thickness at localities I and V, and the average thickness of the sagittal section (localities I, II, III, IV and V) are given. Based on table 6. ○, cortical thickness at locality I. Corrected. ×, Cortical thickness at locality V. Corrected. •, Average thickness of the cortex in the sagittal section. Corrected.

For the horizontal section a different brain was necessarily used. In this instance, table 8, brain III d is first in the Group III. Here the correction-coefficient was obtained by formula (3) and the observed values from each of the five localities IX to XIII were corrected accordingly. It is by the use of the measurements thus recorded that the average thickness of the cortex of a brain about two days of age has been obtained, as shown in table 9.

The entire series has been grouped according to brain weight, beginning with the group 0.1 to 0.2 gram and progressing by increments of 0.1 gram up to 2.0 grams. The normal brain weight at birth lies between 0.2 and 0.3 gram.
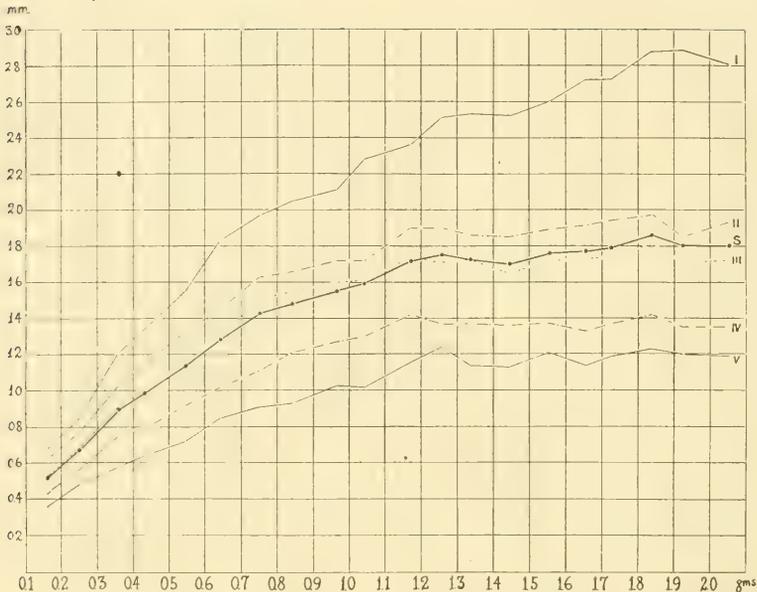
Chart 4 Giving the average thickness of the cerebral cortex for each brain weight group at localities I, II, III, IV and V in the sagittal section and the average thickness for the five localities in the sagittal section, for each brain weight group.   Based on table 6.   ⸻ (above the heavy line) Cortical thickness at locality I.   Corrected.   •—•—• (above the heavy line) Cortical thickness at locality II.   Corrected.   - - - - - - - Cortical thickness at locality III.   Corrected.   •—•—•— (below the heavy line) Cortical thickness at locality IV.   Corrected.   ⸻ (below the heavy line) Cortical thickness at locality V.   Corrected.   •⸻•S Average thickness of the cortex in the sagittal section for each brain weight group.   Corrected.

In each of the groups thus formed (twenty in tables 6 and 7, and eighteen in table 8), there are from one to nine brains in a group.   In tables 6 and 7 the average is nearly four per group and in table 8 it is about two and a half.

For some groups the approximate average age is given in days, but for all of the groups there has been entered the average brain weight, the correction-coefficient and then the corrected thickness of the cortex at each locality in the section. Finally the average thickness of the cortex for the entire section is given.
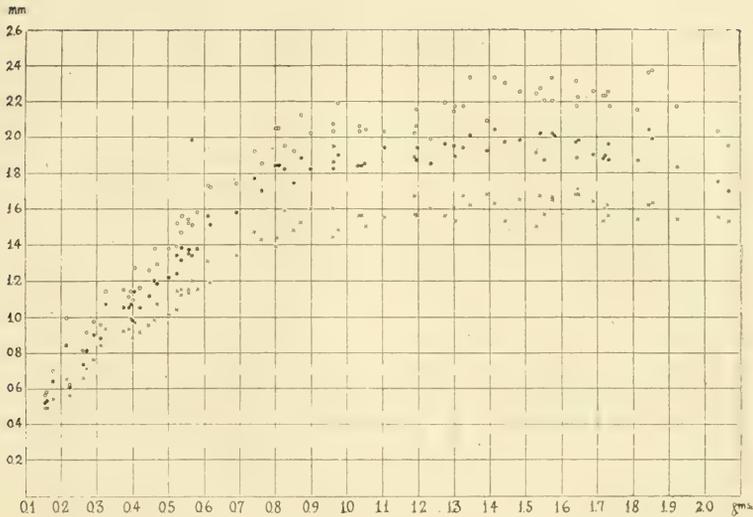
Chart 5 Giving the corrected thickness of the cerebral cortex of the albino rat in frontal section. Individual entries for the cortical thickness at localities VII and VIII, and the average thickness of the cortex in the frontal section for localities VI, VII and VIII are given. Based on table 7. ○, Cortical thickness at locality VII. Corrected ✕, Cortical thickness at locality VIII. Corrected. •, Average thickness of the cortex in the frontal section. Corrected.
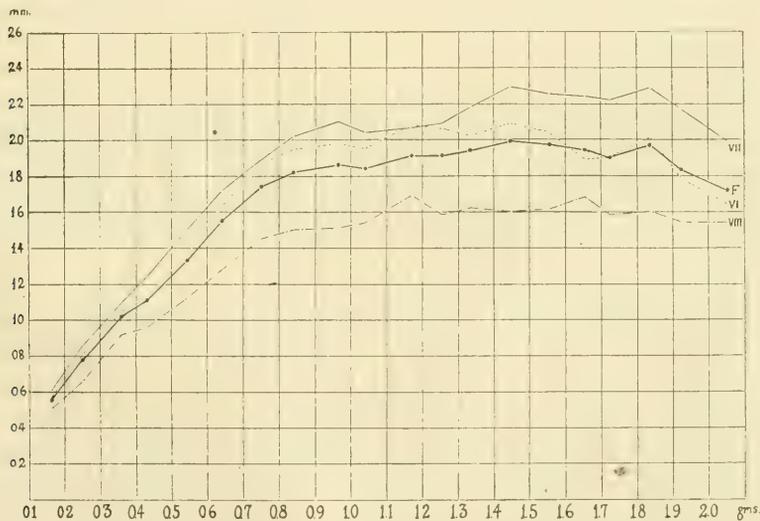
Chart 6 Giving the average thickness of the cerebral cortex for each brain weight group at localities VI, VII, VIII in the frontal section and also the average thickness of the cortex at the three localities combined. Based on table 7. - - - - Cortical thickness at locality VI. Corrected. ——— Cortical thickness at locality VII. Corrected. •—•—— Cortical thickness at locality VIII. Corrected. •———•F Average thickness of the cortex in the frontal section for each brain weight group. Corrected.
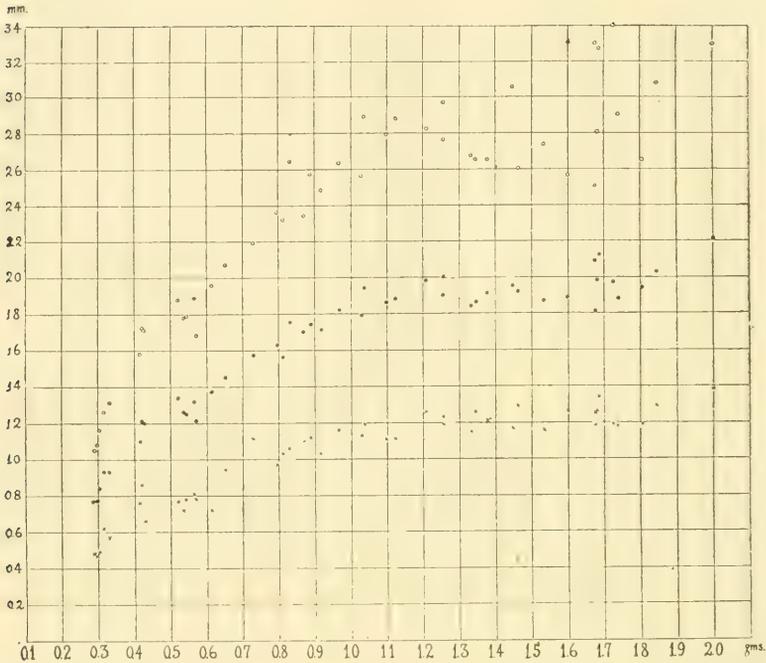
Chart 7 Giving the corrected thickness of the cerebral cortex of the albino rat in horizontal section.   Individual entries for the cortical thickness at localities IX and XIII, and also the average thickness of the cortex of the horizontal section at localities IX, X, XI, XII and XIII combined. Based on table 8. ○, Cortical thickness at locality IX. Corrected. ×, Cortical thickness at locality XIII. Corrected. •, Average thickness of the cortex of the horizontal section. Corrected.

