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THE DEVELOPMENT, TOPOGRAPHICAL RELATIONS AND INNERVATION OF THE EPIPHYSIS CEREBRI IN THE ALBINO RAT

By

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With 42 Figures in the Text

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Introduction

Although a vast literature has accumulated on the pineal organ of mammals we are still far from understanding its function. Physiological, pharmacological as well as clinical data are numerous but not seldom contradictory while some of them do not seem to be substantiated very well. Moreover, the question remains to be answered whether such a function would be common to the organ in all mammals. As has been pointed out especially by KRABBE (1938), the epiphysis is rudimentary in some mammals being even absent in a number of Edentata while in well developed organs the histological structure may vary a good deal. In these respects the epiphysis is very dissimilar to the neurohypophysis.

It would seem appropriate to perform an extensive study of the embryological development, histological structure, vascular relations, innervation and histochemistry of the organ in one single mammalian species showing a well developed epiphysis in order to lay a firm foundation for physiological and biochemical investigations. For that purpose a mammal should be chosen which is easily available and well suited to different kinds of experimental research. The rat fulfils these requirements.

The present paper deals shortly with the embryological development and the topographical relations of the pineal gland in the albino rat. Its main part, however, is devoted to the innervation of this organ.

Material and Methods

The rats, reared at the Department of Anatomy in Groningen, belong to two thoroughly inbred strains. The material on which the embryological investigation is based consists of 24 embryos, aged 13—20 days, all ages being represented by at least two specimens. Embryos or, in advanced stages of development, the brains were fixed in BOUIN's or in ZENKER's fluid. Embedding was in paraffin. Serial sections were cut either in the sagittal or in the transverse plane. They varied in thickness between 8 and 10 μ. For the most part, staining was with hematoxylin-eosin and hematoxylin-erythrosin. Some series were stained with chrome-alum hematoxylin phloxine (GOMORI) or following the azan procedure. The sections of the brain of one specimen, aged 20 days, were treated according to BODIAN's protargol method.

Ten brains of newborn rats were processed in the same way excluding BODIAN's method. Moreover, 10 brains of postnatal rats, aged 1, 4, 7, 8, 14 and 15 days, were fixed in BOUIN's or in ROSSMAN's fluid and the serial sections stained with hematoxylin-eosin, BODIAN's protargol method, GOMORI's chrome-alum hematoxylin phloxine procedure or MCMANUS-HOTCHKISS' periodic acid SCHIFF technique.

From 10 adult rats of widely varying weights the brains or pineal organs including a small part of the brain were fixed in BOUIN's, ZENKER's or ROSSMAN's fluid. Staining of

the serial sections was with the following techniques: gallocyanin-eosin, aldehyde fuchsin after GOMORI-SCOTT, azan, MALLORY, chrome-alum hematoxylin phloxine, MCMANUS-HOTCHKISS and VERHOEFF. Furthermore, 8 adult brains were cut serially in transverse, sagittal and horizontal sections after fixation in BOUIN's fluid or in chloral-hydrate formol 10% and embedding in paraffin. Staining was either with BODIAN's protargol method or with ROMANES's silver chloride ammonia technique. This latter procedure gave excellent results and is, moreover, recommendable because it is cheaper than the protargol method.

For studying the terminal parts of adrenergic fibres frozen sections of 3 epiphyses were stained according to the technique of BIELSCHOWSKY. Serial paraffin sections processed following the method of CHAMPY-COUJARD proved to be very satisfactory for this purpose if the impregnation was successful. The innervation of 6 pineal bodies, taken out together with a small part of the brain was investigated in this way. The time during which the preparations were fixed and stained in the osmic acid-sodium iodide solution varied. Best results were obtained with a fixation-staining time of about 26 hours. A few series were counterstained with saffranine. This, however, proved to be rather superfluous. Sections were 5, 8, 10 and 15 μ in thickness.

For demonstrating lipids a series of frozen sections was cut of one epiphysis and stained according to the Sudan Black B method of LISON (ROMEIS, 1948).

To study the vascularity of the epiphysis and the vascular relations in the epiphyseal region, 7 adult rats were given a perfusion of physiologic saline through the heart immediately followed by slow perfusion of India ink-gelatine, both at body temperature. After fixation in trichloric acid one whole head was cut transversely in 16 μ serial sections after embedding in paraffin. One brain was dissected under the binocular microscope after fixation in alcohol 96%, another brain was cleared according to SPALTEHOLZ' procedure, thick sections being studied under the binocular microscope. The rest of the ink-injected brains were fixed in BOUIN's and the 16 μ paraffin sections stained with hematoxylin and eosin. Some sections of an injected SPALTEHOLZ preparation from the collection of my co-worker Dr. MOLL were also used.

In 8 adult rats the superior cervical ganglion was bilaterally removed by Dr. SMELIK from the Department of Pharmacology and Endocrinology. We are most grateful to Dr. SMELIK for performing these operations. One of these rats was sacrificed on the 8th day after removal of the ganglia. The brain was fixed in BOUIN's, serial sections of 10 μ in thickness were cut and stained according to BODIAN's protargol technique. Two other operated rats were sacrificed after 50 days. The serial sections, 8 μ in thickness, were stained following the method of ROMANES. From one brain of a sympathectomized rat frozen sections of 25 μ were cut and stained with the technique of BIELSCHOWSKY. The rest of the operated material was sacrificed from 2—5 months after ganglionectomy. Small parts of the brains containing the epiphysis were processed according to the osmic acid-sodium iodide method of CHAMPY-COUJARD and cut in serial sections of 5, 8 or 15 μ in thickness.

In all adult rats from which either the entire brain or the epiphysis with small surrounding brain parts was sectioned after fixation in alcohol, formol, chloral-hydrate formol, BOUIN's or ZENKER's fluid and paraffin embedding, the fixing fluid was injected at body temperature into the left ventricle of the heart following on perfusion of the vascular system of the animal with physiologic saline, performed in the same way.

To Dr. WACHTERS from the Division of Medical Photography of the Central Photographical Service of Groningen University we owe sincere thanks for making the microphotographs illustrating this paper.

Observations

In the rat, an epiphyseal plate was not observed. The first anlage of the epiphysis appears as an evagination of the diencephalic roof. In all vertebrates, just rostral to this evagination the habenular commissure is formed in mammals while the posterior or caudal commissure develops caudal to it. In our series no trace of the anlage of the organ was yet observed in embryos aged 13 days. However, in embryos the age of which was between 14 and $14^1/_2$ days, a shallow epiphyseal evagination was seen to be present. This agrees with the age mentioned by

HENNEBERG (1937). According to GARDNER (1949), epiphyseal development in the hooded rat starts somewhat earlier, *i. e.* on the 12th day. During the next days the pineal evagination rapidly develops. In the present paper the embryological development and cytological differentiation during ontogenesis of the pineal organ will not be extensively described. For details of intrauterine histological

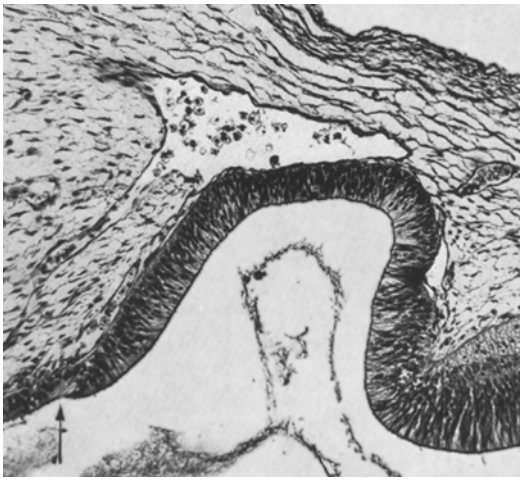

Fig. 1. Embryo, 15 days. Epiphyseal evagination. Dorsal to it anlage of confluens sinuum. Anlage of commissura habenularis indicated by arrow. Well developed anlage of commissura caudalis partly visible to the right. BOUIN, 10 μ, sag., H. E., × 120

Abbreviations for all figures

CA	commissura anterior
CC	corpus callosum
CH	commissura habenularis
CM	corpus mammillare
CO	chiasma opticum
CP	commissura posterior = commissura caudalis
CPH	caudal pole of cerebral hemisphere
CR	colliculus rostralis of lamina quadrigemina
Cr	cerebellum
CS	confluens sinuum
Ep	epiphysis
ES	epiphyseal stalk
GCS	ganglion cervicale superius
ICR	intercommissural recess of third ventricle
LQ	lamina quadrigemina
M	mesencephalon
NH	nucleus habenularis
OSc	organon subcommissurale
OSf	organon subfornicale
PD	pars distalis hypophyseos
PE	pedunculus epiphyseos = epiphyseal stalk
PI	pars intermedia hypophyseos
PN	pars nervosa hypophyseos
SHR	suprahabenular recess of third ventricle
ST	sinus transversus
TC	tentorium cerebelli
Th	thalamus
VCI	vena cerebri interna
VMC	vena cerebri magna
VMP	vena mediana prosencephali
VSI	vena sagittalis inferior
VSS	vena sagittalis superior
III	third ventricle

differentiation in the hooded rat we may refer to the abstract by GARDNER (l. c.). Figures 1—7 offer a survey of the development of the epiphysis in the albino rat.

From these figures it is observed that the rostro-dorsal and the caudo-ventral wall of the epiphyseal evagination which soon forms a epiphyseal sac the axis of which is directed dorsocaudalward, thicken. Then, the ependymal cells by which these walls are constituted start to proliferate forming follicles. Especially the rostro-dorsal wall is involved in this process. The epiphysis of the rat is clearly seen to be built up by these follicles which, however, in advanced stages of development form a compact and dense mass of epithelial cell cords or cellnests. In the adult organ the lumen of many but not of all follicles has disappeared.

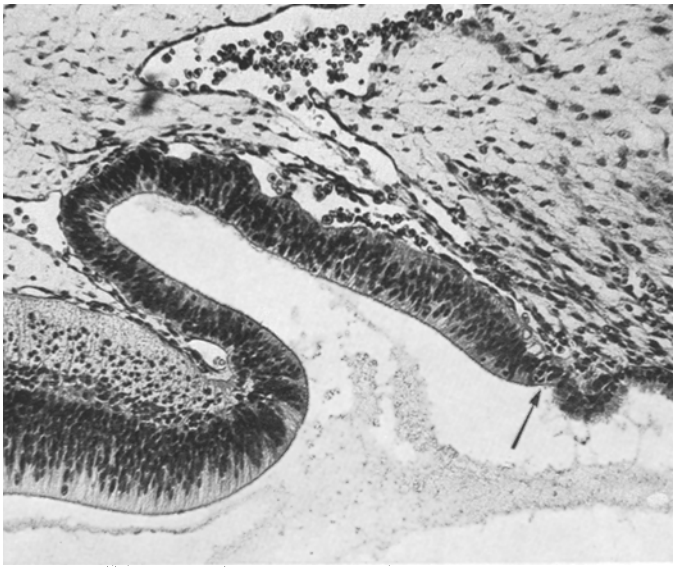

Fig. 2. Embryo, 16 days. Anlage of epiphysis and, dorsal to it, of confluens sinuum. Proliferation starting in rostro-dorsal wall of epiphyseal evagination. Very small habenular commissure indicated by arrow, caudal commissure well developed, subcommissural organ differentiating. BOUIN, 10 μ, sag., H. E., $\times$ 130

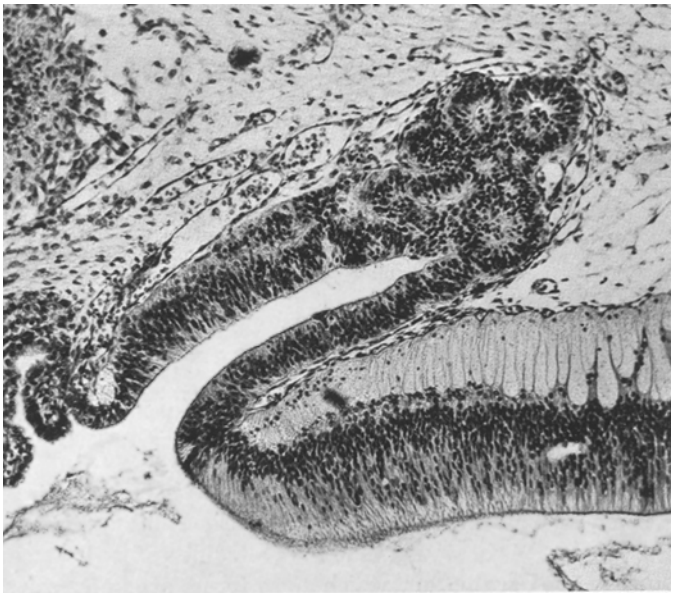

Fig. 3. Embryo, 17 days. Epiphyseal anlage showing follicular proliferation, habenular commissure more clearly distinguished, large caudal commissure and differentiating subcommissural organ. BOUIN, 10 μ, sag., H. Er., $\times$ 110

Nevertheless, a follicular arrangement of the pinealocytes still can be easily distinguished at many places. This holds likewise but somewhat less evident for the pineal stalk or peduncle.

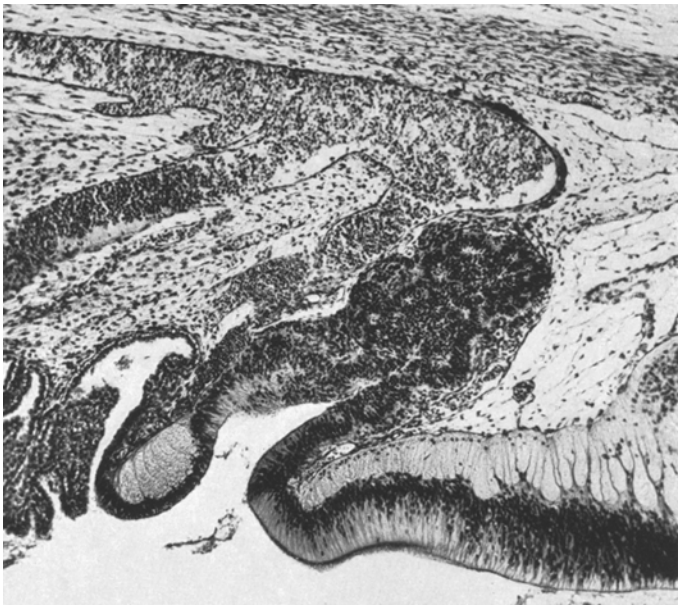

Fig. 4. Embryo, 18 days. Epiphyseal anlage showing follicular proliferation in which the ventrocaudal wall is also involved now. Distal end of pineal recess closing. Habenular commissure much increased in size. Vena sagittalis superior, vena prosencephali madiana discharging in vena sagittalis superior, vena magna cerebri and confluens sinuum. Both latter vessels immediately dorsal to epiphysis. Formation of suprahabenular recess of 3rd ventricle starting. Compare with fig. 10. BOUIN, 10 μ, sag., H. Er., × 72

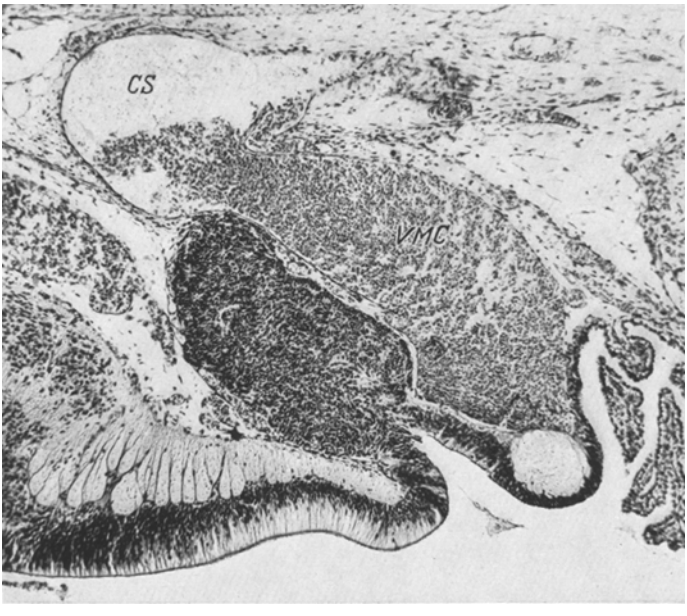

Fig. 5. Embryo, 19 days. Compact epiphysis. Pineal recess of 3rd ventricle still less deep. Habenular commissure still growing in size. Vena magna cerebri and confluens sinuum rapidly developing. BOUIN, 10 μ, sag., H. Er., × 68

During early development mesenchyme, containing vessels, grows in between the follicles providing the epiphysis with a frame of connective tissue strands and an extensive vascular network. In the adult rat the amount of pineal connective tissue strands as seen in our azan series and in the series stained according to VERHOEFF is not very abundant in comparison with the amount of connective tissue in the epiphysis of other mammals as studied from the literature (see for instance BARGMANN, 1943). The nests or lobules of parenchymatous pineal cells

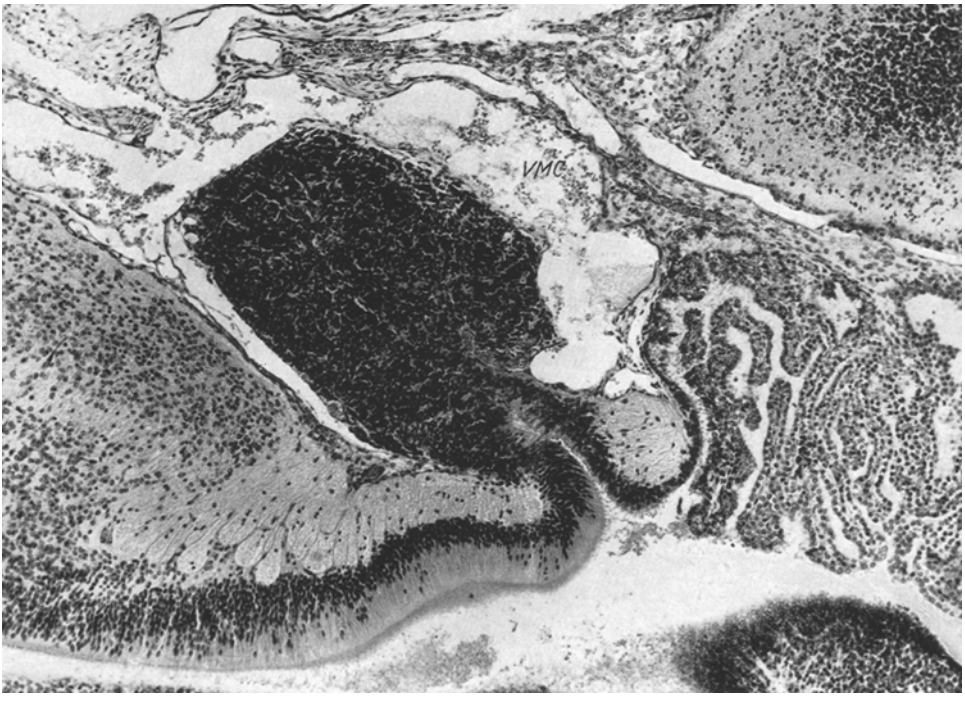

Fig. 6. Newborn rat. Somewhat oblique section. Compact epiphysis. Pineal recess closing. Habenular commissure, caudal commissure and subcommissural organ well developed. Development of suprahabenular recess of dorsal sac containing choroid villi proceeding. Large vena magna cerebri. BOUIN, 8 μ, sag., H. E., $\times$ 80

are surrounded by the strands of connective tissue containing arterioles and venules and by the network of capillaries. In this way the structure of the epiphysis is somewhat similar to that of a gland consisting of acini. We have not been able to observe any ganglioncells in the epiphysis of the rat. The cytology of the pinealocytes will not be dealt with in this paper. For other kinds of cells, sometimes being present, we refer to BARGMANN (1943).

It may, however, be mentioned here that in one single epiphysis of the many adult specimens investigated a few striated musle cells were found (Fig. 8). Striated as well as smooth muscle cells have also been observed in the epiphysis of other mammals, especially in the calf (BARGMANN, 1943; QUAY, 1958), but only extremely rarely. QUAY was first in demonstrating some striated muscle cells in the pineal organ of the rat. Their function, if any, is wholly enigmatical. It may be that these cells are of ectodermal epiphyseal origin. If so, they may be compared with the ectodermal smooth muscle cells originating from the iris. Another possibility, which seems to be somewhat more acceptable, is that these

cells have developed from invading leptomeningeal mesenchymal elements which are partly of ectodermal neural crest origin, partly of mesodermal origin.

It has already been mentioned that the habenular and the caudal commissure develop in close proximity to the epiphyseal anlage. In the embryos, aged 13 days, the structure of the peripheral part of the mesencephalic ependymal wall in the midplane through which fibres of the caudal commissure grow during the next developmental stages had already typically changed. Whether a few

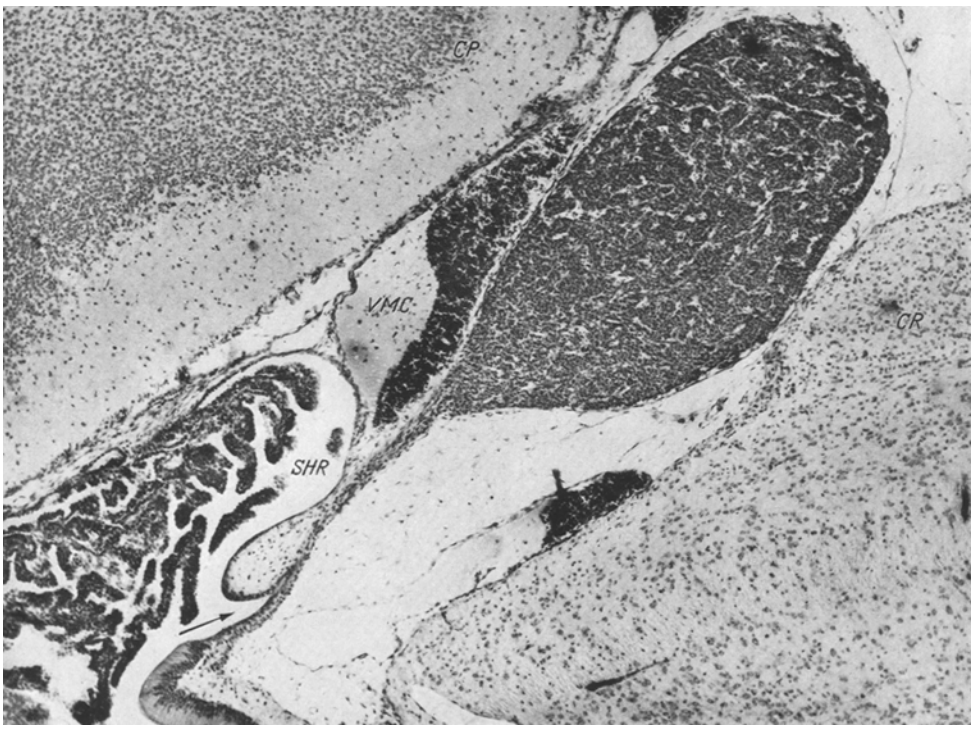

Fig. 7. Rat, aged 7 days. Large and compact epiphysis. An epiphyseal stalk has developed, a true pineal recess disappearing. Arrow indicates intercommissural recess of 3rd ventricle between habenular and caudal commissure. Large supra-habenular recess. BOUIN, 10 μ, sag., BODIAN, × 69,5

commissural fibres were present as early could not be ascertained. In the 14-day stages, however, many fibre bundles of the caudal commissure were clearly shown. The habenular commissure develops at a later stage. The first very small habenular commissural fibre bundle was observed in embryos, aged 16 days. Especially the caudal commissure develops very rapidly.