By making tables 6, 7 and 8 in the form here used and by entering for each brain weight group the data for the correction-coefficient, it is made possible for any one who so wishes to recover from the corrected values here given the values as obtained by direct observation on the slide (table 5), though, in some instances, small discrepancies result between the values thus calculated and the values given in table 5, owing to repeated averaging and the frequent dropping of fractions under 0.005 mm.

To obtain the average thickness in any brain weight group the values for the average thickness in sagittal, frontal and horizontal sections are again averaged, as shown in table 9.   The
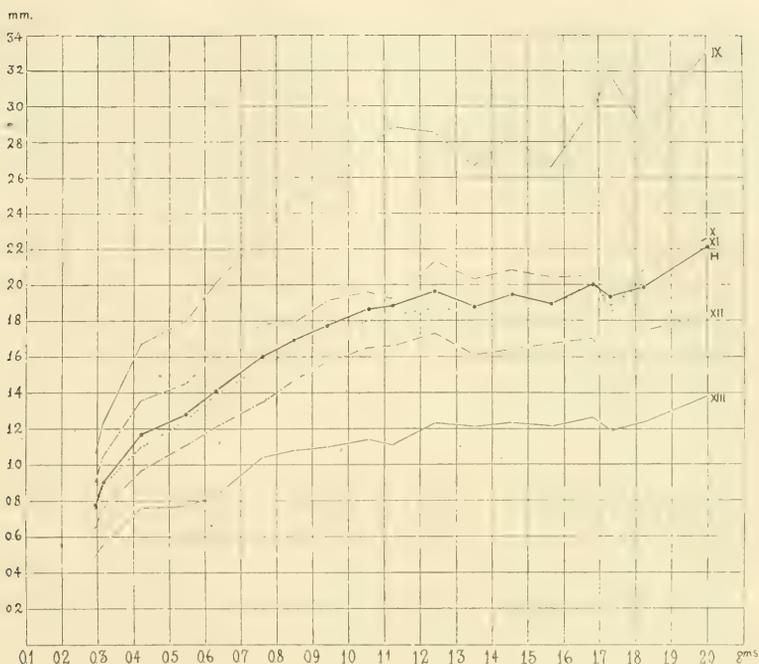
Chart 8 Giving the average thickness of the cerebral cortex for each brain weight group at localities IX, X, XI, XII and XIII in horizontal section and the average thickness for each brain weight group in horizontal section for the five localities combined. Based on table 8. —— (above the heavy line) Cortical thickness at locality IX. Corrested. •—•—• (above the heavy line) Cortical thickness at locality X. Corrected. - - - - Cortical thickness at locality XI. Corrected. •—•—•— (below the heavy line) Cortical thickness at locality XII. Corrected. —— (below the heavy line) Cortical thickness at locality XIII. Corrected. •——•H Average thickness of the cortex in the horizontal section for each brain weight group. Corrected.

general average thickness of the cortex in any brain weight group was obtained by adding the three averages for each brain weight group and dividing the sum by three. The averages of the brain weights were copied from tables 6 and 7 for the sagittal and frontal sections and from table 8 for the horizontal sections. The general average brain weight is obtained by adding the values in table 6 and in table 7 to the value in table 8 and dividing the sum by three.

This table 9 is valuable as a standard for the discussion of the actual growth of the cortex in thickness according to brain growth (weight), because these values have been corrected to the fresh condition and may be regarded as giving a truthful picture of the thickness of the cerebral cortex of the albino rat from birth to maturity.

TABLE 9

Showing the average (corrected) thickness of the cerebral cortex in the albino rat by brain weight groups

BRAIN WEIGHT GROUP	SAGITTAL SECTION		FRONTAL SECTION	HORIZONTAL SECTION		AVERAGE		
	Brain weight	Thick-ness	Thick-ness	Brain weight	Thick-ness	Brain weight	Thick-ness	Approxi-mate age
	grams	mm.	mm.	grams	mm.	grams	mm.	days
I	0.161	0.52	0.56					
II	0.251	0.67	0.78	0.292	0.77	0.265	0.74	B
III	0.358	0.90	1.02	0.317	0.90	0.344	0.94	2
IV	0.432	0.99	1.11	0.419	1.17	0.428	1.09	4
V	0.542	1.14	1.33	0.546	1.28	0.543	1.25	6
VI	0.639	1.29	1.55	0.631	1.41	0.636	1.42	7
VII	0.750	1.43	1.74	0.761	1.60	0.754	1.59	8
VIII	0.841	1.48	1.82	0.848	1.69	0.843	1.66	9
IX	0.964	1.55	1.86	0.939	1.77	0.956	1.73	10
X	1.040	1.59	1.84	1.054	1.86	1.045	1.76	15
XI	1.171	1.72	1.91	1.121	1.88	1.154	1.84	20
XII	1.253	1.75	1.91	1.240	1.96	1.249	1.87	
XIII	1.335	1.72	1.94	1.351	1.87	1.340	1.84	
XIV	1.445	1.70	1.99	1.455	1.94	1.448	1.88	
XV	1.554	1.76	1.97	1.566	1.89	1.558	1.87	
XVI	1.656	1.77	1.94	1.678	2.00	1.663	1.90	
XVII	1.726	1.79	1.90	1.730	1.93	1.727	1.87	
XVIII	1.839	1.86	1.97	1.823	1.99	1.833	1.94	
XIX	1.924	1.80	1.83					
XX	2.054	1.80	1.72	2.004	2.21	2.037	1.91	

The average thickness of the cerebral cortex of the adult albino rat is 1.88 mm., as obtained by averaging the values for the Groups XI to XX, in which stages the cortex may be considered as having reached about its full thickness.