From Figures 1—7 it appears that the relative position of the habenular and the caudal commissure changes during development. In the 16th day embryo, the habenular commissure is situated well rostral to the caudal commissure. In the newborn rat, however, its position is much nearer to the caudal commissure. Moreover, it has shifted its position somewhat in a dorsal direction. In postnatal rats aged 7 days the habenular commissure is situated well dorsal and also somewhat caudal in respect to the caudal commissure. From now on

the habenular commissure, in sagittal sections, shows a drop-like shape, the pointed end of the drop being directed caudalward.

In the newborn a real pineal recess, being the original lumen of the epiphyseal evagination, is still present. The anterior and posterior peduncle of the organ are directed to the broad base of the pineal body which has become very compact by now. Both peduncles are still rather solid. No epiphyseal stalk has been formed as yet (Fig. 6). As early as on the 19th day of intrauterine development

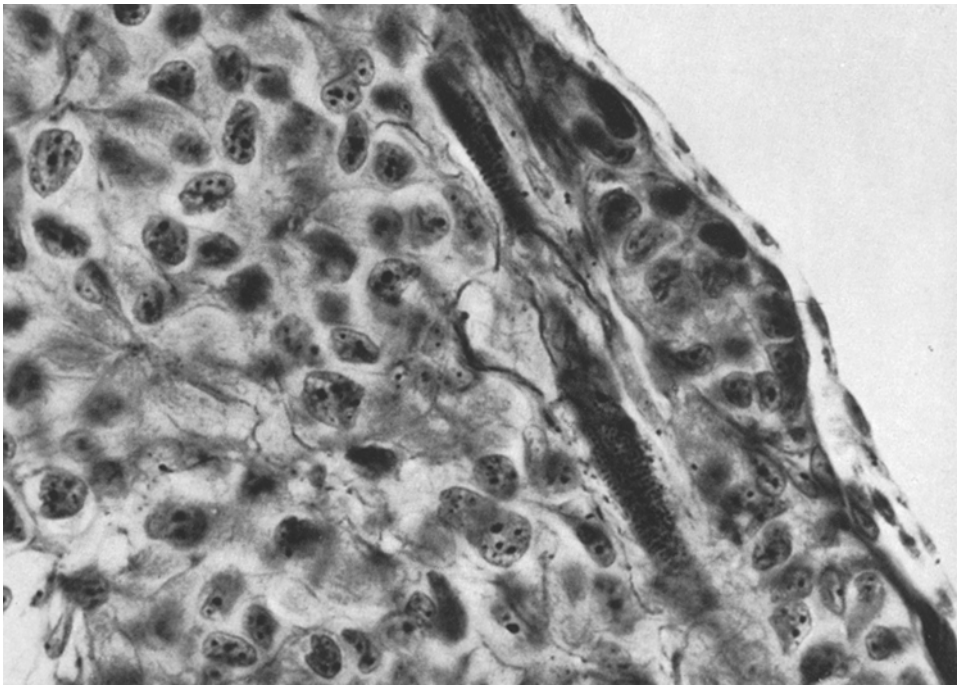

Fig. 8. Striated muscle cells in adult epiphysis. Follicular arrangement of pinealocytes clearly visible at some places. BOUIN, 12 μ, horiz., BODIAN, $\times 750$

the pineal body consists of a very dense and compact mass of cells (Fig. 5). Under low power its follicular structure is no longer apparent. The shape of the epiphysis in rats, aged 4 days, is essentially similar to that in the newborn rat. Then, however, the pineal body is gradually displaced in a caudal and somewhat dorsal direction along the lamina quadrigemina of the mesencephalon. In postnatal rats of 7 days old its position has already shifted considerably. It is now situated between the rostral collicles. A short stalk connects the organ with the commissural region (Fig. 7). During further development this caudalward relative migration is continued until, in the adult rat, the pineal body lies in front of the cerebellum between the caudal collicles. This ontogenetic displacement, most probably, is not due to an active migration but happens by differential growth of the neighbouring parts of the brain.

In the albino rat the pineal organ keeps its connexion with the commissural region by means of a very slender pineal or epiphyseal stalk. This stalk is formed

by fusing and growing out of the anterior and posterior epiphyseal peduncles originating from the most proximal parts of the rostro-dorsal and caudo-ventral walls of the original epiphyseal evagination that are scarcely if at all involved in the process of follicular proliferation. Thus, this stalk is essentially of epiphyseal origin consisting of pinealocytes still showing a follicular arrangement at some places. Especially in its proximal part, however, the stalk contains cells which have not quite differentiated into pinealocytes. They may be pinealoblasts. Moreover, some cells in the stalk are evidently fibrocytes having invaded the stalk from the surrounding leptomeningeal mesenchyme. Vessels do not run in the stalk but nerve fibres are constantly present as will be shown below.

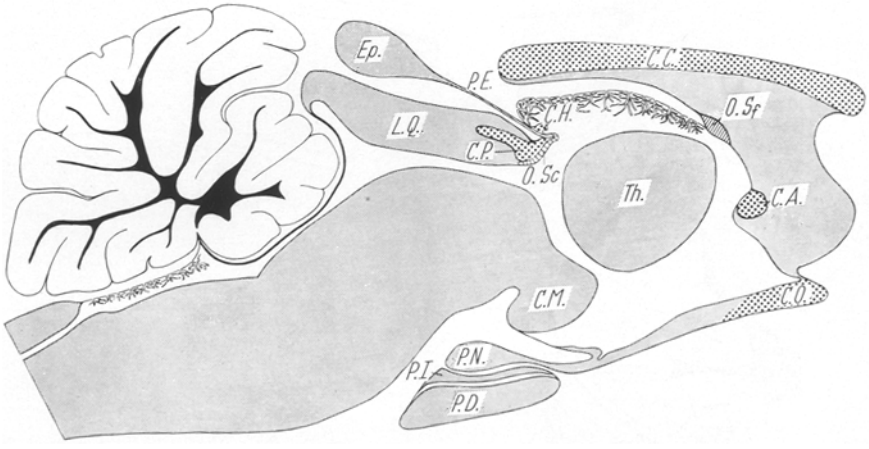

Fig. 9. Diagram of midplane of adult rat brain drawn after a sagittal series. In some specimens the position of the pineal body is even somewhat more caudal

The stalk can be divided in three parts, a proximal part, a midpart and a distal part. The proximal one lies between the habenular and the caudal commissure (Figs 12—14). Then the stalk curves slightly in a dorsal direction along the floor and the caudal border of the suprahabenular recess (see below) where it shows a subependymal position. Often, especially this midpart of the stalk is extremely thin (Fig. 16) and in some specimens pineal stalk cells were found to be even entirely lacking . In these few cases, therefore, the continuity of the stalk is interrupted and the small bundle of nerve fibres runs naked, not enveloped by stalk cells, along a short and varying distance. The distal part of the stalk, which is the longest, is situated in the midline on the lamina quadrigemina between both rostral and both caudal collicles.

Fusion of the original posterior and anterior peduncle of the epiphysis, bringing about the formation of the stalk, causes also the disappearance of the pineal recess. Now the proximal end of the epiphyseal stalk, formed in this way, merges into the hypendyma and ependyma of the subcommissural organ, the ependyma covering the habenular commissure and into the ependymal lamina between both commissures which is the lamina intercalaris (Figs 7, 12 and 13). This lamina is of variable length. Most often it is very short as illustrated in Figures 12 and 13. In some few specimens, however, it was observed to be rather long as

shown in Figure 11 in which part of the intercalary lamina can be seen. This lamina then bounds a fairly deep recess of the third ventricle which, because of its position between the habenular and the caudal commissure, may be termed intercommissural recess. This recess, therefore, is not homologous to the pineal recess originally present which forms part of the lumen of the epiphyseal evagination. A real pineal recess is still found in rats aged 7 days as is illustrated in Figure 7. The intercommissural recess of the third ventricle just mentioned is often erroneously called pineal recess in the literature although it does not lead into the pineal organ. This is specially evident in the rat.

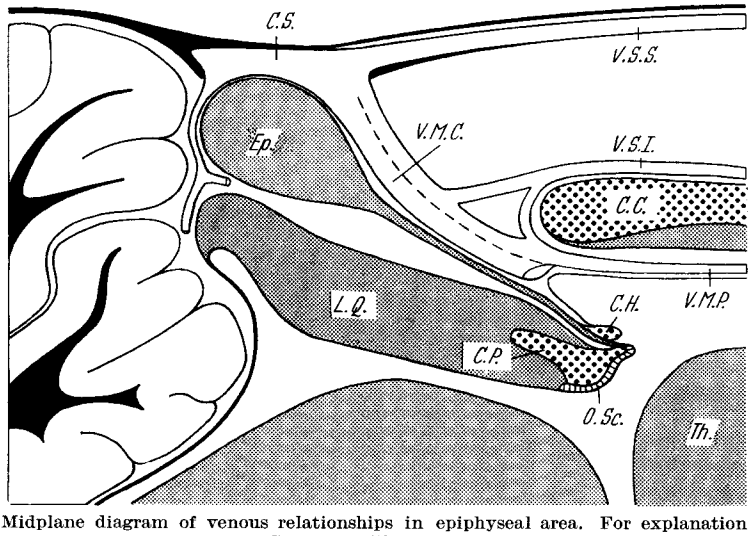

Fig. 10. Midplane diagram of venous relationships in epiphyseal area. For explanation see text. Compare with Fig. 42

The position of the pineal body and the course of the pineal stalk are diagrammatically illustrated in Figure 9. It is seen how from the tapering rostral end of the pyriform pineal organ the stalk runs to the commissural region.

From Figures 1—7 the topographical relationships of some other structures and their developmental change in position in regard to the epiphyseal complex are apparent. The part of the diencephalic roof of the third ventricle rostral to the habenular commissure forms the dorsal sac. The figures show how the caudal wall of this sac, by protruding, forms a pouch which is directed caudalward. From the roof of this pouch choroid villi develop just like they do from the more rostral part of the roof of the dorsal sac. This caudal ventricular recess is homologous to the suprapineal recess of human anatomy. Owing, however, to the caudalward displacement of the pineal organ in the albino rat, the position of this recess is well rostral to the epiphysis and not at all "suprapineal". Therefore, in animals in which a long epiphyseal stalk develops and the position of the pineal organ has shifted along a considerable distance in a caudal direction such as in rabbits and rats, this recess may be better termed "suprahabenular recess" because of its position just dorsal to this commissure. The extension of this recess in a caudal direction is rather variable. The midpart of the epiphy-

seal stalk passes underneath the posterior part of its floor caudal to the habenular commissure as well as along its posterior wall.

In early embryonic stages veins develop just dorsal to the epiphyseal anlage. Here, the confluens sinuum is formed. Its development is shown in Figures 1—7. It is a remarkable fact that, in the rat, the position of the confluens sinuum relative to the pineal body remains constant during ontogenesis, both shifting caudalward. In Figure 10 the topographical relationships of the epiphyseal stalk and the epiphysis to part of the venous system of the brain are diagrammatically illustrated. The pineal body is enveloped by a very thin sheath of connective tissue having been formed by leptomeningeal mesenchyme. The dorsal surface of the pineal body, covered by this sheath, is in immediate contact with the floor of the confluens which is rather thin. At the most rostral end of the confluens at least two and sometimes three large veins, running in the midplane, discharge into the confluens. The most dorsal one is the vena sagittalis superior. It is constantly present. Because only a very small amount of connective tissue and no true falx cerebri is present between the cerebral hemispheres of the rat, this vein is no dural sinus. In some specimens it is more or less fixed to the dura mater covering the hemispheres but in most cases the vena sagittalis superior runs dorsally in the fissura interhemispherica simply enveloped by loose connective tissue. In the midplane dorsal to the epiphyseal stalk either one or two veins may run which discharge into the rostralmost part of the confluens. If two vessels are present the dorsal one is the vena prosencephalica mediana. This vein drains the many venules present in the velum interpositum dorsal to the roof of the third ventricle which, in part, spring from the plexus of this ventricle. The vein present between the vena mediana prosencephalica and the epiphyseal stalk is larger than this latter vein. It drains both venae cerebri internae as well as the basal veins and is, therefore, homologous to the vena magna cerebri of GALEN. If both the vena prosencephalica mediana and the vena cerebri magna are present the venous relationships in this region are very similar to those observed by VON BARTHELD and MOLL (1954) in mice. The only difference is that in mice the vena prosencephalica mediana does not discharge into the rostral part of the confluens proper but somewhat more rostrally into the superior sagittal vein. For a discussion of the phylogeny of the venous system mentioned we may refer to the paper by VON BARTHELD and MOLL.

In the rat, especially in the adult, very often exclusively a single large vein runs dorsal to the epiphyseal stalk and the rostral part of the pineal gland. It may be partly embedded in this organ before discharging into the confluens. Evidently this single vein is a combination of the vena prosencephalica mediana and the vena magna cerebri being brought about by a phylogenetic or even sometimes an ontogenetic fusion of both veins. In the rat the vena prosencephalica mediana is more or less rudimentary. If it is absent or, most probably, fused with the vena magna cerebri, this single large vein drains the blood from the choroid plexus of the third ventricle as well as from the plexuses of the lateral ventricles by means of the internal cerebral veins, and from the basal veins.

Dorsal to the corpus callosum a small vein is present. This is the vena sagittalis inferior. Just caudal to the splenium it drains, sometimes by means of two branches, into the large single midplane vein or into the vena prosencephalica

mediana, if present. In those cases in which dorsal to the epiphyseal stalk and the pineal organ only one large vein occurs the nomenclature of this midplane vein offers some difficulty. It was termed sinus rectus by GREENE (1935). The present author, however, prefers the term vena magna cerebri on the ground that there is some doubt whether this vessel, even caudal to the point of discharge of the inferior sagittal vein into it, is strictly homologous to the sinus rectus in man (quite apart from the fact that, in the rat, it is evidently not a sinus). The only disadvantage in calling this vein vena magna cerebri is that, in this way, this single vein is not quite homologous to the vessel present just ventral to the vena prosencephalica mediana in those cases in which this latter vein occurs. Other problems relating to the phylogeny and the ontogeny of the cerebral venous system will not be dealt with here.

Owing to the fact that the floor of the sinus confluens, covering the dorsal surface of the pineal body, is very thin, the organ can be easily observed through this floor after opening the roof of the sinus, especially in rats from which the blood is drained by vascular perfusion. This position of the epiphysis is well shown by GREENE (1935, Fig. 222). The fact that the epiphysis in the rat is so superficially situated just rostral to the cerebellum and between the occipital poles of the cerebral hemispheres facilitates operation on the organ. Severe disturbance of the vascular relationships in the pertinent region, however, will be unavoidable in removing the epiphysis. This may be of consequence for the interpretation of certain results after the operation.

The present paper will not deal with the meningeal development in relation to the epiphysis. It may only be mentioned that in the adult rat the arachnoid membrane does not cover the dorsal surface of the organ. At the sides of the pineal body, however, arachnoidal laminae are present. They also envelop the caudal parts of the vena prosencephali mediana and vena magna cerebri if both are present, respectively the single large vena magna cerebri if this is exclusively to be found. These arachnoidal laminae are continuous with the common arachnoidal membrane covering the external surface of both hemispheres. Between the hemispheres, in the fissura interhemispherica, an arachnoid membrane is absent. A dural tentorium cerebelli is present. It contains the large sinus transversi, nerve bundles and relatively much connective tissue (Fig. 34). In the adult, ossification of the connective tissue in the basal part of the tentorium occurs. As has been mentioned, no dural falx is present in the fissura interhemispherica, which contains only a small amount of loose connective tissue.

Cerebral pineal nerve fibres. It could be clearly observed that the epiphyseal stalk of the albino rat constantly contains a small number of nerve fibres. These fibres are derived from the habenular commissure as well as from the caudal commissure, the contribution of the former commissure to the amount of stalk fibres being the larger. Figure 11 illustrates a small bundle of nerve fibres running from the caudal tip of the habenular commissure underneath the ependyma of the suprahabenular recess of the third ventricle in which a number of choroidal villi are seen. Figure 12 pictures habenular epiphyseal fibres which leave the habenular commissure at its ventral surface. At the rostral surface of the same commissure, just underneath the ependyma by which it is covered, a nerve fibre can be seen turning in a caudal direction and joining the habenular epiphyseal

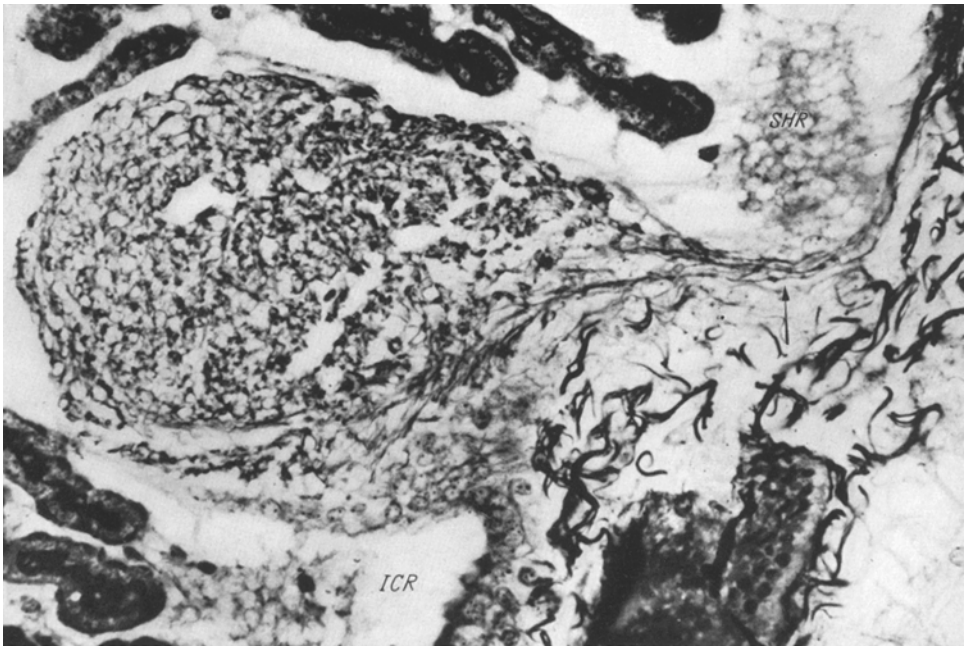

Fig. 11. Habenular commissure. Habenulo-epiphyseal nerve fiber bundle indicated by arrow. BOUIN, 10 μ, sag., ROMANES, $\times 400$

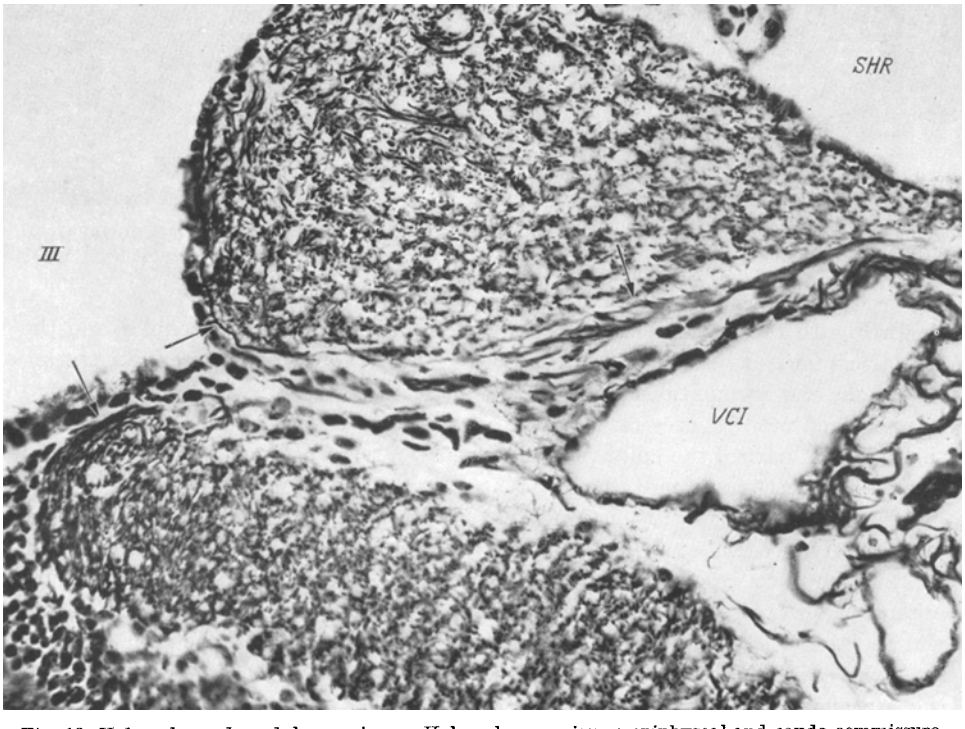

Fig. 12. Habenular and caudal commissure. Habenulo-commissuro-epiphyseal and caudo-commissuro-epiphyseal nerve fibres and bundles indicated by arrows. Chloral hydrate-formol, 8 μ, sag., ROMANES, $\times$ 400

bundle. From the same figure it appears that not a few fibres in the habenular commissure run in a more or less sagittal plane. We will return to this point. In Figures 12 and 13 a small bundle of nerve fibres can be followed which derive from the rostral border of the caudal commissure. They run sagittally through the proximal part of the epiphyseal stalk lying between both commissures and join the nerve fibres of habenular commissural derivation contributing to the formation of the commissural epiphyseal tract.

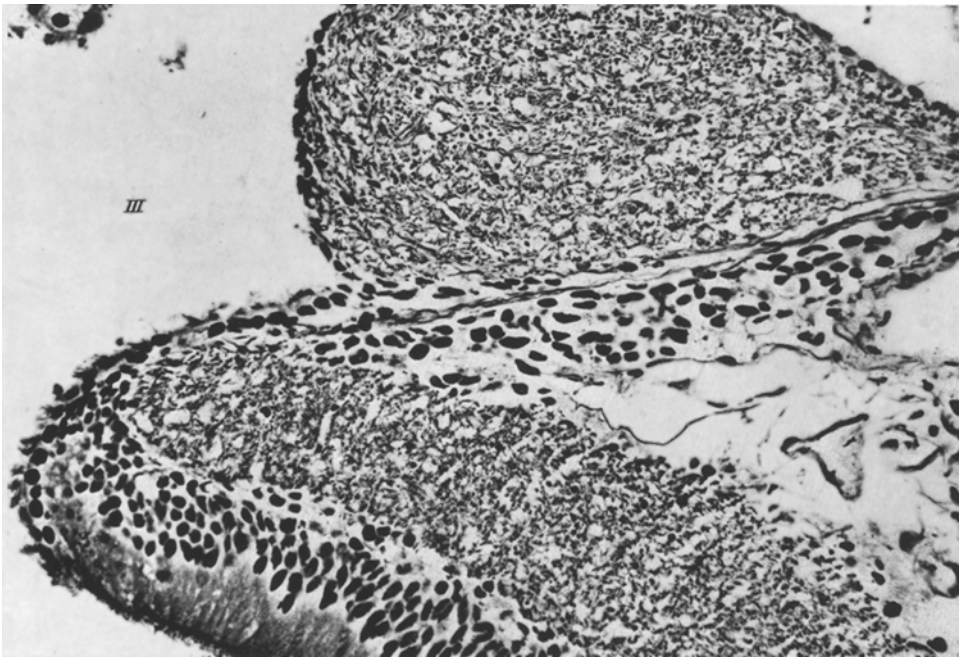

Fig. 13. Habenular and caudal commissure. Between the commissures proximal part of epiphyseal stalk containing commissuro-epiphyseal nerve fibres. Small bundle derives from rostral part of caudal commissure. Chloral hydrate-formol, 8 μ, sag., ROMANES, $\times 362.5$

In Figure 14 a transverse section at the level of both the habenular and the caudal commissure is shown. This as well as the following figures illustrating transverse and horizontal sections of the pertinent region of the rats brain should be compared with Figures 12 and 13. A rather large amount of cells, constituting the proximal part of the epiphyseal stalk, is seen lying between the commissures. The habenular commissural fibres which will run to the pineal organ are indicated by arrows. At this level they course ventral to the bulk of the habenular commissure. The commissure is covered by ependyma constituting the floor of the suprahabenular recess of the third ventricle. The most dorsal part of the commissure is very wide in a transverse direction. In Figure 15, illustrating a transverse section at a somewhat more caudal level just behind the habenular commissure, the epiphyseal stalk is seen lying just ventral to the ependymal lining of the suprahabenular ventricular recess. To the right some commissural fibres can be still seen coursing in a transverse direction in this slightly asymmetrical section. The epiphyseal stalk contains only a very few number of cells and nerve

fibres, forming the commissuro-epiphyseal tract, which are derived form both the habenular commissure as well as the caudal commissure. At still more caudal levels this tract turns somewhat dorsalward behind the ependymal lining of the

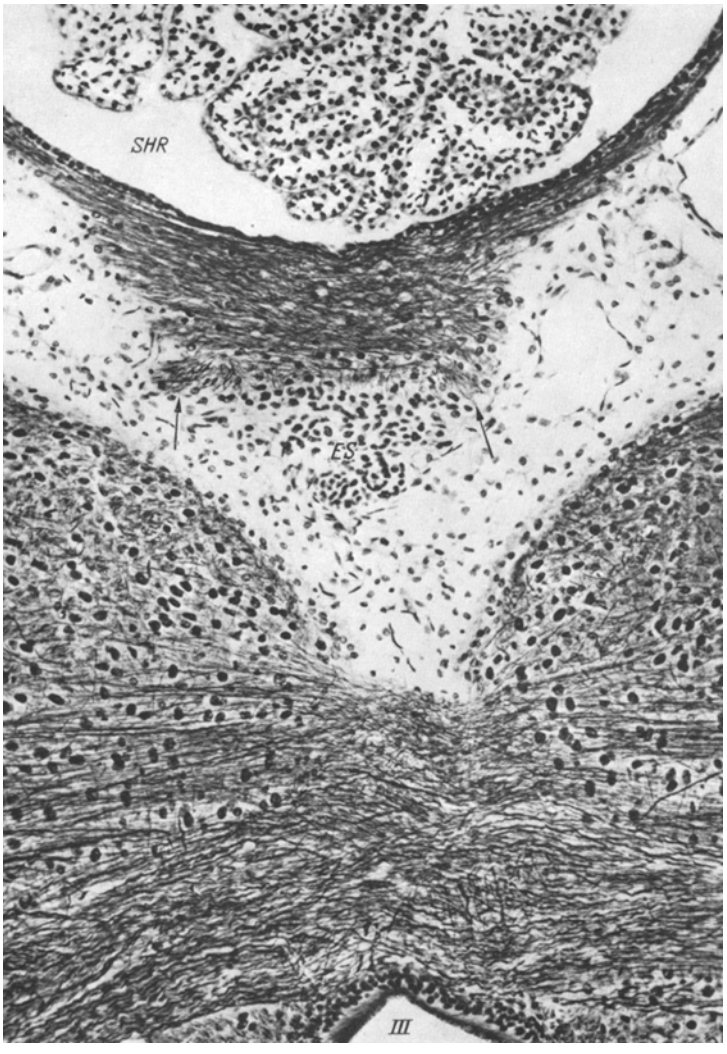

Fig. 14. Proximal part of epiphyseal stalk between habenular and caudal commissure. Commissuro-epiphyseal fibres indicated by arrows. BOUIN, 8 μ, transv., BODIAN, $\times 128$

suprahabenular recess. In this midpart of the stalk cells, in general, are almost absent. This is illustrated in Figure 16. In some few specimens stalk cells were even found to be entirely absent along a short distance. Nerve fibres of commissural origin never failed in the stalk of the rats which were investigated. Their number, however, is often only very small.

The sagittal and transverse sections illustrated are supplemented by Figures 17 to 20 showing horizontal sections at slightly different levels in a ventro-dorsal

direction. In Figure 17 some few nerve fibres deriving from the superior part of the caudal commissure are seen turning in a caudal direction coursing sagittally. These fibres will join the commissuro-epiphyseal tract as is obvious from studying

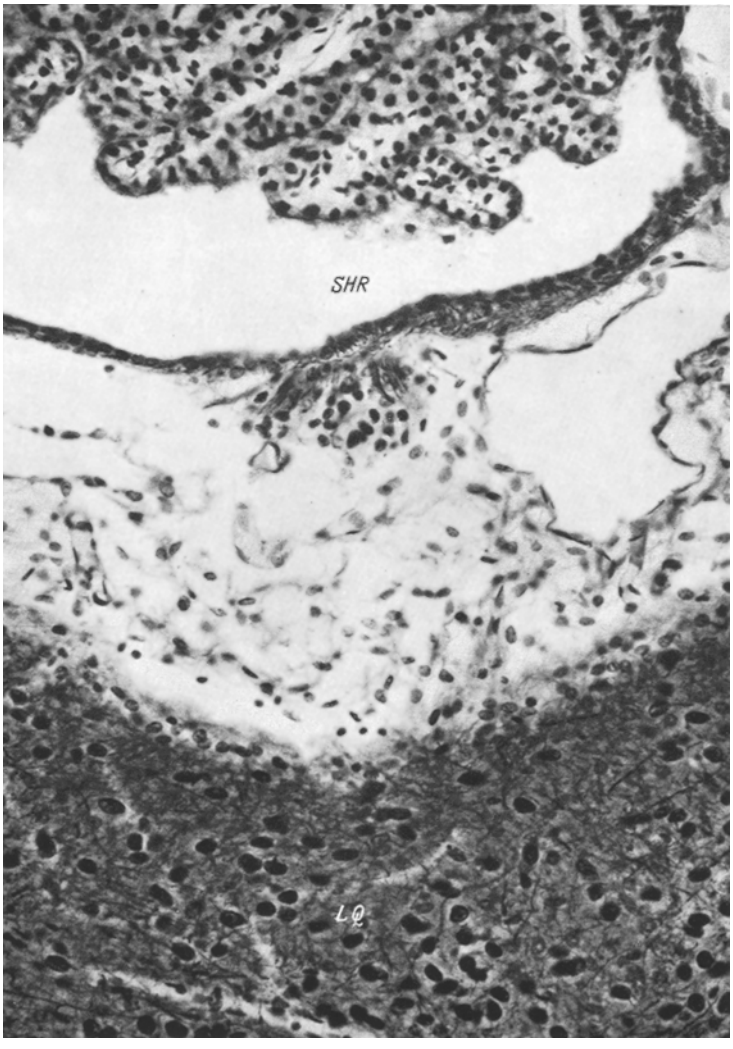

Fig. 15. Epiphyseal stalk containing commissuro-epiphyseal fibres underneath floor of suprahabenular recess. BOUIN, 8 μ, transv., BODIAN × 204

this series. In Figures 18—20 the course of habenular commissural fibres turning in a caudal direction and contributing to the commissuro-epiphyseal bundle in the epiphyseal stalk can be clearly followed. Their number is larger than that of the epiphyseal fibres derived from the caudal commissure.

Figure 21 illustrates a sagittal section of the epiphyseal stalk merging into the rostral end of the pineal body. Nerve fibres of different calibres are seen running in the stalk. Some of them are evidently myelinated. A picture of part

of the distal portion of the stalk with its nerve fibres is given in Figure 22 under higher magnification. Many of the nuclei of the stalk cells are fusiform belonging most probably to fibrocytes. Other cells, however, are clearly of epiphyseal origin. In some stalks even small epiphyseal follicles could be observed. From Figures 21 and 22 it follows likewise that the number of fibres in the commissuro-epiphyseal tract is not very abundant. Figure 21, moreover, suggests that the fibres of this tract do not penetrate very deeply into the pineal body. This

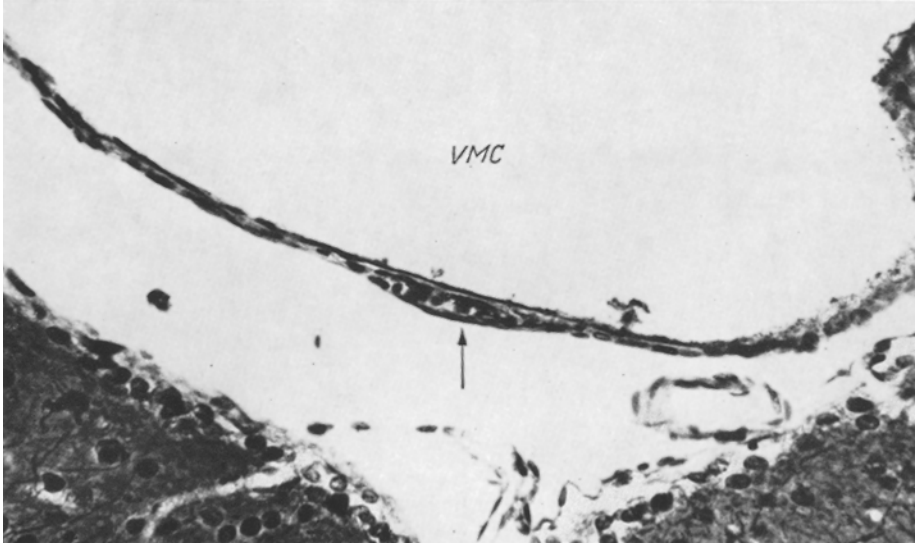

Fig. 16. Thin midpart of epiphyseal stalk, indicated by arrow, underneath large vena cerebri magna. BOUIN, 8 μ, transv., BODIAN, × 320

opinion is corroborated by the study of serial sections. Only some extremely rare single fibres run through practically the whole of the pineal body. This could be observed especially well in pineal organs in which autonomic nerve fibres were absent due to degeneration (see below). Some very few fibres, after having reached the pineal body, may leave the organ running in the adjacent leptomeningeal tissue. Only in one case we were able to observe, in the rostralmost part of the pineal body, a structure which is most probably a knob-like ending of a fibre of the commissuro-epiphyseal tract (Fig. 23). It seems, however, to be questionable whether this ending is a functional nerve terminal as will be discussed later.

A most curious and what we think a most fundamental way of coursing of the commissural fibres is illustrated in Figure 24. This shows the zone of transition from the epiphyseal stalk to the pineal body and is composed of three micro-photographs of two successive sections, two of the photographs being made from one section in different foci. This illustration clearly reveals a nerve fibre making a sharp loop of 180° after having entered the rostral part of the pineal body. Evidently, this fibre runs back in the stalk to the commissural region and should be considered an aberrant commissural fibre having no function whatsoever in the pineal organ. Conditions enabling to follow the course of such

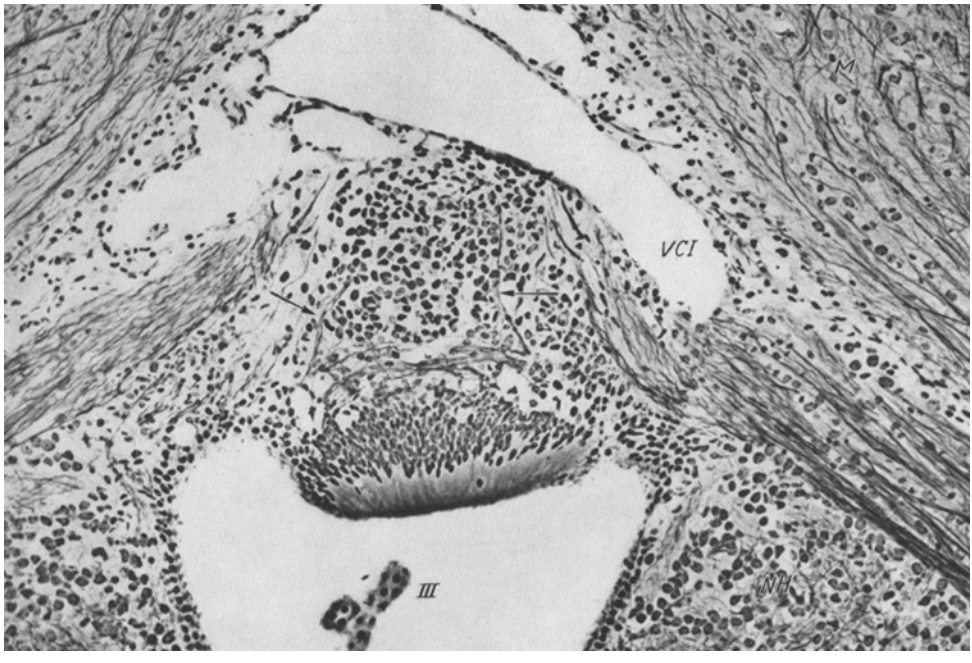

Fig. 17. In midpart of figure from below upward: subcommissural organ, transverse fibres of most dorsal part of caudal commissure, cell mass constituting proximal part of epiphyseal stalk. To the left and right basal fibre bundles of habenular commissure. Some few caudo-commissuro-epiphyseal fibres indicated by arrows. Chloral hydrate-formol, 10 μ, horiz., ROMANES, $\times$ 160

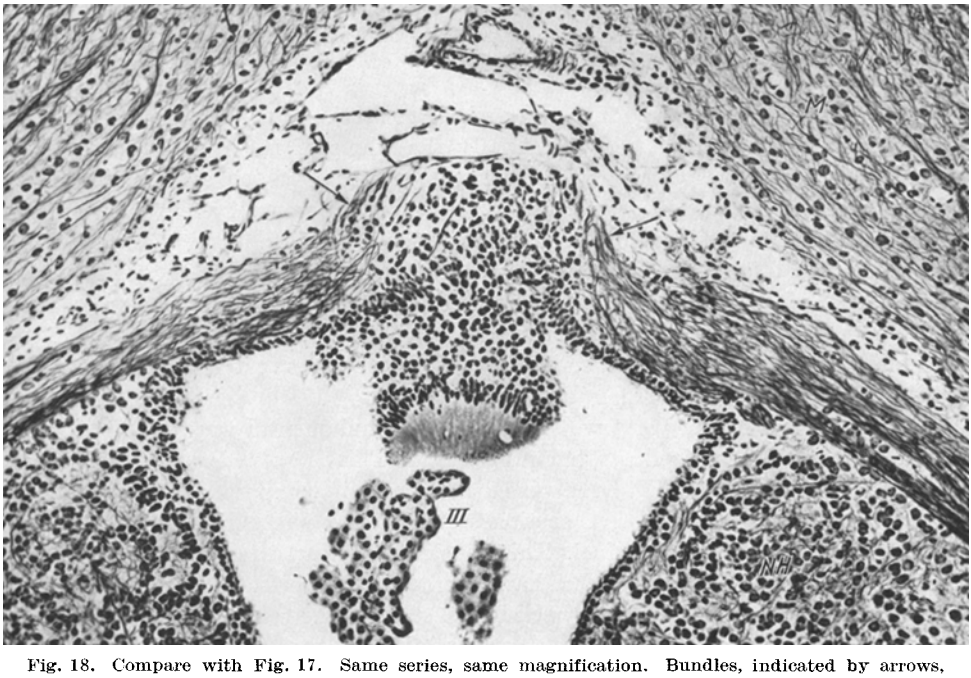

Fig. 18. Compare with Fig. 17. Same series, same magnification. Bundles, indicated by arrows, contain habenulo-commissuro-epiphyseal fibres. A caudo-commissuro-epiphyseal fibre is also seen in centre of epiphyseal stalk

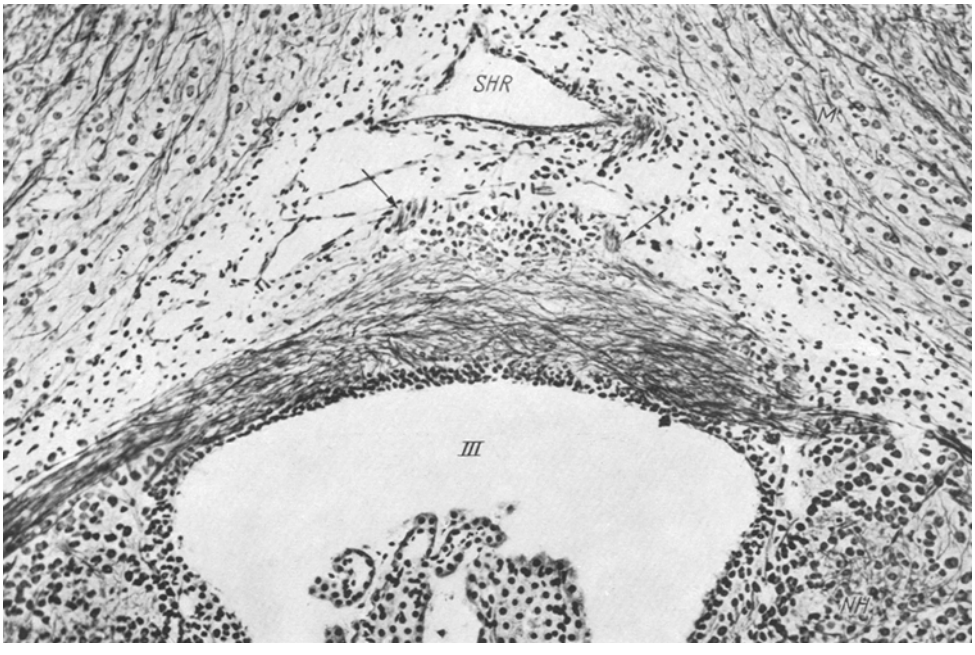

Fig. 19. Compare with foregoing figures. Same series, same magnification. Habenular commissure. Small habenulo-epiphyseal fibre bundles indicated by arrows. Between them part of epiphyseal stalk. Habenular commissure contains transverse as well as oblique fibres

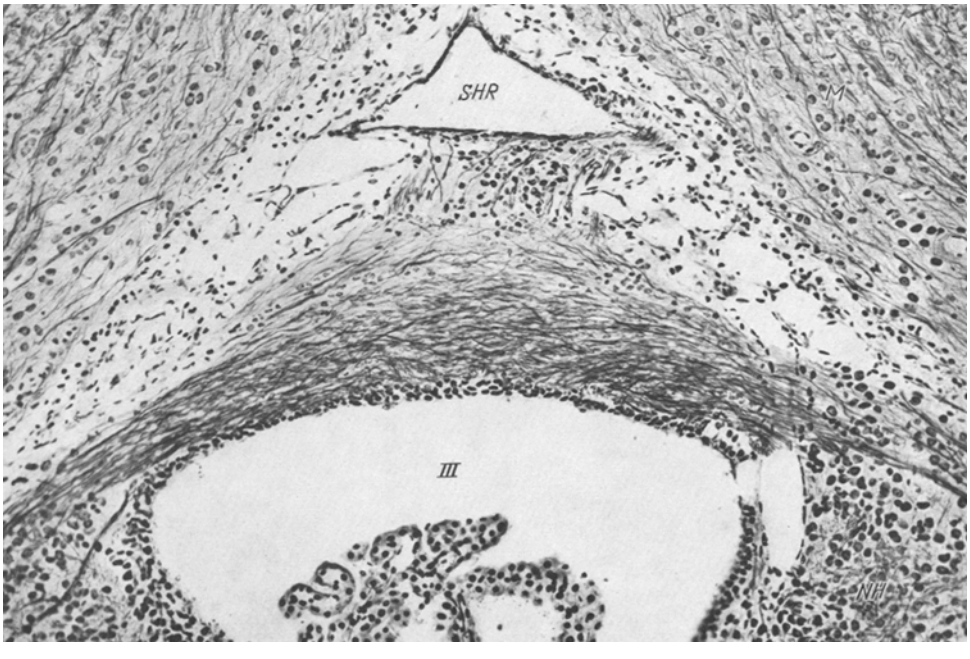

Fig. 20. Compare with foregoing figures. Same series, same magnification. Habenular commissure large in rostro-caudal extension. In epiphyseal stalk, situated between this commissure and supra-habenular recess, commissuro-epiphyseal fibres are seen running in a sagittal direction