The following chart 9 is based on table 9. This chart is very interesting and important for the further consideration as a standard picture of the cortical development and to it I shall refer in the following chapter.

The increase of the cortical thickness according to age, instead of brain weight, is given graphically in chart 10, which was plotted by using the above data, converted by calculations, based on the "Age-Body weight" and the "Body weight—Brain weight" formulas given in "The Rat" (Donaldson, '15). This chart shows in a dotted line also the brain weight curve according to age.
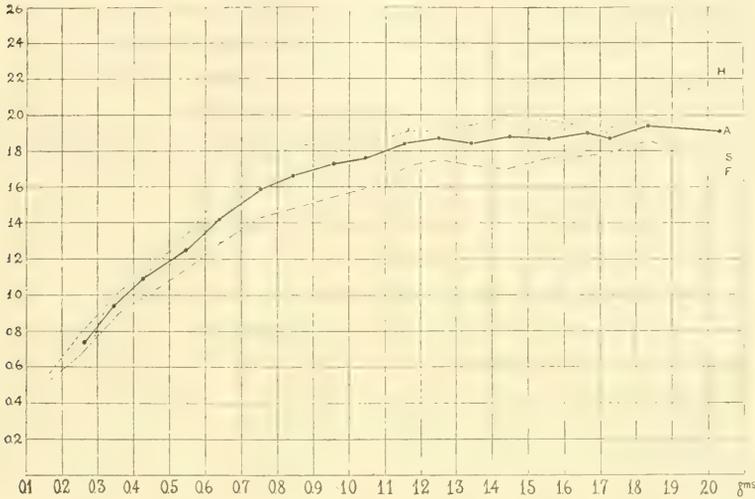
Chart 9 Giving the corrected thickness of the cerebral cortex in sagittal, frontal and horizontal sections and the general average thickness of the cortex for the three sections combined, on brain weight groups. Based on table 9. •—•—•—•—S. Average thickness of the cortex in sagittal section. Corrected. −−−−F. Average thickness of the cortex in frontal section. Corrected. −−−−−H. Average thickness of the cortex in horizontal section. Corrected. •————•A. General average thickness of the cortex for all three sections. Corrected.

On examining chart 9 (for the cortical thickness on brain weight), I was inclined in the first instance to conclude that the course of the cortical growth in thickness should be divided into three phases; that is, a first phase during which the brain weight increases from 0.25 gram (at birth) to 0.75 gram, and during which the increase in thickness is rapid; a second phase during which the brain weight increases from 0.75 gram to 1.15 grams,

when the rate of cortical increase diminishes; and a third final phase of very slow increase. But this division according to the brain weight is not suitable for comparative studies. If, however, we examine chart 10 (cortical thickness on age), it will be readily seen to be more advisable to divide the developmental phases of the cortical growth according to age rather than according to the brain weight; thus, according to age, the first
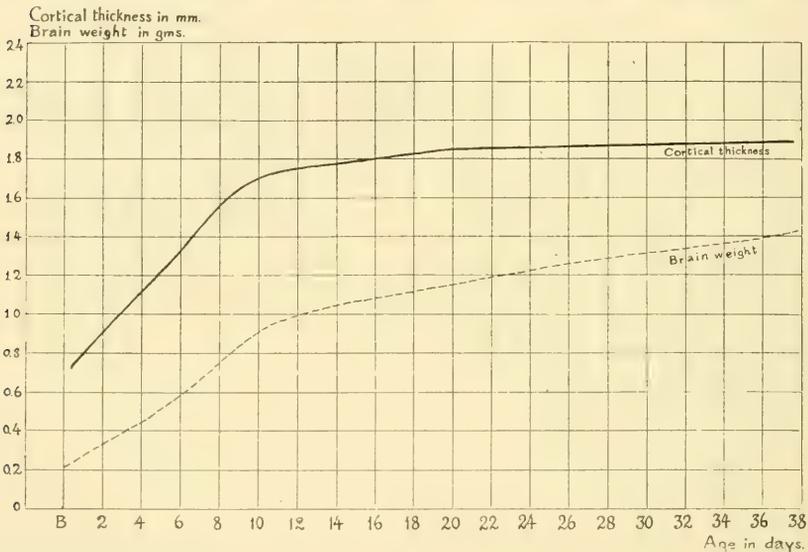
Chart 10 Showing the thickness of the cerebral cortex and the brain weight of the albino rat according to age, up to 38 days. The heavy line gives the mean cortical thickness in millimeters. The dotted line gives the brain weight in grams.

phase of the rapid increase covers the first ten days after birth (brain weight 0.25 to 0.95 gram), the second phase of slower increase covers the following ten days (brain weight 0.95 to 1.15 grams) and the third phase of very slow increase in the thickness of the cortex lasts from twenty-first day to the ninetieth day and, if necessary, a fourth phase for the remainder of the life span might be added.

As is seen in chart 10, the increase of the cortex in thickness according to age follows closely, during the first two phases, the increase of the brain weight according to age.

IX. DISCUSSION

Table 5 and chart 1 show the mean observed values on the slide, while tables 6 to 9 and charts 3 to 9 give in detail the corrected values which are assumed to be the actual thickness of the cortex in fresh condition. The corrected data will be taken as the basis for the discussion which follows.

Brains having almost the same weight rarely show exactly the same cortical thickness, but differ somewhat in this character. This may be due partly to the technical differences during preparation, but it also means probably some individual variation. Generally speaking, the thickness of the cerebral cortex is well correlated with the brain weight, so that, as a rule, the cortex increases its thickness as the brain increases in weight. With the albino rat, the first ten days after birth is a period of rapid growth especially for the central nervous system, so that, at the age of ten days the brain weight has attained nearly four times its weight at birth, growing from 0.25 gram to 0.95 gram, while the body weight has increased only 2.4 times—from 5 grams at birth to 12 grams on the tenth day. Accordingly, the cerebral cortex which follows the brain weight also shows a very rapid increase during this period. In this case, as in other cases of rapid growth, considerable individual variations naturally appear. For example, although 'V a' and 'V b' (table 6) are nearly alike in brain weight, the thickness of the cortex differs on the average as much as 0.10 mm. in the sagittal sections. Again, 'IV d' and 'IV e' with brain weights almost the same, show a difference of 0.12 mm. in the average thickness of the sagittal sections. These variations amount to ±5 per cent of the mean value for the cortical thickness.