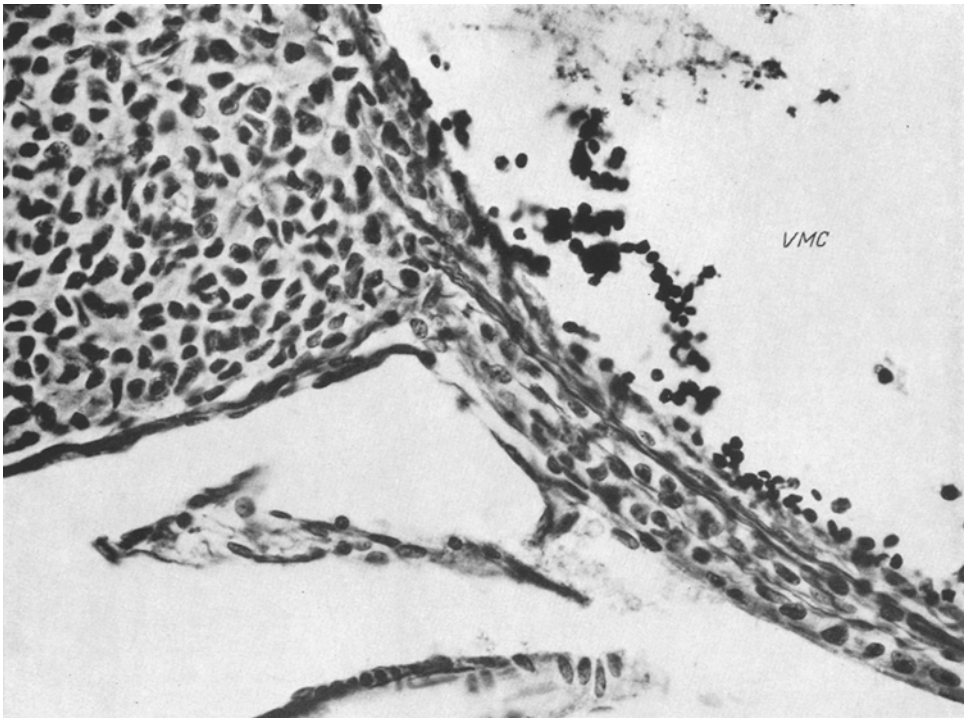

Fig. 21. Distal part of epiphyseal stalk containing small fibre bundles and single fibres of commissuro-epiphyseal tract reaches rostral part of epiphysis. At the base of the organ its leptomeningeal sheath is shown. BOUIN, 8 μ, sag., BODIAN, $\times 320$

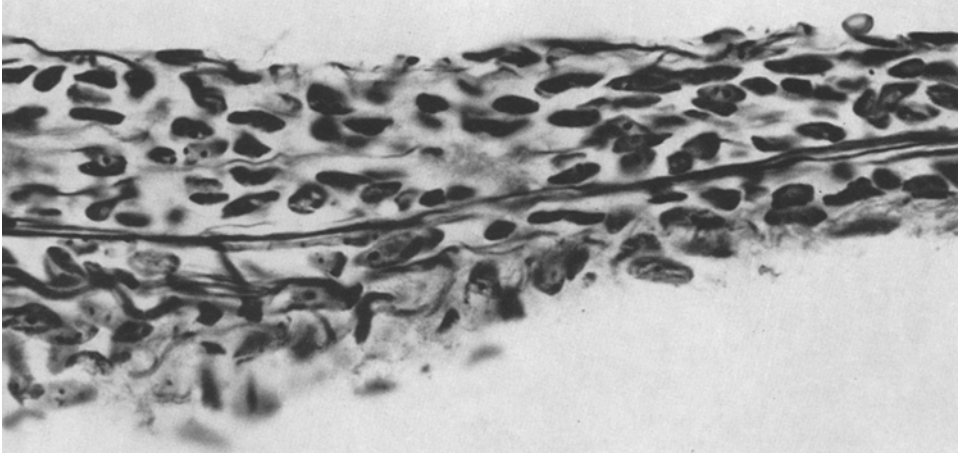

Fig. 22. Distal part of epiphyseal stalk containing fibres of different calibre of commissuro-epiphyseal tract. In stalk nuclei of pinealocytes, one seen to the left above thick fibre showing typical dumb-bell shape, and many fusiform nuclei of fibrocytes. Somewhat to the left of the midpart of the figure very flat and elongated nucleus of probable lemnocyte lying just below and close to thick fibre. Wavy fibres to the lower left are connective tissue fibres. BOUIN, 8 μ, sag., BODIAN, $\times 800$

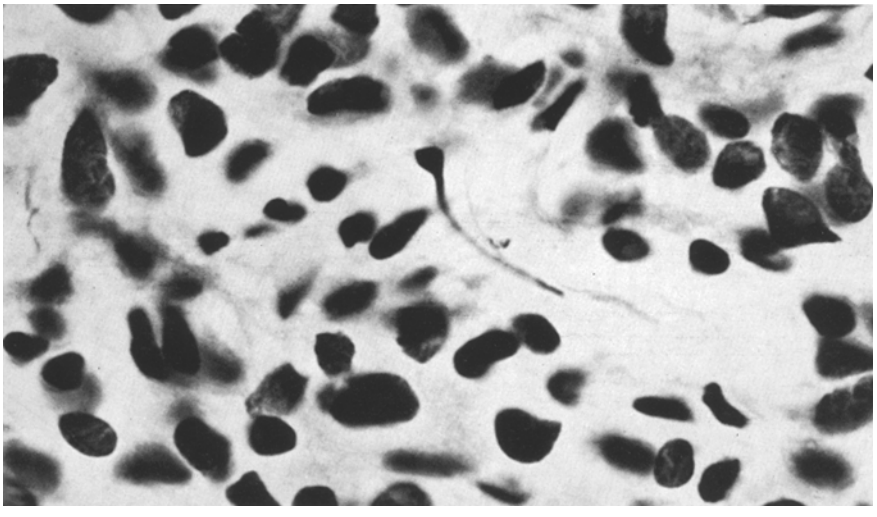

Fig. 23. Ending of commissuro-epiphyseal fibre. Ganglionectomized rat. This ending is quite different from autonomic terminal endings shown in Fig. 40. For explanation see text. BOUIN, 10 μ, sag., BODIAN, $\times$ 1000

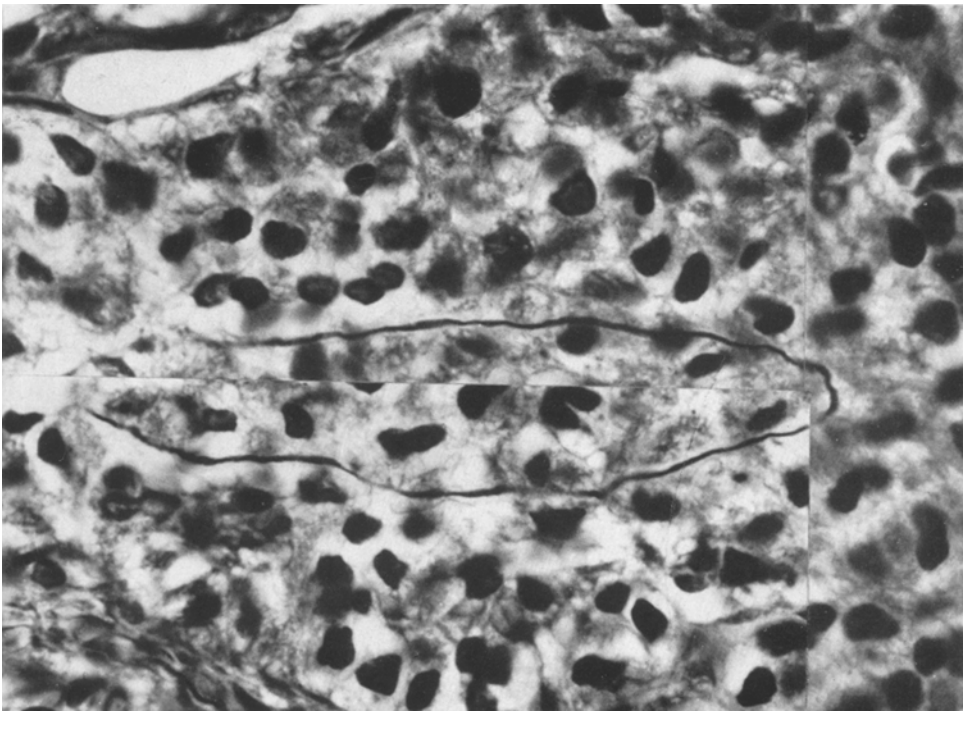

Fig. 24. Looping aberrant commissuro-epiphyseal fibre in most rostral part of epiphysis. Ganglionectomized rat. Distal end of epiphyseal stalk to the left, rostral part of epiphysis to the right. For explanation see text. BOUIN, 10 μ, sag., BODIAN, $\times$ 800

looping fibres will be rarely so favorable as they were in this special instance. It is, however, very probable that many of the fibres of the commissuro-epiphyseal tract do perform these loops after or may be even before entering the pineal body. The consequence is that at least a number of the fibres present in this tract are identical, being seen in their ascending as well as in their descending course. From this it follows that, in reality, the number of individual fibres in the commissuro-epiphyseal tract is still smaller than it seems to be.

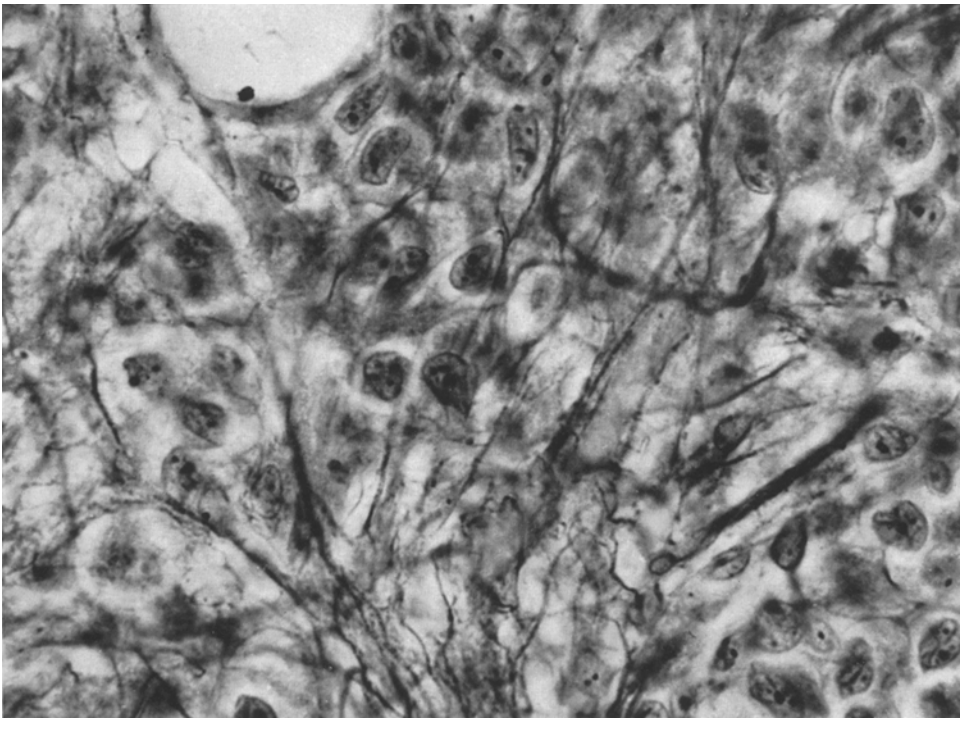

Fig. 25. Intramural plexus of autonomic fibres spreading in epiphysis. BOUIN, 12 μ, horiz., BODIAN, $\times 800$

Autonomic pineal fibres. It has been mentioned above that, in rats, most of the nerve fibres entering the pineal organ by way of the epiphyseal stalk can not be followed very far into the epiphysis. Nevertheless this organ is extremely well innervated as is illustrated in Figure 25. Earlier authors did already suppose that these fibres are of autonomic origin especially because they were seen entering the pineal body by way of its vessels. This suggests that these fibres would be merely extensions of nerve fibres constituting vascular plexuses. This, however, is not the case. In the pineal body large nerve bundles can be seen running along vessels but also quite independently as is illustrated in Figures 26 and 27 and in Figures 28 and 29 respectively. Moreover, in many instances in which nerve bundles can be seen running along vessels they do not surround them, not forming vascular plexuses (Figs 26 and 27). Evidently, at least the large nerve bundles only use vessels as a kind of path-finders in entering the pineal body.

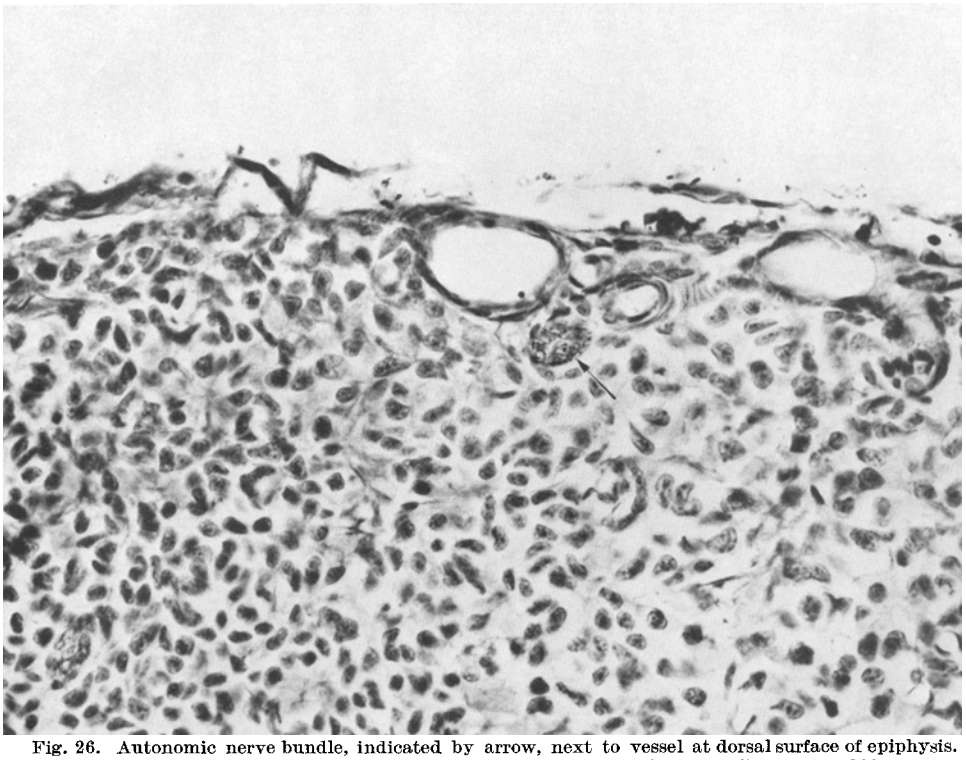

Fig. 26. Autonomic nerve bundle, indicated by arrow, next to vessel at dorsal surface of epiphysis. In the organ some scattered autonomic fibres. BOUIN, 8 μ, sag., BODIAN, × 320

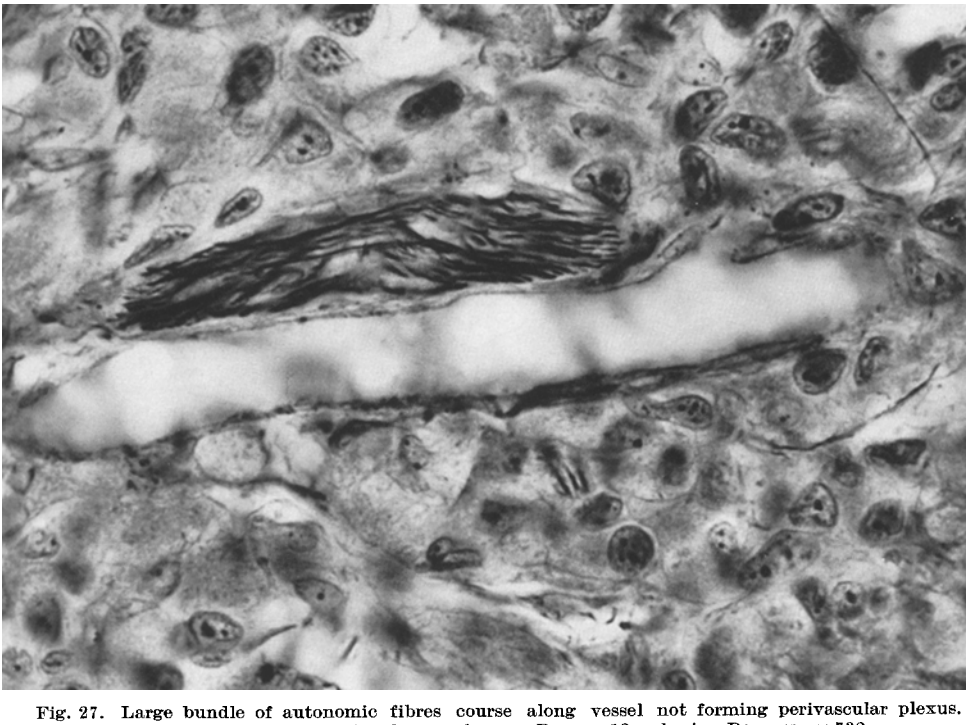

Fig. 27. Large bundle of autonomic fibres course along vessel not forming perivascular plexus. Some single fibres in pineal parenchyma. BOUIN, 12 μ, horiz., BODIAN, × 732

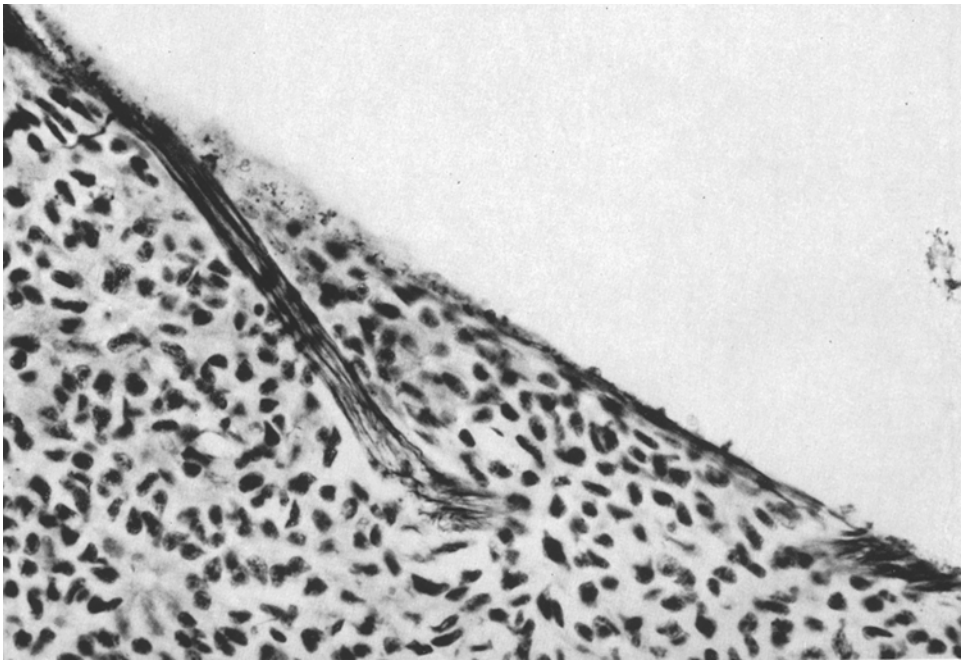

Fig. 28. Intrapineal joining of both nervi conarii below dorsal surface of organ. Course of right nerve only partly shown. BOUIN, 8 μ, transv., BODIAN, × 320

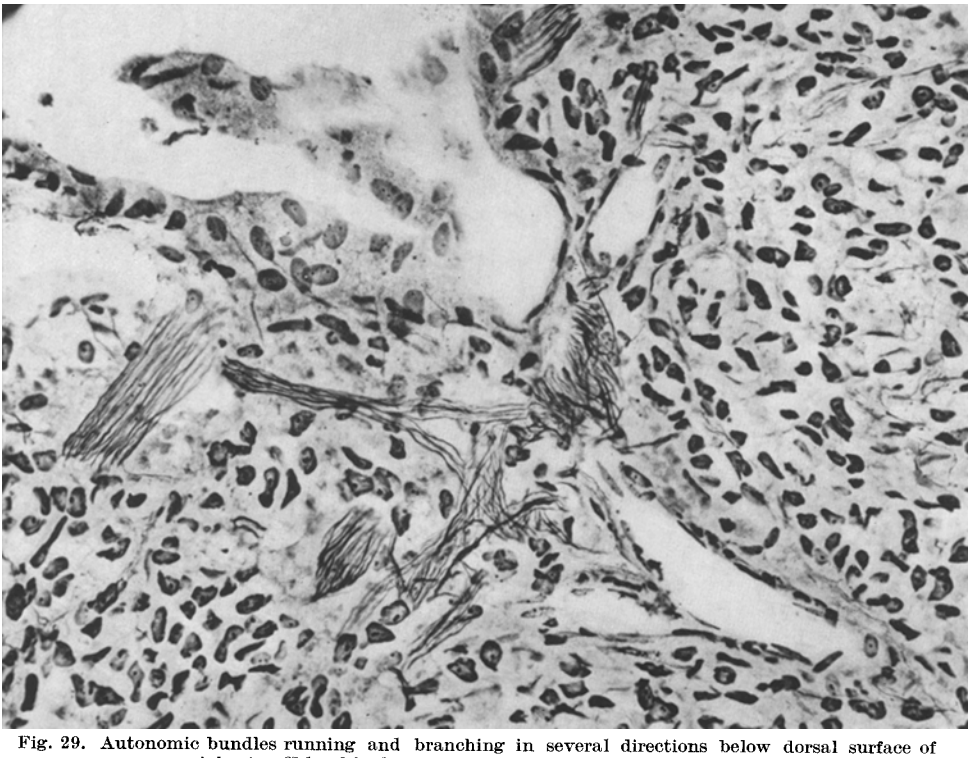

Fig. 29. Autonomic bundles running and branching in several directions below dorsal surface of epiphysis. Chloral hydrate-formol, 8 μ, horiz., ROMANES, × 400

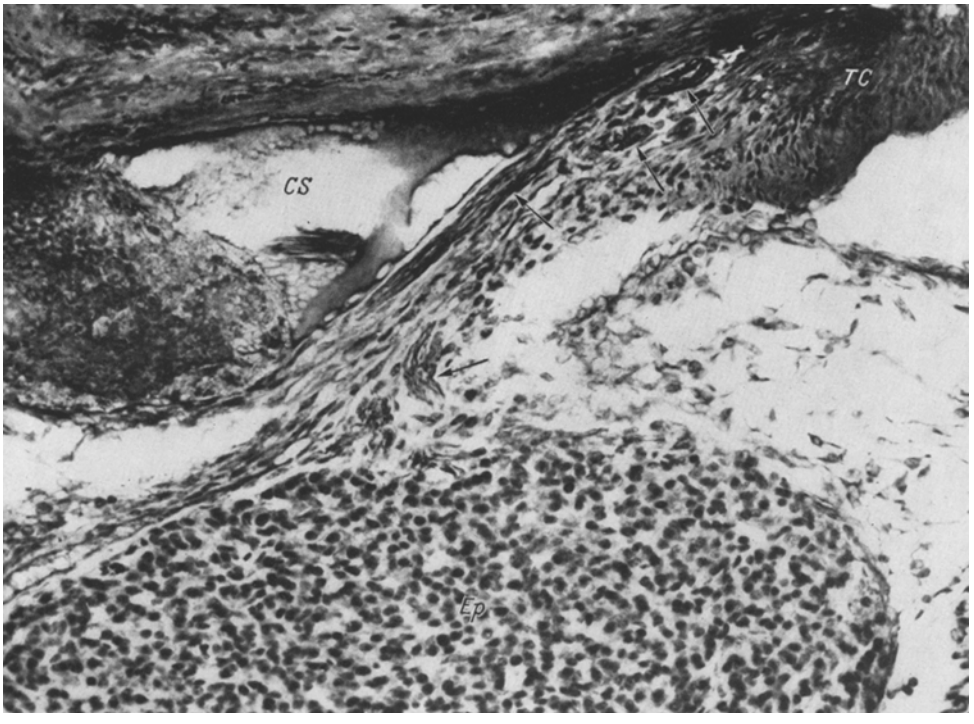

Fig. 30. Autonomic fibre bundles, indicated by arrows, leaving tentorium cerebelli and running below floor of confluens sinuum to epiphysis. Rat, aged 15 days. BOUIN, 10 μ, sag., BODIAN, × 204

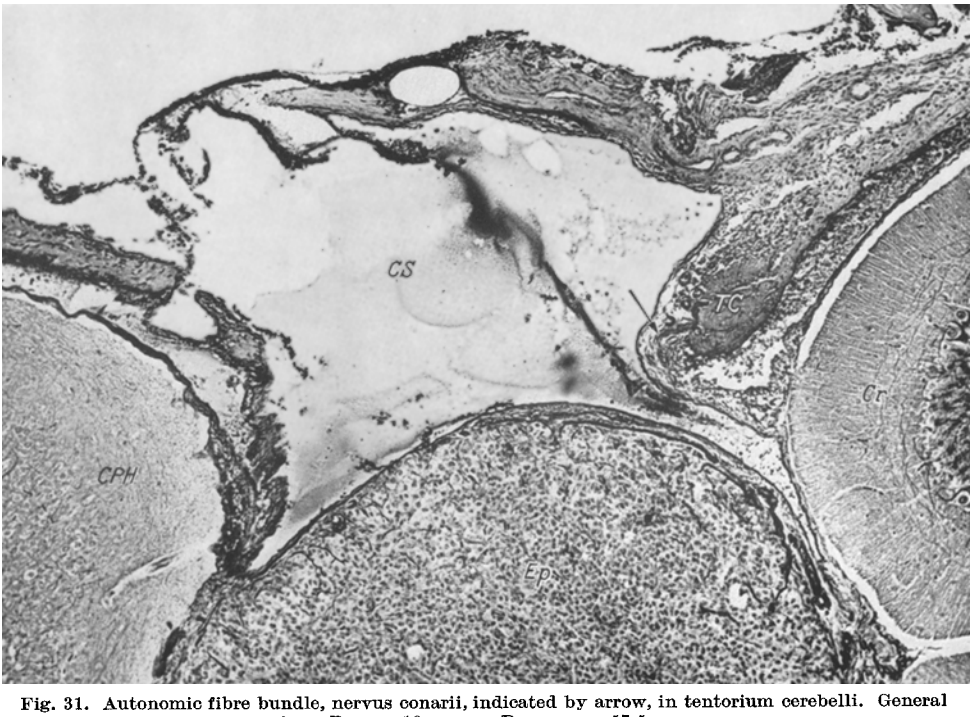

Fig. 31. Autonomic fibre bundle, nervus conarii, indicated by arrow, in tentorium cerebelli. General view. BOUIN, 10 μ, sag., BODIAN, × 57.5

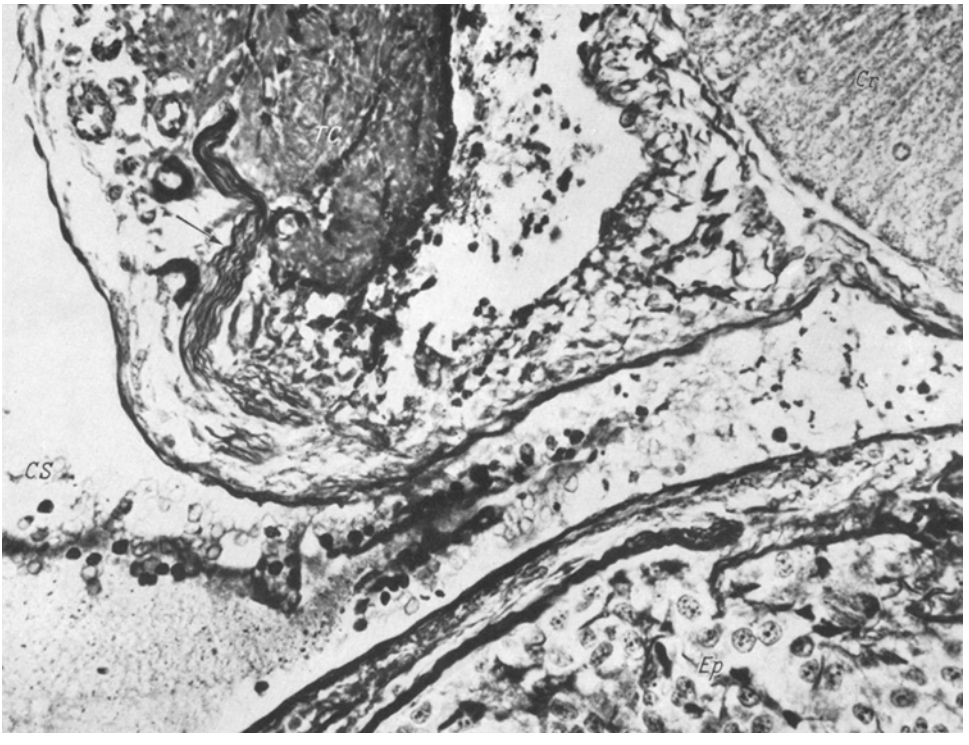

Fig. 32. Same section as illustrated in Fig. 31. Nervus conarii leaving tentorium cerebelli. × 375

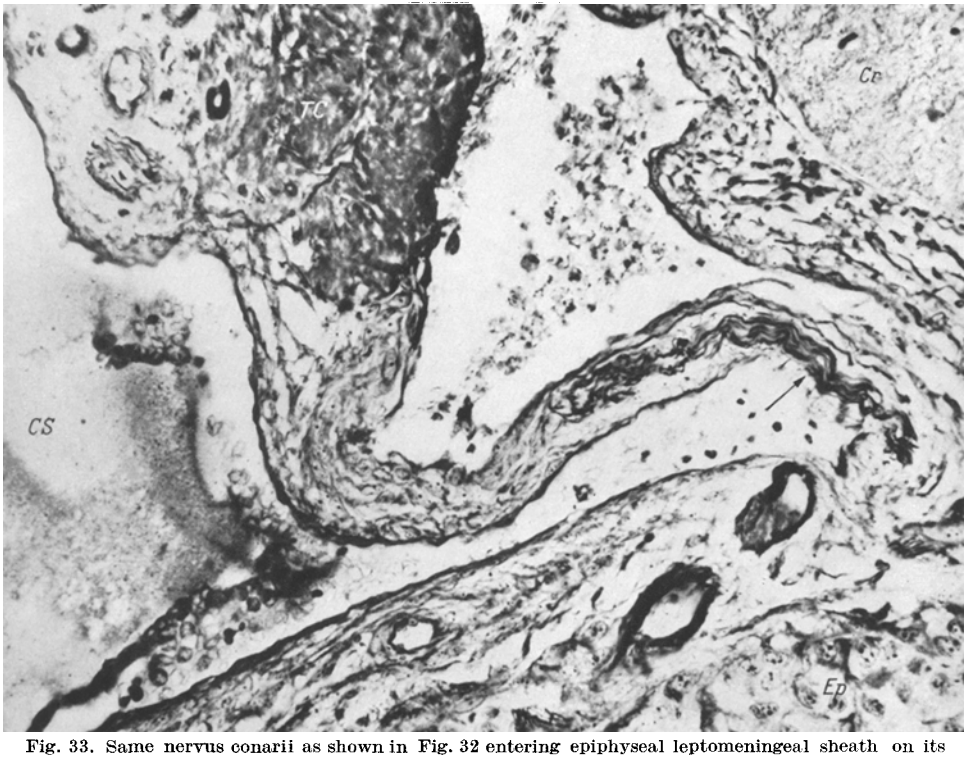

Fig. 33. Same nervus conarii as shown in Fig. 32 entering epiphyseal leptomeningeal sheath on its way to epiphysis. For orientation compare with Fig. 31. × 400

The origin of these fibres can be studied adequately only in serial sections of preparations in which the meningeal relationships are unimpaired. In these it was observed that by far the largest supply of the epiphysis with autonomic fibres is by way of two nerve bundles entering the organ symmetrically at its dorsolateral surface.

In Figure 30, a parasagittal section, the pineal body and, dorsal to it, the confluens sinuum is shown in a rat, aged 15 days. At this stage of development

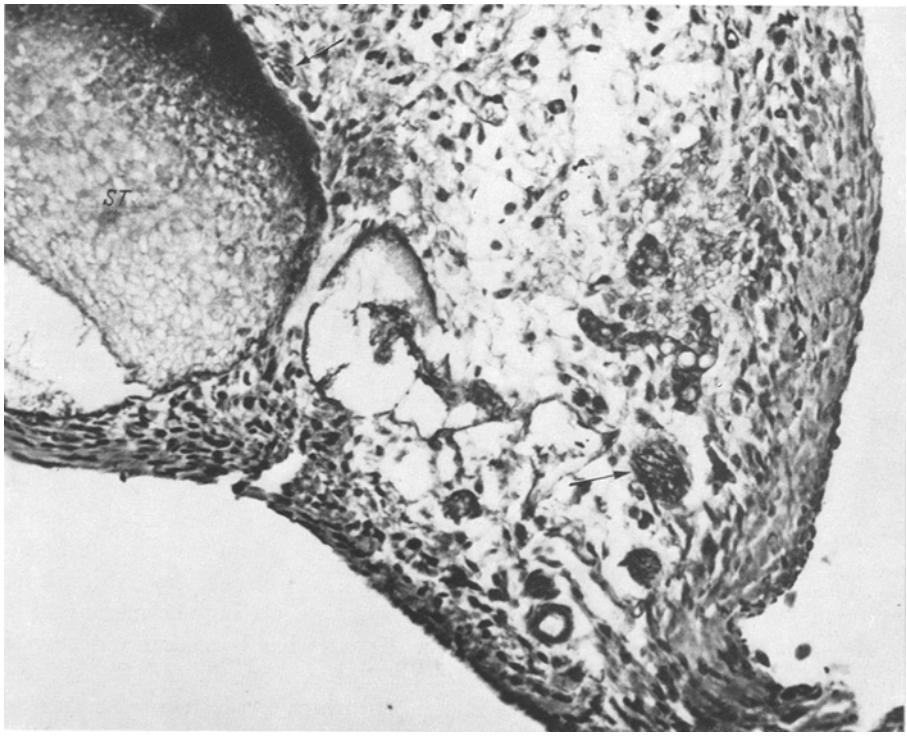

Fig. 34. Tentorium cerebelli showing nerve fibre bundles, some indicated by arrows. Rat, aged 15 days. BOUIN, 10 μ, sag., BODIAN, × 288

the epiphysis has not yet reached its final position just rostral to the cerebellum. In the floor of the confluens some bundles of nerve fibres are indicated and fibres are seen entering the pineal body at its dorsal surface. In next sections many more fibres could be observed. Distalward relative to the pineal organ, these bundles were seen running in the tentorium cerebelli. A similar condition is illustrated in Figures 31—33. Figure 31 pictures the epiphysis of an adult rat. Dorsal to it the large confluens is seen. In this figure it is also well shown that the very thin floor of the confluens is in immediate contact with the dorsal surface of the pineal organ. In this parasagittal section the tentorium cerebelli is illustrated in cross section. It contains a bundle of nerve fibres which is indicated by an arrow. In Figure 32, which illustrates the same section as pictured in Figure 31 but which was taken under higher power, this tentorial nerve bundle can be seen more clearly. In Figure 33 it is shown leaving the tentorium and entering the leptomeningeal sheath of the epiphysis. In next sections it was

seen penetrating into the pineal organ. For orientation Figures 32 and 33 should be compared with Figure 31. The nerve bundle illustrated does not run along a vessel. At a symmetrical level a similar structure is found at the contralateral side.

In some specimens both nerves were seen joining after their entrance in the epiphysis forming a single fascicle running along a short distance in the midplane before branching. This was the case in the epiphysis shown in Figure 28. Here, one of the nerve bundles is illustrated whereas only part of the contralateral one is shown. Joining of both nerves in the organ is, however, not a constant feature. Most often the two nerves were seen branching extensively immediately after entering the epiphysis. The result is that bundles of fibres course higgledy-piggledy just underneath the dorsal surface of the epiphysis, as is illustrated in Figure 29.

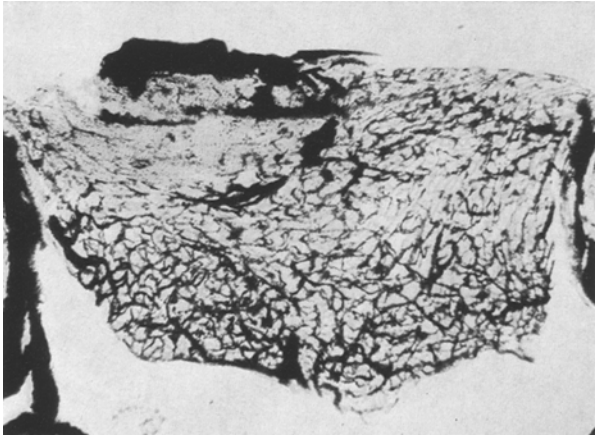

Fig. 35. Spalteholz' preparation (by Dr. J. Moll) showing ink-injected dense vascular network of adult epiphysis. × 68

Figure 34 pictures the tentorium cerebelli containing the large sinus transversus. A number of smaller as well as of larger nerve fascicles is shown in the tentorial connective tissue. Not all of these fascicles leave the tentorium to enter the pineal body. Some of them will also undoubtly be sensory fibres innervating the meninges.

By some previous authors a single nerve fascicle has been seen entering the tip of the epiphysis which has been termed nervus conarii. Le Gros Clark (1940) was able to follow this nerve in the tentorium cerebelli (see Discussion). In our opinion, the two symmetrical nerve bundles mentioned above show that, at least in the rat, there is not a single nervus conarii but two nervi conarii. The problem whether the fibres in the nervus conarii are either afferent or efferent in regard to the epiphysis has not been previously solved. In the series used in the present investigation which were stained according to various methods, no iuxtamural or intramural pineal nerve cells could be demonstrated. This fact suggests that the nervi conarii are afferent in regard to the pineal organ. This suggestion has been proved by bilateral removal of the superior cervical ganglia as will be dealt with below. After passing into the epiphysis the fibres of the nervi conarii, repeatedly branching, form a very extensive and dense network of unmyelinated fibres which are motor in function.

For studying the terminal autonomic innervation the preparations stained according to the osmic acid-sodium iodide technique of Champy-Coujard proved especially useful. As has been argued by Coujard (1943), Champy, Coujard and Coujard-Champy (1945/46), Champy and Hatem (1955) and Champy and Champy-Coujard (1957), especially the preterminal course of adrenergic fibres

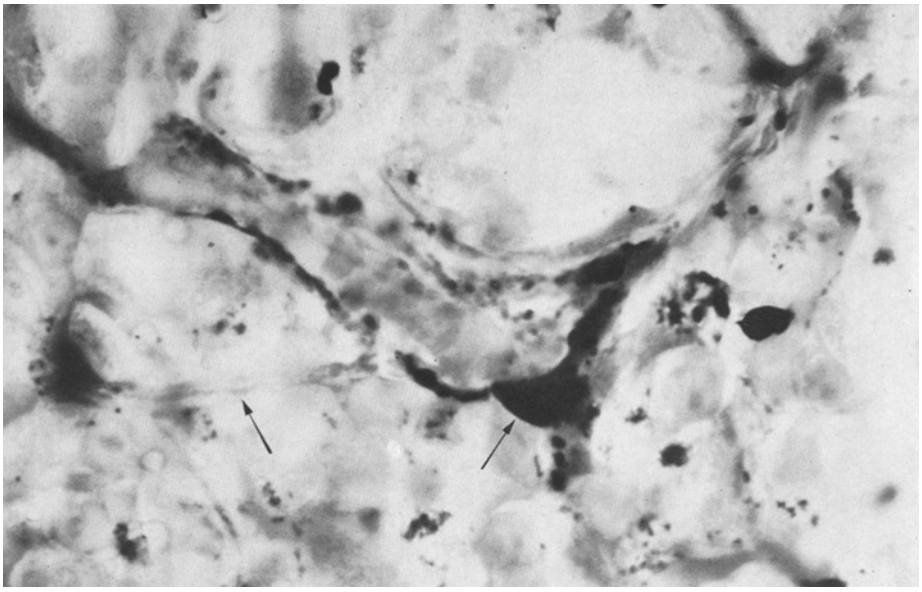

Fig. 36. Terminal strand of autonomic fibres, some showing beads, coursing along capillary. Probable interstitial cell of CAJAL and single thin intralobular fibres branching from strand indicated by arrows. CHAMPY-COUJARD, 8 μ, × 1152

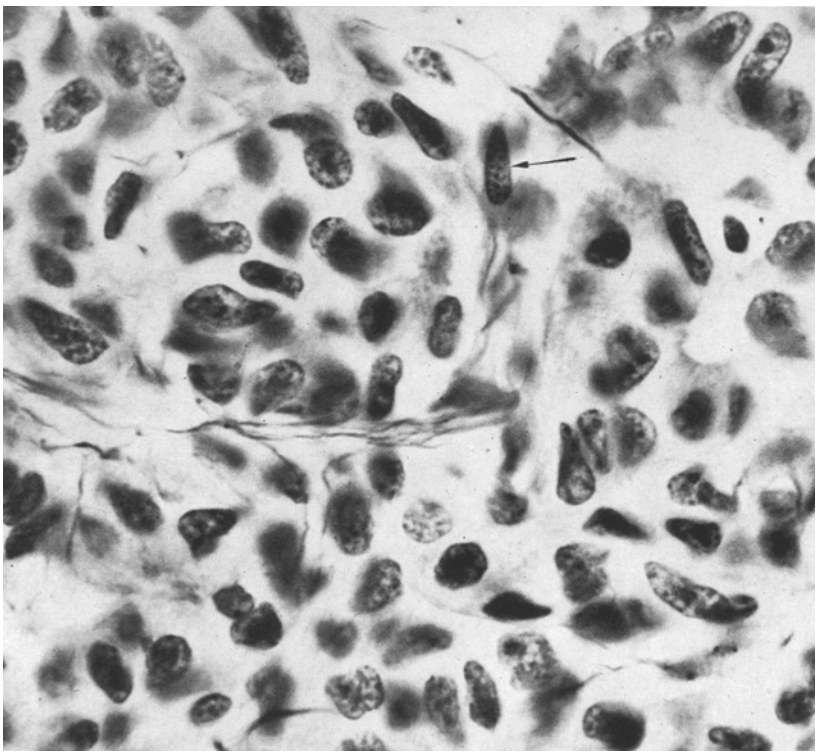

Fig. 37. Interfollicular or -lobular strand of autonomic nerve fibres. Above the terminal strand a pineal follicle. To the right another follicle showing central lumen. Single fibres branching from strand take intrafollicular course in follicles below strand. Nucleus of interstitial cell indicated by arrow. BOUIN, 8 μ, BODIAN, × 1000

as well as adrenergic terminals can be demonstrated by this method which, according to the authors mentioned, would probably stain diphenoles (see, however, the Discussion).