Even thirty days after birth, when the phases of the rapid growth of the cortex have already passed, the individual variation in the cortical thickness is by no means low. During the second and third months after birth, the body weight increases from 30 grams to 150 grams or 400 per cent, while the brain weight increases only from 1.30 grams to 1.70 grams or 30 per cent. Through this period, the thickness of the cortex gains but

2 per cent or less (0.03 to 0.05 mm.). The variations during this period are illustrated by the following records.

Making the determination of the average deviation from the mean thickness of the cortex for Groups XIII to XVII inclusive (tables 6 and 7), it appears that for the sagittal sections this deviation is ±2.9 per cent and for the frontal sections ±2.7 per cent, while for the equivalent groups (table 8) giving the values for the horizontal sections is also ±2.7 per cent. From these results we conclude that after the period of rapid growth the thickness of the cortex follows closely the brain weight.

On the basis of chart 10 (the cortical thickness on age), I have concluded that during the earlier part of the life of the albino rat we may recognize three phases, in the growth of the cortex in thickness, namely;

1) First phase, from birth to the tenth day. (The brain weight increases during this phase from 0.25 gram to 0.95 gram).

2) Second phase, from the tenth day to the twentieth day. (The brain weight increases during this phase from 0.95 gram to 1.15 grams).

3) Third phase, from the twenty-first day to the ninetieth day. (The brain weight increases during this period from 1.15 grams to 1.80 grams).

During these phases the various localities, at which the thickness of the cortex has been measured, grow at different rates. The one which shows the most rapid development in thickness throughout life, is the locality I at the frontal pole (fig. 2). The cortex at locality I attains at the end of the first phase 2.5 times, at the end of the second phase 2.8 times and at the end of the third phase 3.2 times the thickness which it had at birth. At localities II, III, IV, X, XI and XII (figs. 2 and 6), namely those parts of the cortex near the middle of the brain, the development of the cortical thickness is similar. Thus these reach at the end of the first phase on the average 2.3 times, at the end of the second phase on the average 2.5 times and at the end of the third phase on the average 2.6 times the initial thickness at birth. The locality VI, which is situated at the margin of the sagittal fissure, and the locality VII, which represents the parie-

tal region, show a relatively rapid development in the first phase, attaining at the end of that phase 2.45 times the thickness at birth, while at the end of the second phase they have attained

TABLE 10

Giving for the three sections, the thickness of the cerebral cortex in each locality at the beginning and the end of each of the three phases and also the percentages of gain in thickness during each phase. The data on thickness are taken from tables 6, 7 and 8. The average percentage gain in thickness for each section was computed by using the average of the values for the observed thickness for each locality. The general average percentages in the last line of the table were obtained by using the mean of the averages for thickness in the three sections, giving the average values for each section the same statistical weight

SECTION	LOCALITY	THICK-NESS OF CORTEX AT BIRTH (GROUP II)	GAIN DURING FIRST PHASE OF 10 DAYS	THICK-NESS OF CORTEX AT 10 DAYS (GROUP IX)	GAIN DURING SECOND PHASE OF 10 DAYS	THICK-NESS OF CORTEX AT 20 DAYS (GROUP XI)	GAIN DURING THIRD PHASE OF 70 DAYS	THICK-NESS OF CORTEX AT 90 DAYS (GROUP XVIII)
		mm.	per cent	mm.	per cent	mm.	per cent	mm.
Sagittal section......	I	0.85	+148	2.11	+12	2.36	+22	2.88
	II	0.77	+123	1.72	+10	1.90	+ 4	1.97
	III	0.67	+139	1.60	+ 9	1.74	+ 4	1.81
	IV	0.56	+127	1.27	+12	1.42	+ 0	1.42
	V	0.48	+114	1.03	+13	1.16	+ 6	1.23
	Average	0.67	+131	1.55	+11	1.72	+ 8	1.86
Frontal section.......	VI	0.81	+144	1.98	+ 5	2.08	− 3	2.01
	VII	0.86	+144	2.10	+ 0	2.08	+10	2.29
	VIII	0.66	+129	1.51	+ 5	1.59	+ 1	1.60
	Average	0.78	+138	1.86	+ 3	1.91	+ 3	1.97
Horizontal section...	IX	1.07	+139	2.56	+12	2.88	+ 8	3.10[1]
	X	0.91	+110	1.91	+ 5	2.00[1]	+ 5	2.09
	XI	0.76	+124	1.70	+ 8	1.83	+ 9	2.00
	XII	0.65	+141	1.57	+ 6	1.66	+ 5	1.74
	XIII	0.48	+129	1.10	+ 5.	1.16	+ 7	1.24
	Average	0.77	+130	1.77	+ 7	1.90	+ 7	2.03
General average..........		0.74	+134	1.73	+ 6	1.84	+ 6	1.95

[1] As the observed values at these localities in these groups have fallen below those in the foregoing and the following groups, I used in this table the values based on the average of all three groups.

only 2.6 times. But, after this phase locality VI appears even to decrease slightly, while the locality VII is still increasing.

Table 10 gives the rapidity of the gain in the cortical thickness at each locality and for each of the three phases, according to the data in tables 6, 7 and 8. The column designated as "gain during the first phase" shows the percentage value of the thickness at the end of this phase as compared with the thickness at birth. The column designated "gain during the second phase" shows corresponding value for the second phase as compared with the thickness at the beginning of this phase. Similar in arrangement is the column giving the gain during the third phase. This table 10 shows very clearly the relative rapidity of growth at every locality and supports the interpretations presented above.

Speaking generally, the entire cortex, as represented by the thirteen localities from different parts of brain, shows very rapid development during the first phase (see general average in table 10), especially at the frontal pole and along the mid-dorsal aspect of the frontal section (localities VI and VII). The parieto-frontal parts (represented by localities II and X) are somewhat slow in development compared with those on either side of them.

Through the second phase, the parts showing the most rapid development are also the frontal pole (represented by the localities I and IX) and the parieto-occipital parts (represented by the localities IV and V), which were slow in starting, and the slowest growth is by the cortex which appears in the frontal section (represented by the localities VI, VII, and VIII). The growth at the locality XIII is also slow. This latter region has an heterogeneous structure of cortex, displaying a special type of cell-lamination, as shown in figure 6, and this may be associated with the retardation in its development in thickness. Remarkable is the fact that all the localities measured on the frontal sections, namely, the localities VI, VII and VIII, show relatively slow development during the second phase, having already attained nearly their full thickness at the end of the first phase. This is equivalent to saying that they are precocious.