As has been mentioned, the epiphysis of the rat is constructed of a great many cellnests showing the shape of follicles or rosettes in many of which the original follicular lumen still can be observed. Between these follicles a dense

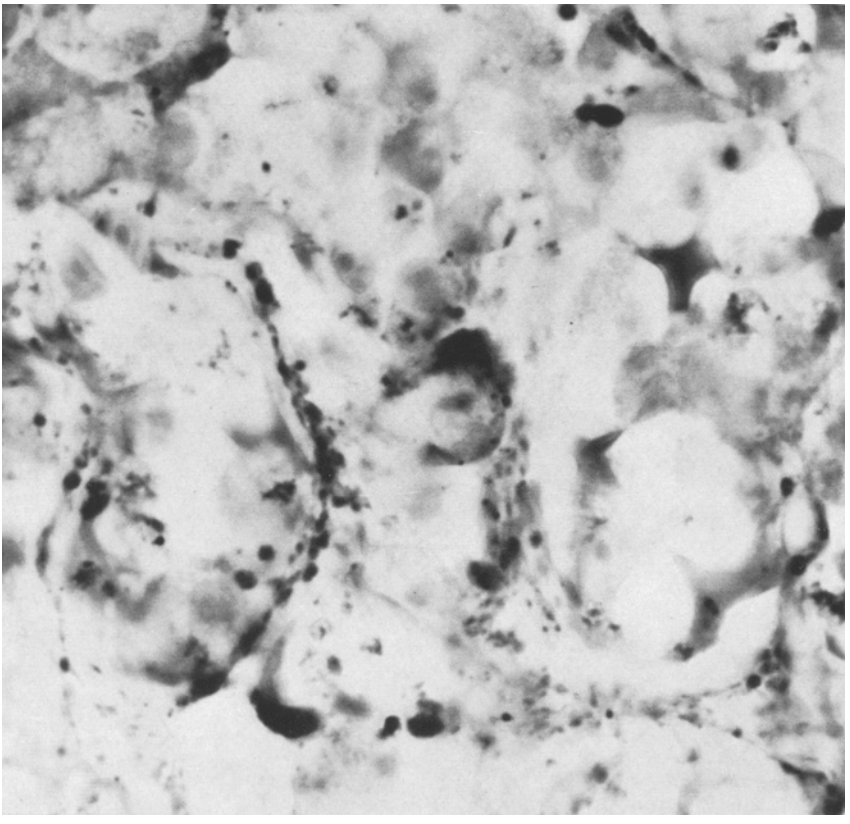

Fig. 38. Interfollicular strands of thin sometimes beaded autonomic terminal fibres. CHAMPY-COUJARD, 8 μ, × 1280

network of anastomosing vessels is present (Fig. 35). The larger ones, arterioles and venules, are enveloped by a scarce amount of connective tissue of mesenchymatous leptomeningeal origin. Beyond, practically no connective tissue cells are present around the anastomosing capillary network. In the interstitial spaces between the follicles and, therefore, partly along the vessels, partly independent of those, strands of extremely thin autonomic fibres were observed in the series stained with the silver techniques as well as in the CHAMPY-COUJARD series. In the latter, particularly, the fine fibres showed many beads. These strands of nerve fibres and some single fibres are illustrated in Figures 36—39. In the bundles, the fibres for the most part were seen running more or less parallel. Seemingly, anastomosing also occurs. In or along these strands cells were recognized of shapes

varying from ovoidal to fusiform. Like the fibres they stained black using the osmic acid-sodium iodide solution of CHAMPY-COUJARD. Most probably these cells are interstitial cells of CAJAL.

It appears that, primarily, the nervous strands run between the follicles. Besides, extremely thin nerve fibres were seen branching off singly or in small bundles from the interfollicular strands, entering the pineal follicles. It is very difficult to decide whether, in the follicles, these fibres show either an inter- or

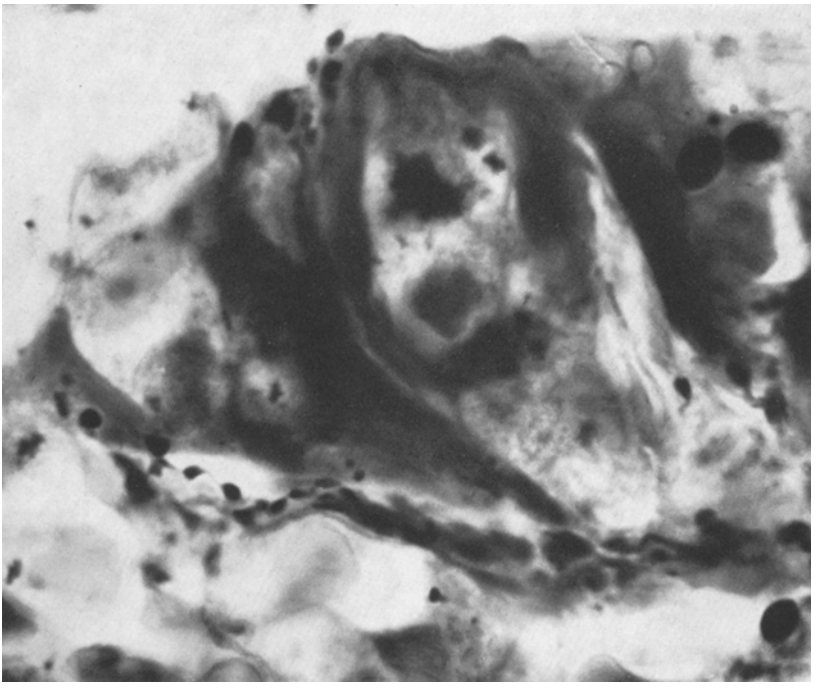

Fig. 39. Beaded terminal autonomic fibres. A large vessel is seen. CHAMPY-COUJARD, 8 μ, $\times$ 2000

an intracellular position. This point will be discussed later. Interstitial cells were not observed in the course of the intrafollicular fibres or fibre strands.

Moreover, curious structures also staining black with the reagent of CHAMPY-COUJARD could be observed in the epiphysis. Their shape is variable but, for the most part, ovoidal, budlike or club-shaped. In some preparations, their number was rather large and they seem to be related to the pinealocytes, not to the wall of the capillaries. In our opinion these structures are terminal endings. Confusion with interstitial cells can be easily excluded. At one side of these structures a single small nerve fibre was seen entering and they neither showed a number of processes as is said to be the case in most cells of CAJAL, nor nuclei. These endings are illustrated in Figure 40. We did not yet succeed in clearly demonstrating them in silverstained series.

In trying to establish the origin of the nerve fibres in the nervi conarii more satisfactorily, both superior cervical ganglia were removed in a number of rats. The results of this operation were striking. In the pineal body of a rat sacrificed

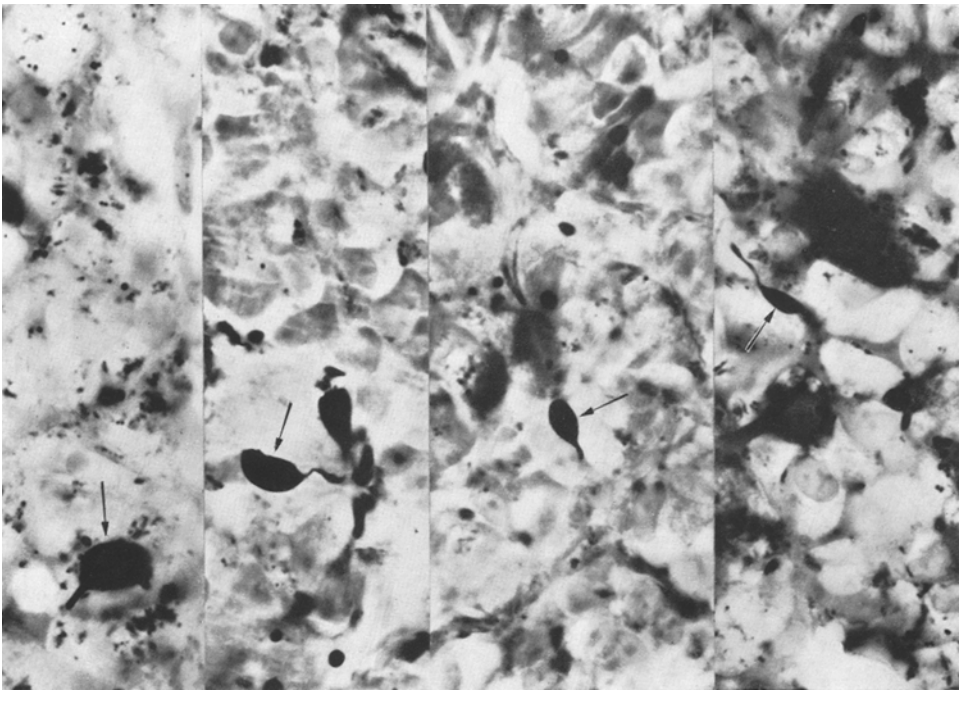

Fig. 40. Autonomic terminal endings in epiphyseal parenchyma indicated by arrows. In the third part from the left of this figure some fibres are also visible. CHAMPY-COUJARD, 8 μ, × 800

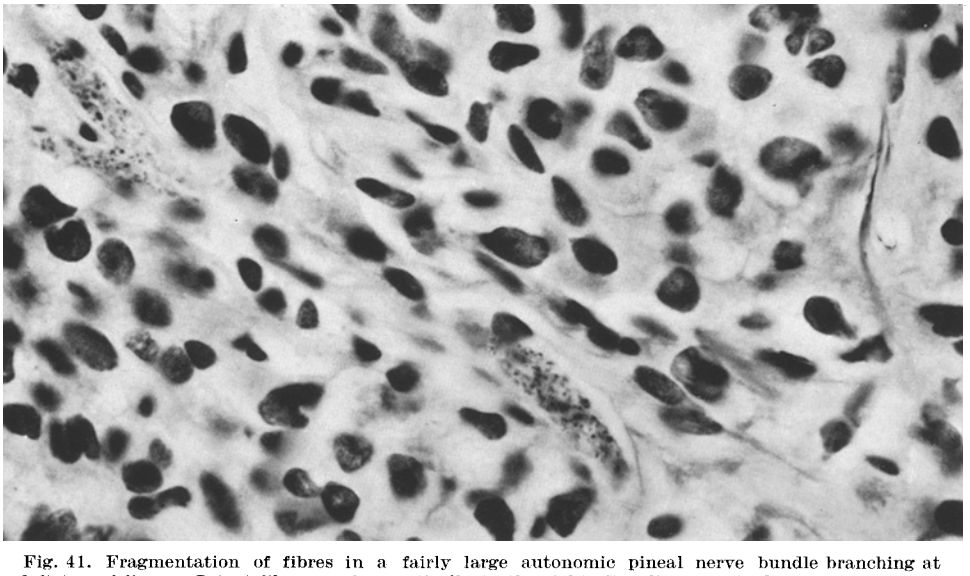

Fig. 41. Fragmentation of fibres in a fairly large autonomic pineal nerve bundle branching at left top of figure. Intact fibre running vertically to the right. Ganglionectomized rat. BOUIN, 10 μ, sag., BODIAN, × 800

on the 8th day after the operation was performed fragmentation of nerve fibres in the branches of the nervi conarii was clearly demonstrated in the epiphysis.

Evidently these fibre bundles were degenerating (Fig. 41). In rats sacrificed after longer intervals, *i. e.* two or more months, the general picture of the epiphysis in silver preparations was characterized by a practically total lack of autonomic fibres, the fibres of the nervi conarii entering the pineal body also failing. Fibres of the commissuro-epiphyseal tract, however, stood out clearly. Obviously, this not only proves that the autonomic fibres had totally degenerated but also that their regeneration was impossible. In a few cases some single fibres, probably not being of commissural origin, were left in the organ. Their presence will be discussed later.

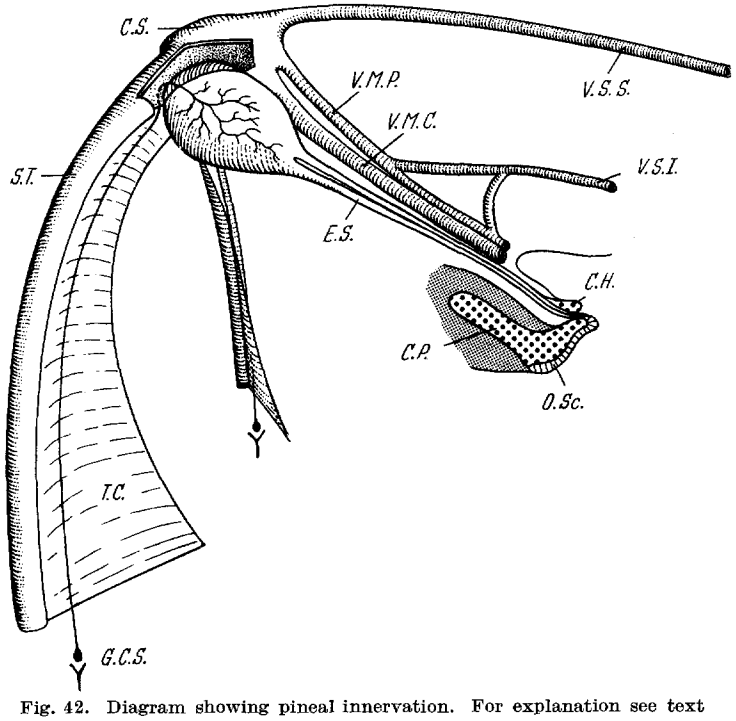

Fig. 42. Diagram showing pineal innervation. For explanation see text

In the pineals of sympathectomized rats which were stained according to the technique of CHAMPY-COUJARD neither interfollicular and intrafollicular autonomic fibre strands nor terminal endings could be demonstrated these having evidently also degenerated.

From this experiment the conclusion may be drawn that the fibres in the nervi conarii, forming after branching the dense nervous network in the epiphysis, are indeed postganglionic fibres having almost exclusively their origin in the superior cervical ganglia, and, furthermore, that most probably the inter- and intrafollicular nervous strands of thin fibres are constituted by the terminal parts of these postganglionic axons.

The results of the present investigation relating to the innervation of the epiphysis in the albino rat are diagrammatically summarized and illustrated in Figure 42. In the epiphyseal stalk exclusively an aberrant commissural fibre performing a loop of of 180° in the rostral part of the pineal body is illustrated.

Neither the extremely rare fibres derived from the commissures which may leave the epiphysis passing to the leptomeningeal tissue nor an ending of such a commissural fibre having, according to the opinion of the author, most probably no functional significance in the epiphysis, are shown in the figure. In the tentorium cerebelli both nervi conarii are indicated, their fibres originating from nerve cells in the superior cervical ganglia and ramifying in the epiphysis after their entrance in this organ under the floor of the confluens sinuum.

Discussion

By most authors it is generally accepted that nerve fibres, present in the epiphysis, are derived from both the habenular and the caudal commissure and that, moreover, also autonomic fibres are to be found in the organ. Regarding the origin, the course and the functional significance of these fibres, however, different opinions are held.

FAVARO (1904) was probably first in giving a fairly exact description of pineal fibres derived from the commissural region in the rat. He distinguished prepineal and pineal fibres. According to him the prepineal are direct stria medullaris fibres. They would not reach the pineal body running in a transverse as well as in an oblique direction under the floor of the posterior part of the dorsal sac which has been termed suprahabenular recess in the present investigation. Among the pineal fibres FAVARO distinguished superior and posterior ones. The superior fibres are derived from the habenular commissure, the posterior running upward from the "tractus medius" by which is meant the rostral prolongation of the caudal commissure. In both the superior as well as the posterior bundles the fibres may run in a transverse and in an oblique direction. Following FAVARO all of his prepineal fibres would disperse in the leptomeningeal tissue.

In the opinion of the present author the bulk of the fibres, lying most dorsally in the striae medullares, cross just ventral to the suprahabenular recess of the third ventricle. These crossing fibres form the caudo-dorsal part of the habenular commissure which is very extensive in a rostro-caudal direction as well as wide in the transverse direction. The course of these fibres may be followed especially well in horizontal sections. From these it can be observed that they are indeed commissural fibres not passing into the leptomeningeal tissue although such a course may be simulated in transverse sections. So far for the transverse prepineal fibres of FAVARO. His oblique prepineal fibres are evidently those which by the present author were seen running directly from the striae medullares into the epiphyseal stalk, contributing to its nerve tract.

HARTMANN (1957) in the horse and the present author in the rat did observe nerve fibres which, on their way through the peduncles (HARTMANN) or through the pineal stalk after entering the rostral part of the epiphysis (rat), pass into the leptomeninx. It may well be that these fibres are similar to those termed oblique prepineal fibres by FAVARO. It is probable that, in the rat, such fibres may leave the epiphyseal stalk or the rostral part of the epiphysis at any level passing into the leptomeningeal tissue. In our opinion, however, it is impossible to decide whether such fibres belong to either the prepineal or to the pineal fibres of FAVARO, most probably belonging to both categories.

Regarding the origin of his fibrae pineales superiores FAVARO holds that part of them are direct fibres from the striae medullares whereas others would originate in the habenular nuclei, in the thalamus and, possibly, from the fasciculus retroflexus. His fibrae pineales posteriores would have their origin in the thalamus

and, less frequently, in the "mesencephalon". In the present investigation we did not try to locate the exact origin of the cerebral epiphyseal fibres. Concerning this origin many speculations have been ventured in the literature (cf. LE GROS CLARK, 1940, and especially BARGMANN, 1943, who gave an extensive survey of previous work done on the innervation of the pineal gland). So far as is known to the present author, degeneration experiments have been only performed by POLVANI (1913). Using the method of MARCHI, this investigator did not observe secondary degeneration in the habenulae of the rabbit after removal of the epiphysis. His conclusion is that there are no fibres which have their origin in the pineal organ and run to the habenulae.

We agree with LE GROS CLARK (1940) that any conclusion pertaining to the origin of the central epiphyseal fibres must be regarded as uncertain because, in sections of normal material, it is impossible to trace individual fibres in continuity along the rather long nerve bundles leaving the habenular and caudal commissure and reaching the pineal organ. We were, however, able to observe that a fairly large number of nerve fibres, running from the habenular commissural region into the epiphyseal stalk, are direct fibres of the striae medullares. In these striae they show a dorsal position. This agrees with the opinion of FAVARO regarding his prepineal and part of his superior pineal fibres. For the nuclei of origin of the fibres running in the striae medullares of the rat we may refer to the paper by GURDJIAN (1925).

PINES (1927), investigating the innervation of the pineal gland in the dog, cat and rabbit, also found central pineal fibres running to the organ from the habenular commissure as well as from the caudal commissure. Likewise to the opinion of this author the bundle, emerging from the habenular commissural region, contains direct fibres from the medullary striae other fibres having their origin in the habenular nuclei.

HERRING (1927), surprisingly, was not able to observe any nerve fibres, either myelinated or unmyelinated, in the epiphyseal stalk of the rat although mentioning fibres of habenular origin in the epiphysis of the cat, monkey and man. HERRING, however, did observe the epiphyseal stalk in rats which he describes as an extremely delicate strand of neuroglial tissue connecting the pineal organ with the habenular commissure. GLADSTONE and WAKELEY (1940) also erroneously maintained that central fibres do not reach the epiphysis in the rat even holding that the pineal gland in the adult is completely separated from the central nervous system.

According to GARDNER (1953), in the hooded rat the epiphysis is not continuous with the diencephalon. It is supported by connective tissue strands which connect the organ with "the tela chorioidea rostrally and with the inferior surface of the transverse sinus caudally". No such relationship was observed in the adult albino rat. GARDNER, however, constantly found nerve fibres running from the commissural region to the pineal body. He holds that, in the hooded rat, most of these fibres are derived from the habenular commissure, the contribution of the caudal commissure being only small. This is in accordance with our findings in the albino rat. The cause may be that in both species the epiphysis for the most part develops from the antero-dorsal wall of the epiphyseal evagination which is related to the habenular commissure and only for a minor part from the postero-caudal wall of this evagination which is related to the caudal commissure. In other mammals sometimes the reverse ist the case.

In all our specimens, fibres from the commissural region could be followed to at least the rostral part of the pineal body.

ROUSSY and MOSINGER (1938) and GARDNER (1953) termed the bundle of nerve fibres connecting the commissural region with the epiphysis "epithalamo-epiphyseal tract". This designation, however, is not entirely justified if the caudal commissure is considered to belong to the mesencephalon as is usually done.

For this reason, the present author suggests the term "commissuro-epiphyseal tract" or, somewhat more specified but also more cumbersome, "habenulo-caudo-commissuro-epiphyseal tract" for this fibre bundle. This designation has the advantage that it is not committed to the location of the cells of origin of the fibres which is not exactly known as yet.

For a long time the functional significance of the fibres derived from the commissures and reaching the epiphysis has presented a problem. In the normal silver-impregnated series studied in the present investigation their distribution in the pineal organ could not be easily followed because of their running through the complicated network of autonomic fibres. It is, however, certain that, at least in the albino rat, by far the most of these central fibres do not penetrate very deeply into the organ. This was very clearly shown in the epiphysis of the sympathectomized rat from the brain of which a Bodian-stained series was made three months after the operation. In this preparation the extensive autonomic pineal nervous network had disappeared by degeneration, the fibres of commissural origin now standing out clearly. It was also in this specimen that the loop, made by one of the commissural fibres in the rostral part of the epiphysis could be observed (Fig. 24). In this same series a blunt ending of what is evidently likewise a commissural fibre (Fig. 23) and a fibre leaving the rostral part of the organ and penetrating into the leptomeningeal tissue were shown. Very rarely, similar fibres passing to the leptomeninx were seen in other preparations. That at least myelinated fibres of commissural origin are very scarce indeed in the central and caudal parts of the epiphysis could, moreover, be demonstrated in the series which was exclusively stained with osmic acid as well as in the many series stained with osmic acid-sodium iodide after CHAMPY-COUJARD, the myelin sheath of these fibres staining black with the osmic acid. In these preparations only exceptionally a myelinated fibre was seen running straight through the epiphysis to its caudal part.

LE GROS CLARK (1940), in his careful study, surveys the different opinions of previous authors concerning the course and meaning of the central fibres of commissural derivation reaching the epiphysis. A somewhat speculative theory regarding the origin, course and function of these fibres is to be found in a paper by ROUSSY and MOSINGER (1938).