In the third phase, there is some growth, except at the localities IV and VI. The locality VI, as remarked above, appears to decrease somewhat in thickness during this phase and the locality VII, which represents typical extra-limbic type of cell-lamination of the parietal part of hemisphere, makes a considerable progress throughout the last phase. The most marked growth is made, however, at the frontal pole, in the localities I and IX (charts 3, 4, 7 and 8).

From chart 2, which shows the development of the entire brain in each diameter, it will be seen that, after the brain has attained 1.0 gram in weight, the rapidity of growth in length (the sagittal diameter) largely surpasses that in breadth (the frontal diameter), a phenomenon possibly associated with the growth changes in the central nuclei, and with the rapid and continuous development of the cortex at the frontal pole.

At the locality III, the growth in thickness follows very closely the average thickness for the sagittal sections, and in the same way at the locality XI the growth in thickness follows the average thickness for the horizontal sections.[3]

Table 11 shows the localities arranged in the order of their cortical thickness at birth. In five cases, two localities close to one another and similar in structure are grouped together and the average thickness given. The order of the localities thus arranged by increasing cortical thickness remains unchanged at maturity. In this table, the ratios in both groups between the values of the same locality at birth and at maturity are roughly similar and

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[3] Incidentally, I made a series of sections from the rat fetus of 18 days (body length from neck to buttocks average 1.95 cm., body weight average 1.0 gram) by the uniform technique above presented. When examined in the sagittal sections of the entire body, four main layers in the entire ventricular wall of the brain are distinguished, for example, (1) the lamina zonalis ('Randschicht'), (2) the lamina corticalis ('Rindenschicht'), (3) the lamina intermedialis ('Zwischenschicht') and (4) the matrix. The average thickness of the entire wall of the hemisphere is 0.38 mm., and the lam. cort., which does not yet show any cell lamination, measures only 0.06 mm., consisting of five or six rows of the cells (the matrix 0.13, the lam. intermed. 0.16, the lam. cort. 0.06, the lam. zon. 0.03 mm. on the average in the sagittal sections). But in the newborn the thickness of the cortex has increased already to 0.6 mm. on the average in the sagittal sections (on slide), namely about ten times in thickness during the last four days of gestation.

the ratios between values of any two localities in the same age
group are all almost equal.   This indicates that the proportional
thickness at any region of the cerebral cortex to that of any other
region is quite constant throughout the growth of the brain.
Moreover it indicates that the thickness of the cortex at ma-
turity is directly related to the thickness found at birth and from
this we infer that the process of thickening is similar in the
several localities but that the amount of material (number of
cells) involved in the process differs.

TABLE 11

*Showing the relation between the initial and final thickness of the cortex at the several
localities.   The order of thickness at maturity is the same as the order of
thickness at birth*

LOCALITY	GROUP II (AT BIRTH)		GROUP XVIII (AT MATURITY)	
	Thickness of cortex at each of localities	Average by locality group	Thickness of cortex at each of localities	Average by locality group
	mm.	*mm.*	*mm.*	*mm.*
V and XIII	0.48 and 0.48	0.48	1.23 and 1.24	1.24
IV	0.56	0.56	1.42	1.42
XII and VIII	0.65 and 0.66	0.66	1.74 and 1.60	1.67
III and XI	0.67 and 0.76	0.72	1.81 and 2.00	1.91
VI	0.81	0.81	2.01	2.01
II and X	0.77 and 0.91	0.84	1.97 and 2.09	2.03
VII	0.86	0.86	2.29	2.29
I and IX	0.85 and 1.07	0.96	2.88 and 3.10	2.99

Thickness of cortex according to sex

No sex difference in the thickness of the cortex has been de-
tected.   Among the 125 rats, employed in this study, there are
28 females, as indicated in tables 1 and 2.   Table 12 shows the
comparison of the thickness of the cortex, grouped by sex.   Exam-
ining this table, it is seen that in Groups III to XIII, on all
kinds of sections, there can be detected no difference due to sex,
because the differences, greater than the probable error, in the
figures within each group can be explained by the differences in
the average brain weight.   But, Group XVII on the sagittal sec-
tions and Groups XIII and XVII on the frontal sections show

Showing the thickness of the cerebral cortex according to sex, within each brain weight group. Data taken from tables 6, 7, and 8. The average thickness of the sagittal and frontal sections and that of the horizontal section are separately given, with the average brain weight for each brain weight group examined

		MALE			FEMALE		
		Brain weight	Sagittal	Frontal	Frontal	Sagittal	Brain weight
		grams	*mm.*	*mm.*	*mm.*	*mm.*	*grams*
Sagittal and frontal sections.................	III	0.349	0.87	1.01	1.07	0.99	0.395
	V	0.539	1.13	1.32	1.36	1.15	0.553
	VI	0.612	1.25	1.54	1.58	1.37	0.690
	VIII	0.829	1.47	1.82	1.82	1.52	0.898
	IX	0.966	1.53	1.86	1.86	1.58	0.960
	XII	1.234	1.73	1.85	1.96	1.76	1.273
	XIII	1.320	1.72	1.95	1.92	1.75	1.392
	XV	1.530	1.72	1.91	1.98	1.77	1.560
	XVI	1.666	1.79	1.94	1.93	1.74	1.645
	XVII	1.725	1.83	1.93	1.88	1.77	1.726
		Brain weight	Horizontal		Horizontal		Brain weight
		grams.	*mm.*		*mm.*		*grams*
Horizontal sections........	III	0.317	0.86		0.93		0.316
	VIII	0.835	1.67		1.74		0.884
	X	1.066	1.90		1.79		1.028
	XII	1.233	1.94		2.00		1.255
	XIII	1.338	1.85		1.91		1.377
	XIV	1.461	1.92		1.95		1.448
	XVI	1.679	1.97		2.04		1.678
	XVII	1.738	1.88		1.97		1.723
	XVIII	1.844	2.03		1.94		1.802

some excesses in favor of male. Groups XIV, XVI and XVII on the horizontal sections also show some excesses in favor of female. These differences, however, lie within the limits of individual variation, and hardly can be regarded as suggesting a difference in cortical thickness due to sex. Therefore, I conclude that there exists no sex difference in cortical thickness when brains of like weights are compared. But, if either body weight or body length are taken as the standard for comparison, a sex difference in cortical thickness appears in favor of male, because the brain weights under such conditions are higher in the males than in the females.