LEGROS CLARK, in his silver-impregnated macaque material, was able to observe that individual fasciculi, traced section by section, in some instances arched and then runned down leaving the gland again and rejoining the habenular commissure on the opposite side. He, then, was first in observing central fibre bundles which, most certainly, do not have terminal relations to the pineal parenchymatous cells. According to this author these fibres are merely aberrant commissural fibres derived from both the habenular as well as from the caudal commissure being "drawn up" in the pineal peduncles in his macaque series. LE GROS CLARK also gives arguments suggesting that indeed most if not all of the central fibres entering the epiphysis are to be regarded as such aberrant commissural fibres not penetrating deeply into the organ but merely forming elongated commissural loops. HARTMANN (1957) mentions similar bundles crossing over to the contralateral side in the apex of the pineal organ of the dog giving a diagrammatic illustration of these bundles. This author likewise suggests

that these fibre bundles take a contralateral course returning to the commissural region.

The present observations corroborate the opinion of LE GROS CLARK and HARTMANN. Figure 24 illustrates a separate aberrant commissural nerve fibre looping in the rostral part of the epiphysis. Certainly, conditions for observing this fibre were exceptionally favourable in this series of the brain of a ganglionectomized rat. It can not wonder that such looping fibres can be only rarely seen.

Interpretation of the nerve ending illustrated in Figure 23, observed in the rostral part of the epiphysis in the same sympathectomized rat in which the looping commissural fibre was so clearly seen, is difficult. Most certainly it belongs to a nerve fibre derived from the commissures not being an ending of an autonomic nerve fibre. The present author is inclined to the opinion that this is no functional nerve terminal but much more similar to endings which can be found in neurinomata or in transsected and regenerating peripheral nerves not finding their proper way to the effectors. It may well be that this structure represents the ending of a commissural aberrant fibre which, during its growth, "has lost its way" not being able to find its way back through the epiphyseal stalk to the commissural region. Very possibly, this fibre had grown into the stalk after this latter had already gained some length. In this connexion it should be realized that the development of the brain in the rat and, therefore of its fibre systems, proceeds for a considerable time after birth, even after the formation of the epiphyseal stalk, in which a small number of commissural fibres is "drawn out", has started.

We are in complete agreement with LE GROS CLARK in supposing that, in the rat as well as in the macaque, most if not all of the fibres of the commissuro-epiphyseal tract are aberrant commissural fibres. Some of these fibres will reach the rostral part of the epiphysis and, behaving as real commissural fibres, return to the commissural region ending at the contralateral side. Other fibres, however, after reaching the epiphysis, probably at later stages of development, will loose their way ending meaningless in the organ. Still other fibres will leave the stalk before having reached the epiphysis passing into the enveloping leptomeningeal tissue whereas a few fibres derived from the commissural region will reach the rostral part of the epiphysis only then passing into the leptomeninx. From this it follows that ascending as well as descending parts of commissural fibres looping in the pineal organ run in the epipyhyseal stalk. Therefore, the number of individual fibres in this stalk is, as a matter of fact, still less than is suggested by microscopical examination of the stalk.

From the above it can, moreover, be concluded that, most probably, the central cholinergic fibres derived from the habenular and caudal commissures do not have functional terminals in the epiphysis. This conclusion is enhanced by the fact that, in the epiphysis of the rabbit, GEREBTZOFF (1959) was not able to find any cholinesterase activity.

In the light of these observations the conclusions drawn by GARDNER (1953) from experiments performed in the hooded rat seem somewhat dubious. In the summary of his paper this author states that "in rats in which nerve fibres from the habenular and posterior commissures to the pineal gland had been interrupted the nerve terminations to the pineal gland underwent complete degeneration.

In rats in which the superior cervical sympathetic ganglion was removed bilaterally no change was observed in the nerve terminations related to the gland cells". This quotation suggests that the pinealocytes would be innervated by the commissural fibres and by these fibres exclusively, autonomic fibres not being related to their function. It appears that the opinion of GARDNER is incompatible with that of the present author in regard to the distribution and function of the fibres of commissural origin as well as to the distribution and function of the autonomic fibres. In the present investigation the epiphyseal stalk and, therefore, the fibres derived from the commissures were not cut so that the degeneration of their peripheral parts could not be studied. However, the facts that, in our series, terminal endings of commissural fibres on pinealocytes could not be demonstrated, that commissural fibres after entering the epiphysis by way of the stalk do not or only very rarely penetrate very far into the organ, many of them even returning to the commissural region, and, furthermore, that the extensive autonomic pineal network including its endings promptly disappears after bilateral removal of the superior cervical ganglion give, in our view, a rather solid foundation to the opinion that the cells of the pineal parenchyma are innervated by autonomic nerve fibres and not by the few nerve fibres of commissural derivation which enter the epiphysis by way of the epiphyseal stalk.

Turning now to the discussion of the autonomic innervation of the epiphysis it will be necessary to mention previous investigations dealing with this matter to obtain a fuller background for the present findings in the rat which may contribute to the solution of at least some problems involved.

HENLE, quoted by PINES (1927), seems to have been first in observing sympathetic fibres in the epiphysis. CAJAL (1904) demonstrated a dense network of nerve fibres in the pineal of the mouse which, according to him, is not of commissural origin being constituted by fibres derived from sympathetic bundles coursing along the vessels in the tela chorioidea. Following CAJAL these bundles enter the pineal body over its whole surface accompanying the vessels, especially at its base, invading the parenchyma and branching extensively. CAJAL, describing also nerve terminals belonging to this network, denied that the epiphysis would receive any nerve fibres of different origin. FAVARO (1904), in rodents, observed similar nerve terminals as seen by CAJAL doubting, however, their autonomic origin. He is inclined to the opinion that they belong to fibres, derived from the habenular commissure. JOSEPHY (1920) was unable to find the extensive intrapineal nerve plexus at all. WALTER (1913, 1922, 1923) denied any endocrine function of the epiphysis. On the ground of the relationships of the pineal vessels and their innervation with the vessels in the tela chorioidea and, therefore, in the choroid plexuses, this author speculated about a supposed reflectory function of the organ which would regulate the intracranial vascular system and, more specially, the blood-flow in the vessels of the choroid plexuses. In short, WALTER was of the opinion that the epiphysis is a reflex organ regulating the production of the cerebrospinal fluid in the choroid plexuses evidently holding that the pineal sympathetic fibres are efferent in regard to the epiphysis giving, however, no arguments.

ANTONOW (1926), investigating the pineal organ of the dog, cat, rabbit and cattle, mentions many nerve fibres of equal thickness running in various directions. He is of the opinion that they would derive from the pia, referring to the view of DÉJÉRINE that these fibres run from the pineal organ to the vessels of the tela chorioidea. Therefore, ANTONOW also holds that the autonomic fibres are efferent in regard to the epiphysis. He was, however, unable to observe the dense network described by CAJAL, even suggesting that this latter author took the connective tissue fibres by which the pineal cellnests are enveloped for nerve fibres. HERRING (1927) was not able to find any nerve fibres at all in the epiphysis of the rat, whether myelinated or unmyelinated.

PINES (1927), in the dog, cat and rabbit, once more saw the dense network of ramifying nerve fibres earlier observed by CAJAL and described nerve terminals of various shape. Following this author the nervous network does not show any direct relationship with the pineal vessels and, therefore, would be a nervous apparatus serving the specific function of the parenchymatous cells of the epiphysis. He suggests that the fibres from which this network originates enter the organ by way of the pial vessels and, furthermore, that the nerve cells of these fibres would be localized in the superior cervical ganglia. Therefore, the fibres would be afferent in regard to the pineal organ.

In man and a number of mammals including rodents, MAZZUCCHELLI (1950) observed a dense pineal nervous network constituted of thin fibres originating from bundles present in the connective tissue sheaths of the vessels. Moreover, fairly large tracts of similar fibres were seen by this author, sometimes taking a wavy course. According to him most fibres end on vessels some of them, however, on pineal cells. Thus, vasomotor as well as secretory nerve fibres of autonomic origin would be present in the pineal organ. MAZZUCCHELLI is very critical concerning the interpretation of the terminals seen, suggesting that some of them may not be functional endings but products of degenerative as well as of regenerative processes occurring in the epiphysis.

Following TUBAHARA (1955) the epiphysis of the cat and dog is innervated by nerve fibres derived from the habenular commissure. In the rat, however, sympathetic fibres invading the organ along the pial vessels are most numerous. In the glandular parenchyma a nerve complex, similar to an autonomic terminal reticulum is described by this author.

Thick fibres of commissural origin showing secretory terminals in the glandular folliculi of the epiphysis in the japanese monkey are mentioned by HOSAKA, NAKAI and KUSHIMA (1957). Moreover, these authors observed autonomic nerve fibres accompanying the pial vessels entering the epiphysis. From this nervous plexus fine fibres were seen branching off reaching medium-sized pineal cells.

An interstitial pineal nerve plexus has been also described by HARTMANN (1957) in the cat. Its fibres are derived from perivascular plexuses ramifying extensively. Perivascular plexuses also enter the pineal organ at its apex. The author illustrates a nerve bundle which is very similar to the nervus conarii, described by KOLMER and LÖWY (1922), which will be dealt with below. Fibres, forming plexuses, were also seen by HARTMANN in the pineal organ of the dog and of cattle. He is not sure whether the interstitial plexus, which would be perivascular, represents a nervous terminal formation. In some rare cases small terminal rings were observed by HARTMANN in the pineal of cattle.

From the foregoing survey it appears that by a number of authors fibres of commissural derivation as well as of autonomic origin have been observed in the epiphysis. Some investigators described also terminal endings of either commissural or autonomic fibres. It has been generally thought that the autonomic fibres enter the organ along with the leptomeningeal vessels. There is no agreement concerning the question whether either the commissural fibres or the autonomic network provide for the innervation of the pinealocytes. On the strength of the arguments, discussed before, the present author is of the opinion that, at least in the rat, the fibres of central orgin are merely aberrant commissural fibres having no functional relationship with the parenchymatous cells of the epiphysis.

A somewhat new aspect regarding the autonomic innervation of the organ was introduced by KOLMER and LÖWY (1922) describing a nerve bundle connecting the epiphysis with the venous system discharging into the vena magna cerebri of GALEN. They termed this bundle "nervus conarius" (better: nervus conarii) and were able to observe this fascicle in man, Cercopithecus, in the dog and goat but not in the rat. In a young goat the authors could follow the nerve, containing unmyelinated fibres, into the tentorium cerebelli. It did run next to the origin of the great vein of GALEN. According to KOLMER and LÖWY, in adult specimens of the mammals mentioned the fibres of the nervus conarii were myelinated. They were not sure whether this nerve is afferent or efferent in regard to the epiphysis. The fact,

however, that they observed nerve cells in the pineal organ in near topographical relationship with the nerve led them to the hypothesis that the nervus conarii is probably efferent. Although KOLMER and LÖWY, on theoretical grounds, did not accept WALTER's theory mentioned before, their conclusion that the epiphysis is an organ regulating the outflow of blood from the choroid plexuses, and, therefore, the production of the cerebrospinal fluid, is very similar to WALTER's. They based this equally speculative conclusion on the impression that the nervus conarii would be connected with that part of the venous intracranial system into which the veins of the choroid plexuses discharge. KOLMER and LÖWY, on the other hand, do not deny that in addition to the supposed function mentioned, the epiphysis might have another, *i. c.* an endocrine function.

In connexion with the investigation of KOLMER and LÖWY and the present study the observations of LE GROS CLARK (1940) are of importance. In his monkey as well as in his human material the nervus conarii was recognized and illustrated by this author. In the rhesus macaque it ramified in a plexiform manner underneath the endothelium of the straight sinus. In man the nerve was seen by this author emerging from the tip of the epiphysis running an uninterrupted and unbranched course to reach the dura mater of the tentorium cerebelli. From here, according to LE GROS CLARK, it turned back in the floor of the sinus rectus occupying a subendothelial position. Its destination could not be determined. In man a structure was demonstrated somewhat resembling a large arachnoid granulation. This was termed by the author the "suprapineal arachnoid body". He suggests that this structure may provide a ball-valve neuro-vascular mechanism whereby the venous return from the great vein of GALEN is regulated and controled. In some cases the human nervus conarii was seen to pass through this suprapineal arachnoid body traversing one of its blood sinuses. Nerve terminals, however, could not be observed. In the macaque no such structure was recognized. On the other hand, the author found a rich vascular plexus in the pial tissue on the ventral surface of the great cerebral vein suggesting that this might play a role similar to that of the suprapineal arachnoid body in man. The description and arguments in the paper by LE GROS CLARK suggest that the nervus conarii, containing pineal fibres efferent in regard to the organ, may be possibly instrumental in innervating the reflex mechanism mentioned. The author, however, is very careful in his conclusions saying that this problem requires further investigation. Evidently, his theory is somewhat similar to that advanced by WALTER and by KOLMER and LÖWY.

Regarding the question whether the pineal autonomic fibres are either afferent or efferent, observations in the literature concerning pineal nerve cells are of interest.

In 1930, PASTORI described a "ganglion conari" (better: ganglion conarii) which she observed at the posterior pole of the epiphysis in man and several mammals. In the sheep and donkey this ganglion contains about 20—30 nerve cells. Their fibres would run to the wall of the great cerebral vein, in the other direction entering the epiphysis along a number of vessels. Following PASTORI the fibres which are directed to the wall of the vena magna cerebri show varicosities and do form a bundle which she takes for the nervus conarii, earlier described by KOLMER and LÖWY (1922). PASTORI is of the opinion that this nerve bundle and the ganglion from which it originates favour the theory of the supposed regulatory function of the pineal organ on part of the intracranial vascular system. It is interesting to note that MARBURG, as early as 1909, described a small ganglion in a newborn human, lying on top of the epiphysis. A nerve passed caudally from this ganglion. MARBURG considered this structure to be analogous to the parietal ganglion and nerve in reptiles which, however, seems rather improbable.

Neither in his human nor in his macaque material, LE GROS CLARK (1940) was able to observe this ganglion of PASTORI. GREVING (1931) did not find it in dogs.

Besides this iuxtapineal ganglion the existence of which seems doubtful, intrapineal nerve cells have been recognized by several authors. Apparently, they are constantly but exclusively present in the epiphysis of various species of monkeys and in the pineal organ of man (in monkeys: KOLMER, 1929; LEVIN, 1938; LE GROS CLARK, 1940; HOSAKA, NAKAI and KUSHIMA, 1957; HARTMANN, 1957; in man: JOSEPHY, 1920; BARGMANN, 1943; see also the discussion in BARGMANN's paper). Their number is rather variable. LE GROS CLARK suggests that the

intrapineal nerve cells in monkeys would represent the ganglion conarii which, then, has been secondarily incorporated within the gland. The same author mentions that the general appearance of the nerve cells in the epiphysis of the macaque monkey is very similar to that of the cells in the peripheral autonomic ganglia. HOSAKA, NAKAI and KUSHIMA, moreover, described small multipolar nerve cells in the autonomic plexuses accompanying the epiphyseal vessels in the japanese monkey. From these plexuses nerve fibres were seen running to medium-sized pineal cells. Both these authors and LE GROS CLARK are of the opinion that the pineal nerve cells observed probably belong to the autonomic system. The question, however, remains whether the small multipolars seen by the Japanese authors were either typical nerve cells or, may be, interstitial cells of CAJAL.

In non-primate mammals pineal nerve cells have either been found extremely rarely or are stated to be absent. FAVARO (1904), JOSEPHY (1920) and PINES (1927) deny their presence in subprimate material. PASTORI (1928) was only able to observe one single nerve cell in her series of about 100 pineal organs. To her opinion this must have been a heterotopic cell element. GREVING (1931) did not see nerve cells in the dog's pineal.

During some time the theory has been brought forward that the pineal cells themselves would show a nervous structure, although not being real nerve cells, on the ground of their processes which may be rather long in some mammals, of their affinity to silver and gold-chloride, of the appearance of the endings of their processes which would show some resemblance to nerve terminals and of the relationship and behaviour of the pineal neuroglia to the pinealocytes which would be very similar to the relation, topographical as well as functional, of neuroglia to nerve cells (cf. PASTORI, 1928). For some time, however, this theory has been abandoned.

In the present investigation it has been shown that a dense network of unmyelinated fibres is present in the epiphysis of the albino rat. This network is clearly shown in the silver preparations as well as in the series which were stained with the osmic acid-sodium iodide mixture according to CHAMPY-COUJARD. It could be demonstrated that the epiphysis of the albino rat is, for the most part, supplied with autonomic fibres by way of two symmetrical nervi conarii and not exclusively by perivascular plexuses entering the organ along the pial vessels as has been thought by some previous authors. Observation of the two nerves, running in the tentorium cerebelli and entering symmetrically the dorsolateral surface of the organ, was possible only by examination of serial sections of material in which the meninges were left intact. In the rat, therefore, not a single nervus conarii as has been shown before by KOLMER and LÖWY (1922) in some mammals, excluding the rat in which these authors were not able to observe the nerve, and by LE GROS CLARK in man and the macaque, but two of these nerves are clearly present. Quite often the nerve bundles were seen entering the epiphysis at the same spot at which also two symmetrical branches of the posterior cerebral artery, the main artery for the vascular supply of the epiphysis, enter the pineal body. In these cases, however, it is evident that the nerves are not concerned in innervating these vessels. As is well known, nerve bundles during embryological development or in tissue culture experiments tend to grow into an organ along bundles of connective tissue fibres or along vessels, using these structures as "path finders".

The fact that the present author was able to observe, in his rat material, two symmetrical nervi conarii in stead of one may be due to the fact that in the albino rat the tip of the epiphysis next to which the nervi conarii enter the organ is situated very near to the tentorium cerebelli through which these nerve bundles course. This is not the case in those mammals in which a single nervus conarii has been previously described. Very probably, the single

nerve observed in these cases consists in fact of two nerve bundles having joined and running jointly along some distance before entering the tip of the epiphysis simulating a single nerve. In this connexion it is remarkable that in Figure 2 of the paper by LE GROS CLARK (1940) illustrating the nervus conarii in a rhesus macaque, this nerve is seen running from the tip of the epiphysis as a single bundle then, after some distance, dividing in two large branches which are interconnected by smaller branches. This figure, therefore, also suggests a bilateral origin for the single nerve described by LE GROS CLARK. In the rat, in which the tip of the epiphysis is so near to the tentorium cerebelli, the nervi conarii do not have the opportunity to join before entering the epiphysis. In some cases, however, they were observed to do so not outside but inside the organ (Fig. 28) showing a short joint course as has been described before. Apart from the facts mentioned it stands to reason that there are indeed two bilateral symmetrical nervi conarii because of the fact, shown in the present investigation, that their fibres originate in the superior cervical ganglia, degenerating after removal of these ganglia. In regard to the epiphysis, therefore, the fibres of the nervi conarii are afferent.

It has been mentioned before that in rare cases some very few nerve fibres evidently being of autonomic nature were left in the organ after removal of both superior cervical ganglia. This may be due to the fact that not all of the autonomic ganglion cells, present in the ganglia, were removed. Another possibility is that these fibres originate from leptomeningeal nerve cells which have been observed by some authors in rare cases (CLARK, 1931; SCHALTENBRAND, 1955; STÖHR, 1957; COOPER, 1958). It is often difficult to determine whether these cells belong to the cerebrospinal or to the autonomic nervous system. As has been also suggested by LE GROS CLARK, the ganglion of PASTORI being located at the tip of the epiphysis, if indeed occurring at all, could possibly represent a local accumulation of such leptomeningeal nerve cells of autonomic origin.

Accepting the view that the intrapineal nerve cells observed by several authors in primates as well as the cells constituting the somewhat doubtful ganglion of PASTORI are autonomic, one might suggest that some cells originally located in the superior cervical ganglia would show a peripheralward phylogenetic migration along the nervi conarii in the direction of the epiphysis, in some mammals constituting the iuxtapineal ganglion of PASTORI finally obtaining in primates an intrapineal position. If this hypothesis is true, this phylogenetic peripheralward migration of autonomic nerve cells would parallel a similar ontogenetic migration of nerve cells. As has been mentioned, neither iuxtapineal nor intrapineal nerve cells have, so far, been observed by the present author in the rat.

We will turn now to the discussion of the terminal autonomic innervation of the epiphysis which has been studied in silver preparations as well as in series which were stained with the osmic acid-sodium iodide mixture according to CHAMPY-COUJARD.

It has been claimed by COUJARD (1943), CHAMPY, COUJARD and COUJARD-CHAMPY (1945/46), CHAMPY and HATEM (1955) and by CHAMPY and CHAMPY-COUJARD (1957) that this reagent, originally introduced by CHAMPY (1913), would probably stain diphenoles. This might form the histochemical basis for the fact that by this method the adrenergic ground plexus, containing noradrenaline, as well as adrenergic terminals can be demonstrated. It must, however, be mentioned that the reagent of CHAMPY-COUJARD is not specific for diphenoles as will be dealt with in a next paper. Moreover, in sections treated according to this method

lipids are stained by the osmic acid component. This made careful checking of our results, obtained in the epiphysis of the rat which contains relatively much lipids, very necessary. Recently, HILLARP (1959b) even denies that in using the technique of CHAMPY-COUJARD noradrenaline in adrenergic fibres is demonstrated at all observing that adrenergic fibres deprived of at least this neurotransmitter by means of reserpine stained as well as normal nerve fibres. This author, therefore, concludes that noradrenaline can not be the substance responsible for the staining of autonomic fibres with the osmic acid-sodium iodide mixture. The fact, however, remains that the ground plexus can be easily and beautifully demonstrated by this technique.