The increase in the average thickness of the cortex

According to charts 9 and 10, the growth curve of the cortical thickness shows many features in its course. In the first phase, previously mentioned, during which the brain weight increases from 0.25 gram to 0.95 gram, the curve rises rapidly and steadily. If we assume that, throughout this phase, the specific gravity of the brain substance remains unchanged and the various parts of the brain grow similarly in all dimensions, then the cerebral cortex should increase in thickness in proportion to the cube root of the brain weight (cf. also table 14 and chart 11). But in fact, the relative thickness of the cortex at the end of this phase is 2.34 or almost exactly equal to the square of the cube root (1.53) of the brain weight (volume). If the fact is

TABLE 13

Giving the absolute increase in thickness of the cerebral cortex during each phase of development, accompanied by the average increase per day during each phase and the ratios of this increase in the three phases. Data from table 10

PHASE	ABSOLUTE INCREASE IN THICKNESS	INCREASE PER DAY	RATIOS
	mm.	*mm.*	
First phase (Birth to 10th day)..........	0.99 (1.73–0.74)	0.0990	62
Second phase (10th to 20th day).........	0.11 (1.84–1.73)	0.0110	7
Third phase (20th to 90th day)...........	0.11 (1.95–1.84)	0.0016	1

recalled that in this first phase the increase of the brain weight according to age has been comparatively rapid, it will be seen that the growth of the cortex in thickness is very rapid indeed.

In the second phase, during which the brain weight increases from 0.95 gram to 1.15 grams (from the tenth day to the twentieth day after birth), the slope of the curve diminishes markedly, and, in the third phase, after the twentieth day, it runs almost parallel to the base line, showing but a slight gain during further brain growth.

If the rates of increase of the cortical thickness in the three phases are compared in the terms of absolute increase per day, the following values appear (table 13).

Thus the rate of increase during the first phase is 62 times as rapid as that during the third phase and the rate during the second phase 7 times as rapid as that during the third phase.

Three changes are occurring during these phases: (1) cell multiplication and immigration, (2) cell enlargement, represented by the growth of the cell body, and (3) the production of dendrites and of the axon, the latter representing the larger mass of substance.

Allen ('12) has given the following figures as to the number of mitoses per cubic millimeter in the cerebrum of the albino rat at certain levels, selected in frontal sections. These show that cell production runs down rapidly between the first and second phases as follows.

	FIRST PHASE			SECOND PHASE		
Age (days).............................	1	4	6	12	20	20
Number of mitoses.......................	430	447	193	37	27	18

We may conclude from this that many more new cells are contributed in the first phase than in the second, but the data do not permit us to judge of the absolute amount of increase from this source.

By far the most important contribution to the cortex comes from the transitional layers of cells, the elements of which are rapidly added to the cortex during the early days of post-natal life.

According to another series of my studies, the cell size of the pyramids, for example, increases during the first phase in the length of cell body from 17 micra to 20 micra; about 18 per cent gain. Furthermore, the intercellular structures are also steadily increasing at this phase, and the cells become more and more separated from each other. These facts taken all together fit very well with the rate of 62 as given by my data.

As seen from chart 9, the cerebral cortex reaches within about 4 per cent of its full thickness at the end of the second phase (weaning time), when the brain weighs 1.15 grams or somewhat more than half its mature weight. After the end of the second

phase, the cortex gains in thickness very slowly though continuously throughout the first year of life, while the brain continues to increase in weight according to age much more rapidly, so that, in the full grown rat, whose body weight amounts to more than 200 grams, the cortical thickness attains on the average nearly 1.94 mm. The general average thickness of the groups XI to XX is 1.88 mm. (table 9).

TABLE 14

Showing (column B) brain weights, (column C) their ratios to the initial weight at birth, (column D) the cube roots of the numbers given in column C, indicating roughly the rate of increasing size, (column E) the average thickness of the cerebral cortex corresponding to the given brain weights and (column F) their ratios to the initial cortical thickness at birth, in every group

A	B	C	D	E	F
Group	Brain weight	Ratio	Cube root	Average thickness	Ratio
	grams			*mm.*	
II	0.265	1.00	1.00	0.74	1.00
III	0.344	1.30	1.09	0.94	1.27
IV	0.428	1.62	1.17	1.09	1.48
V	0.543	2.05	1.27	1.25	1.69
VI	0.636	2.40	1.34	1.42	1.92
VII	0.754	2.85	1.42	1.59	2.15
VIII	0.843	3.18	1.47	1.66	2.25
IX	0.956	3.61	1.53	1.73	2.34
X	1.045	3.94	1.58	1.76	2.38
XI	1.154	4.36	1.63	1.84	2.49
XII	1.249	4.72	1.68	1.87	2.53
XIII	1.340	5.07	1.72	1.84	2.49
XIV	1.448	5.47	1.76	1.88	2.54
XV	1.558	5.88	1.81	1.87	2.53
XVI	1.663	6.28	1.85	1.90	2.57
XVII	1.727	6.52	1.87	1.87	2.53
XVIII	1.833	6.92	1.91	1.94	2.62
XX	2.037	7.68	1.97	1.91	2.58

Table 14, which is based on table 9, presents the relations existing between the increase of the brain weight and the increase of the cortical thickness. The figures in column B of table 14 show the average brain weights by groups. Column C shows the ratio of the brain weight of each group compared with the initial brain weight at birth. Column D shows the cube roots of the
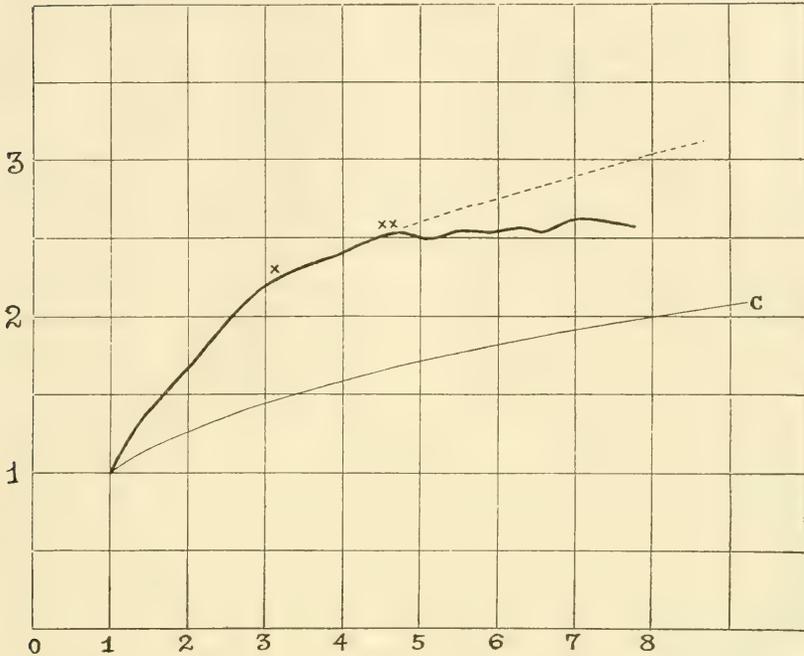
Chart 11   A comparison of the growth curves of the cortical thickness, as observed, with the theoretical growth curve (c) of one diameter of the brain of the albino rat, (c) being the cube root of the ratio of the brain weight (Table 14).   The cortical thickness and the length of one diameter of the brain at birth were taken as unity on the ordinate.   On the abscissa the ratio of brain weight is entered.   The dotted line is a theoretical curve which the cortical thickness would have followed if the cortical thickness at 20 days of age were taken as the starting point and it had increased at the same rate as the growth of the brain in diameter.   X and XX mark respec tively the ends of the first and the second phases of development in cortical thick ness.   Based on table 14.

ratios given in column C, values which may be taken as representing the rate of increase in one dimension of the brain, if we neglect the slight increase in the specific gravity of the brain substance with advancing age and assume that the brain form is similar throughout the series.   Column E shows the absolute thickness of the cortex in each group and column F the ratios of the cortical thickness for each group, compared with the initial cortical thickness at birth.

On comparing the data in column F with those in column D, it
will be seen at once that the increase in the thickness of the cor-
tex is much more rapid than the increase in the diameters of the
brain. Chart 11 visualizes the relations given in table 14, × and
×× showing respectively the ends of the first and the second
phases. The graph marked with 'C' was plotted to show the
cube root values given in column D. During the first phase, the
cortex develops very rapidly, while during the second phase the
increase of the cortex in thickness is similar to the increase of
the brain in one diameter. If the thickness of the cortex at the
end of the second phase were taken as the starting point of the
third phase, and if the cortex continued to grow in thickness as
during the second phase, then the increase in cortical thickness
would have taken the course given by the dotted line. But the
course of the actual increase, as shown by the heavy line graph,
is very slow indeed. The cortex at this phase grows mainly in
area. This agrees with the graph in chart 9 by which it has been
shown that the average thickness of the cortex has nearly ceased to
increase at the end of the second phase.

According to another series of studies, it has been found that
the cells of which the cortex consists do not all reach their full
size at the end of the second phase. Though a number of them
have reached their full size before this time, most of the remainder
are about midway in their development. Though some of the
pyramids in the third layer have in this phase already attained
their full size, yet their protoplasmic structure is not mature,
as is shown by their staining reaction. The large ganglion cells in
the fifth layer have not yet reached their full size, many of them
being yet only midway in enlargement, and increasing in volume
continuously through the third phase. Allen ('12) has shown
that in the second phase the number of mitoses is already very
small. I assume therefore that, in the second phase, the in-
crease in the thickness of the cortex is caused chiefly by the en-
largement of cell bodies, the production of cell branches and to
a small extent by the deposition of myelin. Through the third
phase, the brain continues to increase in volume according to
age, and the cortex, which has almost ceased to grow in thickness,

must however increase in area, as the hemispheres enlarge. Mitosis having almost ceased at the end of the second phase, this increase in area must be due mainly to an increase in the diameters of the cell bodies and to the increase in the number and the size and the myelination of the fibers. This increase in area after the end of the second phase is very considerable. When the weight of the brain increases from 1.15 grams to 2.03 grams (table 14), the area increases some 46 per cent and it is this extension of area which is accomplished by the cortex, after growth in thickness has come nearly to an end. A detailed study of the manner in which this extension is accomplished must be reserved for another occasion. A word may be said however regarding the age relations of myelination in the rat's brain.

In his study on the myelination of the central nervous system of the albino rat, Watson ('03) found in the cerebrum the first myelination or investment of the axons with myelin sheaths, as indicated by the substance which stains with the Weigert-Pal's method, to begin at the ages given below.

Localities in cerebrum	Age of the beginning of myelination
Capsula externa	11th day.
Stria olfactoria lateralis	14th day.
Corpus striatum	14th day.
Corpus callosum	14th day.
Radiation into the cortex	14th day.
Commissura anterior	17th day.
Thalamus	17th day.

The fibers radiating into the cortex myelinate very slowly, however, so that but few are to be seen till after twenty-fifth day. This age, the twenty-fifth day, would correspond to the early part of the third phase.

The fact that the cortex attains nearly its full thickness before the radiating fibers are myelinated should mean that the organization of the cortex occurs while growth in thickness is in progress. After this organization has been made, then myelin begins to appear around the axons, increasing their diameter. The increase in cortical area during and after the third phase must, therefore, be caused principally by the enlargement of the cell-

bodies, the increase in the diameter of the axons and the formation and enlargement of the myelin sheaths.

This conclusion is supported by the table 74 in "The Rat" (Donaldson, '15) which gives the percentage of water in the albino rat brain. From the values in that table, the mass of dry substances has been calculated and results are plotted according to brain weight in chart 12. In the first phase of the cortical development, the solids of the brain increase proportionally to the brain weight, but after the middle of the second phase (brain weight 1.00 grams) and through the third phase, they increase much more rapidly, again decreasing in rate at about 35 days after birth (brain weight 1.40 grams). This means clearly that from the middle of the second phase (about 15 days of age) some new substances begin to be deposited rapidly in brain, i.e., myelination is in progress. Looking at chart 26 given in "The Rat" (Donaldson, '15), we see that the percentage of water in the brain decreases comparatively slowly during the first ten days after birth, then, from the tenth day, decreases rapidly till the thirtieth day, after which the decrease becomes slow again. At the sixtieth day after birth, the brain (weight 1.60 grams, percentage of water ca. 79 per cent) has reached nearly to the stage of the adult in percentage of water. This indicates that myelination is going on energetically between the tenth and the thirtieth day after birth. The myelination in the cortical radiation begins late, accelerating its rate after the twentieth day.

Bringing together the foregoing observations and inferences, we may make the following statements concerning the growth changes in the cerebral cortex during the three phases which have been recognized.

The first phase covers the first ten days after birth. This phase represents the period in which the thickness of the cortex increases rapidly by means of both cell immigration and multiplication and cell enlargement. If the data of the Group I (tables 1, 3, 5, 6, 8, 9) from rats before normal birth are also taken into consideration, it is evident, as shown by the first entries on Charts 1, 3, 4, 5, 6 and 9, that the increase of the cortical thickness is equally rapid just before birth.

The second phase extends from the tenth day to the twentieth day after birth. During this phase the cortex receives but few new cells, but the thickness of the cortex increases chiefly by enlargement of cells already present and the developmental lengthening of axons, the rate of the increase in cortical thickness being just about equal to the increasing rate of brain size in one
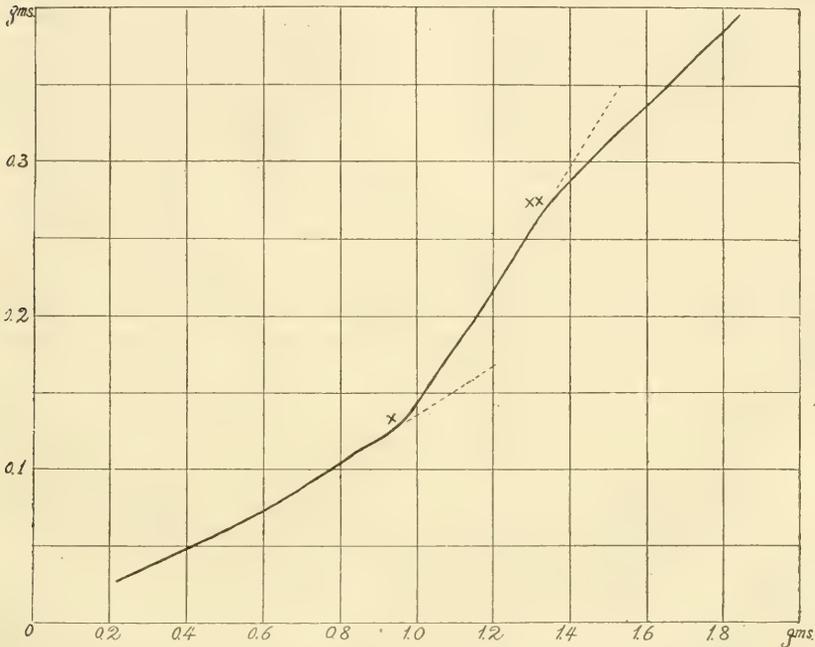
Chart 12 Showing the absolute weights of the dry substance (in grams) in the brain of the albino rat according to the brain weight (in grams). Based on the data given in table 74 in "The Rat" (Donaldson, '15). ×, the middle of the second phase; ××, the early part of the third phase.

diameter. During this phase, the cortex becomes provided with nearly all of the cellular elements necessary to it at maturity. By the end of this phase, the cortex has attained almost its full thickness, the brain weight, however, being only a little more than one-half that of the mature brain. Myelination has been in progress since the first few days of this phase, but in the cortex the amount of myelination is still very slight.

The third phase begins at the twentieth day after birth and lasts to the ninetieth day after birth when the rapid growth of body decreases. During and after this phase the cortical thickness remains almost fixed, despite the increase in brain weight or brain size. Rapid myelination of the cortical fibers first appears at the beginning of this phase and is also present in the corpus striatum or in other central nuclei. Thus the increase in brain mass during this phase is due principally to the deposition of the myelin sheaths. The fact that the cortex has reached its full thickness before the rapid development in myelination begins, leads us to conclude that the formation of the myelin sheath on any axon does not begin until the axon has established functional connections with one or more other neurons. During subsequent growth, both the axon and its myelin sheath lengthen and enlarge without disturbing their relative volume relations. It appears that the very considerable increases in the area of the cortex, after its thickness is attained, is accomplished largely by the formation of the myelin sheaths.

X. SUMMARY

1. The postnatal increase in the thickness of the cerebral cortex according to body growth has been systematically investigated, employing as material 96 male and 28 female albino rats, representing every stage of postnatal life.

2. The material was fixed, imbedded and stained by a uniform technique devised for the present investigation. The cortical thickness was measured on sagittal, frontal and horizontal sections, 10 micra thick, all of which were taken from the fixed levels of the brain. Two brains were required for the three sections. On these sections, thirteen localities in all were measured. These localities were accurately determined and represent regions well distributed over the surface of hemisphere. The values thus obtained from the measurements on the slide were later converted into the actual thickness of the cortex in fresh condition, by the use of correction-coefficients based on observation. This series of determinations is the first we have

on the cortex of any mammal, which aims to represent the thickness of the cortex when fresh.

3. The data are presented in tables and in charts. The cortical cell-lamination of the albino rat is critically reviewed, and the characteristic appearance of the cortex in the newborn is described.

4. The cortex at the frontal pole of the hemisphere is the thickest and that at the occipital pole is the thinnest. Speaking in general terms, the cortex diminishes in thickness from the frontal to the occipital pole and from the dorsal to the ventral aspect.

5. The cerebral cortex of a newborn rat is somewhat less mature than the cortex of man at birth. In the newborn rat, the general average thickness of the cortex is 0.74 mm., corresponding to a brain weight of 0.25 gram.

6. After birth, the general average of the cortical thickness increases very rapidly during the first ten days, thickening to 1.73 mm., or more than twice the thickness at birth, while the brain weight increases to 0.95 gram during the same phase. This is designated by me as the first phase of the cortical development in the albino rat in its postnatal life.

7. Between the tenth day and twentieth day after birth, the cortical thickness increases more slowly, attaining at twenty days to within 4 per cent of the full thickness of the cortex, namely 1.84 mm., or about 2.5 times the thickness at birth, while the brain weight increases to 1.15 grams. This is designated the second phase of the cortical development in the albino rat.

8. From the twentieth to the ninetieth day, the cortical thickness increases but little on the average, attaining at about 90 days the thickness of 1.93 mm. or 2.6 times the thickness at birth, while the brain weight has increased to about 1.80 grams. This is designated the third phase of the cortical development in the albino rat.

After the ninetieth day, there is no significant change in the thickness of the cortex, but the area of the cortex increases as the brain weight rises towards two grams.

9. On comparing the rate of increase in the cortical thickness during the three phases, we find that during the first phase the

average daily increase is 62 times and in the second phase 7 times as rapid as in the third phase (table 13). This corresponds well with the rate of mitosis in the cerebrum and cell immigration in each phase. Furthermore if the ratio of the increase of cortical thickness is compared with that of the increase of one diameter of the brain, the former is much greater than the latter in the first phase, almost equal in the second phase, and much less in the third phase. It appears, therefore, that in the first phase the cortex increases its thickness by receiving newly formed cells from the matrix and at the same time by the enlargement of the cell bodies; in the second phase, however, mainly by the enlargement of the cell bodies and the growth of the axons and dendrites; while in the third phase it almost ceases to thicken, but extends in area as the result of the formation of the myelin sheaths.

10. On comparing the rapidity of growth in the several localities of the hemisphere, it is easily seen that the cortex at the frontal pole increases its thickness very rapidly and continuously, even after the end of the second phase, while at all the other localities the cortex thickens by similar steps, so that at the end of the second phase all the localities reach nearly the full thickness. The localities heterogeneous in their cell-lamination show some deviation in their courses of thickening from the localities which are typical.

11. If the brain weight is taken as the standard of comparison, no sex difference is to be detected in the cortical thickness.

12. We conclude that the cortex generally attains nearly its full thickness before myelination in the cortex, as shown by the Weigert staining method, has begun.

13. The cortex has nearly its mature thickness at 20 days, just before the young rat is weaned. The growth of the cortex in thickness is therefore precocious.

14. If the relative growth rates of the rat and man are as 30 to 1 (Donaldson '08), and the development of the human brain at birth coincides with that of the rat at five days of age, then at about the age of fifteen months the human cortex should have attained nearly its full thickness.

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