On the whole, our findings concerning the peripheral vegetative innervation of the epiphysis agree with the results obtained in different organs, especially in glands, by previous authors using the lightmicroscope. In the pineal organ, an extensive interstitial network consisting of loosely built bundles of extremely fine fibres was observed. The larger bundles show an interfollicular position. From them smaller bundles and single fibres were seen branching to penetrate into the follicles as has been described. In the interfollicular bundles, but not in the intrafollicular ones, interstitial cells could be demonstrated. The bundles are evidently identic with the "reticular cytoplasmatic nervous strands" observed by many previous authors (ground plexus of BOEKE, preterminal reticulum of STÖHR, distal nervous syncytium of JABONERO). The literature regarding this peripheral autonomic network is very extensive. The morphological as well as the functional interpretation of these structures which have been studied in silver, methylene blue and CHAMPY-COUJARD preparations vary a good deal according to different authors. On the ground of our own observations we feel compelled to define our standpoint in this most difficult question referring first to some summarizing papers by experienced and eminent authors in this field using the lightmicroscope and surveying such problems as the nature of the interstitial cells of CAJAL, the structure of what has been called the "peripheral autonomic neurencytium" and the way in which the peripheral sympathetic efferent stimulus is transmitted to the effector cells (HILLARP, 1946, 1959a; BOEKE, 1949; STÖHR, 1954, 1957; CLARA, 1955; MEYLING, 1955; JABONERO, 1952, 1952/53, 1954, 1955).

Many authors agree that the processes of the interstitial cells, supposed to be of a nervous nature, constitute a syncytial reticulum forming a "synaptic field" (BOEKE) which transmits the stimulus to the effector cells by means of neurohumors. In this connexion JABONERO speaks of a "plexiform synapse à distance" in which the interstitial cells functioning like neurosecretory cells would play an all-important functional role in producing chemical transmitters (also MEYLING). According to JABONERO this syncytium of interstitial cells is a special organ intercalated between the postganglionic nerve fibres and the non-nervous tissue. This conception includes that there would not be any synaptic relationship in the classical sense between this distal neurencytium consisting of anastomosing „neurofibrils" and the effector cells, this being true at least in most organs showing a sympathetic innervation. Following this author, the neurencytium is to be regarded as a closed terminal formation in which individual nerve endings do not occur. STÖHR is not quite convinced that the interstitial cells would indeed be nerve cells. According to him a second syncytial system, containing neurofibrils and constituted by the interstitial cells, is intercalated in the terminal part of the syncytial meshwork which, for that matter, consists of Schwann cells. STÖHR, like some other authors cited by him, is of the opinion that the interstitial cells are much more similar to lemnocytes than to nerve cells, many transitional forms occurring between Schwann cells and interstitial cells. Also according to HILLARP (1959a) the interstitial cells are but neurilemma cells.

The opinions concerning the transmission of the nervous stimulus to the effector cells of BOEKE, MEYLING and JABONERO have been mentioned above. STÖHR holds that extremely

thin neurofibrillary processes leave the preterminal reticulum, which is conformable to the ground plexus of BOEKE, forming a terminal reticulum which is ubiquitary, surrounding the cells of all tissues and penetrating into the plasm of the effector cells. In this way, continuity would be present between the plasm of his terminal reticulum and the plasm of the effector cells.

HILLARP (1946, 1959a) is of the opinion that the innervation of autonomic effector cells happens by means of a nervous ground plexus consisting of a plexus of terminal axon ramifications running in a finely-meshed network of anastomosing strands formed by the terminal Schwann plasmodium and superimposed on the effector cells. According to HILLARP, all of the effector cells would probably be in direct contact with this ground plexus. He was not able to prove the existence of any free either intercellular or protoplasmatic nerve endings and holds that the construction of the ground plexus indicates that it is a closed terminal formation. From his papers it is not clear whether any axons would end at all in this "closed" terminal formation and, if so, in which way. Following a personal communication of HILLARP to the present author he, however, admits that, according to his conception, there have to be individual endings of axons in the ground plexus. Furthermore, HILLARP holds that each axon within the plexus innervates in its course a certain number of cells which react as a functional unit, the "neuro-effector unit". One axon may supply one or more of such units. On the other hand, one neuro-effector unit would not be innervated by one axon only but by several, the terminal ramifications of these axons running within the same strands of the ground plexus converging to the neuro-effector unit. HILLARP emphatically refutes the arguments brought forward by BOEKE and his collaborators and by MEYLING in regard to the supposed nervous nature of the interstitial cells.

Before giving our own opinion regarding the terminal autonomic innervation three more points will be discussed first, *viz.* the results of electronmicroscopical investigations, the degeneration of the autonomic terminal network and our observation of pineal autonomic terminal structures.

The literature on electronmicroscopic investigations concerning the peripheral part of the autonomic system and its relation to the effector cells is not yet very extensive but of the greatest interest. We will deal only with a few papers here. RICHARDSON (1958), studying the small intestine of the rabbit, agrees that the existence of the ground plexus (BOEKE) or the preterminal reticulum (STÖHR) is a fact. Its structure, however, is somewhat different from that described by workers using the lightmicroscope. According to RICHARDSON the ground plexus consists of exactly the same components in miniature as large autonomic fibre bundles do, *i. e.* nucleated Schwann cells or a Schwann syncytium, investing groups of nerve fibres. The Schwann syncytium envelops bundles of true axons and not of neurofibrils. RICHARDSON still is of the opinion that interstitial cells and Schwann cells, in the ground plexus, are quite distinct. He, however, holds that the relationship between the interstitial cells and the autonomic ground plexus, examined with the electronmicroscope, does not support the theory that the interstitial cells form the final link between axons and muscles. Various details have led RICHARDSON to the interpretation that the interstitial cells would be connective tissue elements, probably fibroblasts, rather than primitive nerve cells as has been advocated by authors like BOEKE, MEYLING and JABONERO. From this citation it is evident that RICHARDSON's argument is in favour of those authors who do not agree that the interstitial cells would be of a nervous nature playing a role in the transmission of the sympathetic stimulus to the effector cells.

In the epiphysis, the present author did clearly observe interstitial cells strewn in between the fibres constituting the ground plexus. Especially in the series stained with the silver techniques it was obvious that some of these cells showed oval nuclei whereas the nuclei of other interstitial cells were fusiform resembling the nucleus of fibroblasts but also those of Schwann cells. Some authors, holding that the interstitial cells are but lemnocytes as has been mentioned before, were able to observe transitional forms between the nuclei of Schwann cells and those of interstitial cells. We also agree with the arguments brought forward by

HILLARP (1959a), contradicting those of BOEKE and his school which had been used as proofs that the interstitial cells would be of a nervous nature. According to our opinion, there is no reason whatever to accept the theory that the interstitial cells are primitive nerve cells. In series, stained by the CHAMPY-COUJARD technique, the interstitial cells are blackened just like the nerve fibres. This might speak for a difference existing between these cells on the one hand and lemnocytes as well as ordinary fibroblasts on the other. On the other hand, ELFVIN (1958, see below) observed in Schwann cells granular structures similar to those present in the adrenergic nerves with which these lemnocytes were associated. This might explain why these nerves as well as their lemnocytes (= interstitial cells ?) stain black using the technique of CHAMPY-COUJARD. Black-stained cells showing processes, moreover, were also observed in the sheath enveloping the nervi conarii which consist, as a matter of fact, of bundles of postganglionic fibres. Therefore, it is obvious that these latter cells cannot be interstitial cells and may be better classified among the lemnocytes or perhaps among the connective tissue elements. Moreover, the staining of these cells may the more prove that the osmic acid-sodium iodide mixture is rather unspecific, most certainly not exclusively staining diphenoles.

It seems somewhat difficult to decide whether the finest threads in the ground plexus, seen in silver, methylene blue and CHAMPY-COUJARD preparations using the lightmicroscope are either axons or "neurofibrils" as has been thought by many previous authors. Also on this point the electronmicroscope sheds the light which can not be given by the lightmicroscope.

From electronmicroscopic investigations it is well known that in the axons submicroscopic neurofilaments are present, which, in fixed preparations, may artificially form "neurofibrils" (FERNANDEZ-MORAN, 1950; CAESAR, EDWARDS and RUSKA, 1957; SCHULTZ, MAYNARD and PEASE, 1957; ELFVIN, 1958 and other authors). The question now rises whether the "neurofibrils" mentioned by previous investigators to be present in the ground plexus are either composed of single neurofilaments (JABONERO, 1953) or consist of individual axons (HILLARP, 1946, 1959a; SZENTÁGOTHAI, 1957; CAESAR, EDWARDS and RUSKA, 1957; RICHARDSON, 1958; CAESAR, 1959). The axons, in their terminal course, are often as thin as 200 Å as is pointed out by CAESAR, EDWARDS and RUSKA who suggest that axons of this size can be easily misinterpreted under the light microscope as "neurofibrils". The latter authors as well as CAESAR (1959) did observe that the terminal axons are suspended within cisterns of lemnocytes formed by the infolding plasma membrane of these cells. The cisterns communicate with the space between the plasma membrane and the basement membrane of the lemnocytes by way of the mesaxon which is known to consist of a duplicature of the plasma membrane. These relationships are well illustrated diagrammatically by ELFVIN (1958).

According to ELFVIN who investigated the splenic nerve, to CAESAR, EDWARDS and RUSKA and to CAESAR who dealt with the innervation of smooth muscle, the Schwann sheath of unmyelinated fibres is not to be regarded as a syncytium. This sheath consists of a series of individual lemnocytes in every one of which a number of axons is embedded. The authors mentioned do not want to commit theirselves to the problem whether there exist any specialized lemnocytes, *i. c.* the interstitial cells.

From the discussion in the paper of ELFVIN it appears that in nerves, containing noradrenaline, this substance may be attached to corpuscular or granular structures in these nerves. Similar granular structures were observed in the Schwann cells associated with these axons. It may well be that the reason why some lemnocytes or/and interstitial cells stain black in using the technique of CHAMPY-COUJARD is that they are catechol-containing just like the adrenergic fibres. This, however, is still mere speculation as it is not yet quite known which chemical substances stain with this technique. We will not deal here with the literature

concerning the catechol-amine containing structures, present in the medullary cells of the adrenal and related questions.

The present author is convinced that the results accumulating from electronmicroscopic research should be taken into account in interpreting the structure as well as the function of the sympathetic terminal formation. These results may and indeed have already changed profoundly the previous conceptions concerning the ground plexus obtained by lightmicroscopists.

We will now turn to the discussion of our observation that, after removal of both superior cervical ganglia, not only nerve bundles evidently containing postganglionic fibres degenerate but that likewise the interstitial ground plexus was seen disappearing after performing this operation. This latter observation is inconsistent with the view, held by authors like BOEKE, MEYLING and JABONERO, that the ground plexus is a structure formed by the interstitial cells of CAJAL and, as such, independent from the postganglionic fibres, a synaptic connexion being present between the postganglionic fibres and the interstitial network (JABONERO, MEYLING). If, however, the ground plexus is constituted by individual axons being the terminal parts of postganglionic fibres, enveloped by lemnocytes or, peripherally, by interstitial cells belonging to the same cell category as the lemnocytes do or being even similar to the latter, the present findings are not astonishing.

According to JABONERO (1953), at least in organs showing iuxta- and intramural ganglia, the postganglionic orthosympathetic nerve fibres show synaptic connexions with ganglion cells of type II of DOGIEL the processes of which would unite with the distal nervous "syncytium". Preganglionic parasympathetic fibres would end in the ganglia on nerve cells of type I of DOGIEL the processes of which would be likewise synaptically connected with the type II cells of DOGIEL. JABONERO holds that the terminal network is common to the ortho- and to the parasympathetic system. In this matter the opinion of MEYLING (1955) is very similar to JABONERO's. Following MEYLING the system of efferent pre- and postganglionic fibres is a synaptically constructed reflex apparatus, synaptically connected with the peripheral autonomic "network" being constituted by the nervous interstitial cells. This "network" would be neither exclusively ortho- nor parasympathetic but is played on by both counterparts of the autonomic nervous system. JABONERO agrees that his conception is more or less hypothetical and the same may be said of MEYLING's theory. Any objective demonstration of synaptic connexions between, for instance, postganglionic orthosympathetic fibres and the fibres in the strands of the ground plexus will be most difficult if not impossible by means of lightmicroscopical investigations. Till now such proofs of the conceptions of MEYLING and JABONERO are wanting.

From the disappearance of the pineal ground plexus after removal of both superior cervical ganglia, only a few single fibres having been left in some preparations, the conclusion can be drawn that the pineal organ, for the far-most part, is innervated by postganglionic fibres originating in these ganglia, the terminal interstitial strands consisting of the terminal parts of these postganglionic axons. This conception does not take into account the slender and merely theoretical chance that transsynaptic degeneration might possibly occur via synaptic connexions between postganglionic fibres and a nervous network constituted by the interstitial cells.

On the ground of the present observations as well as on that of the results obtained from electronmicroscopical examinations it is not felt that there is any need to accept the hypotheses of MEYLING and JABONERO.

Our conclusion is in accordance with the opinion of some lightmicroscopists like SZENTÁGOTHAI (1957) and HILLARP (1959a) as well as of the electronmicroscopists mentioned earlier in this paper. Moreover, the observation that degeneration of the ground plexus occurs after elimination of postganglionic fibres has been also reported by other investigators. Therefore, this conclusion is corroborated.

NELEMANS and DOGTEROM (1955), for instance, found rapid degeneration of the interstitial ground plexus in the iris of rats and in the rabbits ear after thorough ortho- as well as parasympathetic denervation. Thus, these authors were likewise unable to confirm the supposed independence of the "interstitial network" with respect to the ortho- and parasympathetic systems. SZENTÁGOTHAI (1957), in his comprehensive paper, observed degeneration of the terminal autonomic nervous strands in a number of organs, proving that these may contain ortho- as well as parasympathetic postganglionic fibres and sensory fibres as well. HILLARP (1959a) cites investigations following which the autonomic ground plexus degenerates in the submandibulary gland and in the pars intermedia of the pituitary after section of the innervating nerves. This same author mentions degeneration of fine varicose fibres in the endnet enclosing the smooth muscle layer of the small vessels in the iris after sympathectomy. These fibres disappeared completely although interstitial cells were faintly visible at some places.

Finally, we will turn to the discussion of the structures observed in the epiphysis in the rat which, in our opinion, are probably autonomic efferent endings. The following gives a short historical survey on nerve endings in the pineal organ and glands in general.

Terminals on pinealocytes have probably been first mentioned by CAJAL (1904) who observed them in mice. According to him they are of autonomic origin. FAVARO (1904) found similar structures in rodents, doubting, however, whether they were endings of autonomic fibres. Following WALTER (1913), CAJAL also demonstrated arborizations, ending with terminal knobs, in the epiphysis of the rabbit, cat and dog. BOEKE (1934), in his beautiful study on the innervation of glands, described intracellular that is intraplasmatic endings of "neurofibrils" extending from the ground plexus. From his more recent publications (*e. g.* 1949), however, it does not clearly appear whether he is true to this previous opinion defending a closed terminal formation of the ground plexus and the conception of chemical transmission of stimuli by the ground plexus to the effector cells. According to CHAMPY, COUJARD and COUJARD-CHAMPY (1945/46), the innervation of the salivary glands is mainly sympathetic and adrenergetic. These authors describe an interacinous ground plexus from which extremely fine branches penetrate between the cells of the acini probably likewise forming networks which show tiny endings. These terminals do not have an intraplasmatic position, though this is suggested by some sections, ending on the surface of the cells. The findings of BOEKE and of CHAMPY and collaborators obtained in glands are very similar to ours in the pineal organ in the rat this being the reason why we mention them here. It is of importance that CHAMPY and his co-workers hold that the ground plexus, apart from exerting a secretory function, would have at the same time a trophic influence (see also COUJARD 1955). This opinion is related to the question whether denervation of the epiphysis would stop secretion of the pineal cells. This problem will, however, not be dealt with in the present paper.

In the epiphysis of many mammals, MAZZUCCHELLI (1950) was able to observe ovoidal, spherical, lobiform and fusiform endings of probably autonomic origin showing relations with the vessels as well as with the pinealocytes. He suggests that at least some of these terminals may be sensory, especially those "quale le grosse capocchie". This would prove the theory mentioned before that the epiphysis is a regulatory organ, at least according to MAZZUCCHELLI. This author, however, is most careful in regard to the interpretation of the endings observed, saying that they could possibly be the result of degenerative and regenerative phenomena. GARDNER (1953) found nerve terminals in the epiphysis of the hooded rat interpreting them as endings of nerve fibres derived from the commissures. Earlier in this paper it has been shown that this opinion is rather improbable. Some few annular autonomic nerve endings were seen by HARTMANN (1957) in the pineal organ of cattle.

As has been mentioned before, the epiphysis of the albino rat was found to contain a very extensive autonomic interstitial network showing, for the most part, an interfollicular position. From this network extremely fine single fibres or small bundels of few fibres were seen branching and penetrating between the cells constituting the follicles. So far, our results are in good accordance with those of BOEKE and of CHAMPY and coworkers investigating the terminal innervation of glands. Intrafollicular interstitial cells could not be observed. This agrees with the description of CHAMPY and collaborators describing and illustrating interstitial cells exclusively in the interacinous nervous strands.

Although some thin fibres in the terminal strands seem to show small endknobs we hesitate to accept their actual existence. Most probably, the cut end of fibres will have simulated these endings. Neither intraplasmatic fibres nor endings of such fibres could be clearly demonstrated in the pinealocytes. The results of recent electronmicroscopical investigations, moreover, are incompatible with the concept, held by some previous authors, that continuity would exist between nervous plasm and the plasm of the effector cells.

On the other hand, the rather large structures seen in the series stained according to the technique of CHAMPY-COUJARD, described and already discussed in the previous chapter (Fig. 40), certainly show the appearance and characteristics of nerve terminals. The fact that they stain black using the osmic acid-sodium iodide mixture suggests that they are not of a sensory nature but most probably autonomic efferent terminals ending on pinealocytes. Most certainly, they are not related to the fibres of commissural origin and confusion with equally blackened interstitial cells or with beads of autonomic fibres is out of the question. Till now, we have not been able to demonstrate these endings beyond doubt in silver preparations.

The possibility of their demonstration and their differing size and shape may be related to a varying functional state of these terminal structures. GABRIELESCO and BORDEIANU (1959), for instance, observed that the terminal parts of autonomic fibres as well as their terminal arborizations in the wall of the uterus of rabbits react on physiological stimulation of the organ by hormones or drugs. Using vital staining with methylen blue, they showed a. o. an accentuation of the varicose disposition of the fibres and a hypertrophy of their terminal endings presenting an increased stainability.

According to the description of MAZZUCCHELLI (1950), the pineal endings observed by this author are very similar to those seen in the epiphysis of the albino rat. They show also some resemblance to the large endings of autonomic fibres mentioned and illustrated by STÖHR (1957; Figures 78, 269 and 270) in the adrenal medulla which are, however, synaptic endings of preganglionic fibres on nerve cells. After removal of both superior cervical ganglia the endings in the epiphysis of the rat disappeared as the terminal autonomic innervation did. That, in the albino rat, a practically total denervation of the epiphysis can be obtained after performing this operation strongly suggests that the autonomic network in the pineal organ is exclusively of orthosympathetic origin no parasympathetic fibres being involved.

Summarizing, the discussion of the descriptive results obtained by examination of the autonomic innervation of the epiphysis in the albino rat, of the degeneration of the terminal innervation after sympathectomy and of the results of recent electronmicroscopical investigations leads to the conclusion that the interstitial

terminal fibre strands are constituted by the axons of postganglionic fibres originating in the superior cervical ganglia. These fibres are embedded in lemnocytes and, especially in their peripheral terminal parts, by interstitial cells which, most probably, belong to the same category of cells as the lemnocytes do. Apart from the rather large endings described, terminal endings of the axons could not be demonstrated. This is not astonishing as these endings will be so small that they are submicroscopic and exclusively demonstrable using the electron-microscope. A closed terminal network is not conceivable regarding the results of electronmicroscopical investigations. This conception does not exclude the possibility that the stimulus, finally reaching the effector cells, is transmitted by one or more neurohumors, produced in the terminal parts of the post-ganglionic axon ramifications constituting the terminal bundles.

Summary

In this paper, a description is given of the development of the epiphysis cerebri of the albino rat and of some structures showing topographical relations with this organ. Furthermore, the innervation of the pineal body is examined in normal adult rats as well as in a number of specimens in which both superior cervical ganglia were removed. The main conclusions can be summarized as follows.

1. The anlage of the epiphysis first appears on the 14th day of embryonic development.

2. The epiphysis develops by follicular proliferation of the wall of the epiphyseal evagination. In the rat, especially the rostro-dorsal part of this wall is involved in the process.

3. Leptomeningeal mesenchyme containing vessels grows in between the pineal follicles. The adult organ shows a dense vascular network. It is a compact structure consisting of cellnests the follicular structure of which can be still distinguished at many places.

4. In embryos, aged 14 days, fibres could be clearly demonstrated in the caudal commissure. The first habenular commissural fibres were shown in embryos of the 16th day.

5. During development the true pineal recess of the third ventricle disappears by fusing of the epiphyseal peduncles. In the adult, a small recess bordered by the intercalary lamina is sometimes present between the habenular and the caudal commissure. This recess of the third ventricle has been termed intercommissural recess.

6. During early postnatal development the pineal body gradually shifts in a caudal and somewhat dorsal direction. In the adult brain it shows a superficial position just in front of the cerebellum between the caudal collicles. Its lateral parts are sometimes covered by the caudal poles of the cerebral hemispheres.

7. The epiphysis is enveloped by a sheath of leptomeningeal origin. The floor of the confluens sinuum covers the dorsal surface of the organ.

8. The pineal body keeps its connexion with the commissural region by means of a thin and slender avascular epiphyseal stalk consisting of pinealocytes, pinealoblasts and fibrocytes. This stalk can be divided topographically in three parts. Dorsal to it either two veins, vena prosencephali mediana and vena cerebri magna, are present or a single large vena cerebri magna. These veins

discharge into the confluens sinuum. The vena prosencephali mediana may fuse either phylogenetically or ontogenetically with the vena cerebri magna originally present. This, then, results in the formation of a single large vena cerebri magna draining, a. o., the choroid plexuses of the third as well as those of both lateral ventricles and the basal veins.

9. In the epiphyseal stalk nerve fibres could be constantly demonstrated. Most of the fibres derive from the habenular commissure the contribution of fibres from the caudal commissure being only small. The nerve bundle in the epiphyseal stalk has been termed commissuro-epiphyseal tract.

10. The fibres of the commissuro-epiphyseal tract are aberrant commissural fibres. They are of no functional significance for the innervation of the pinealocytes. They may leave the stalk or the rostral part of the epiphysis passing into the surrounding leptomeningeal tissue. Other fibres, after having entered the pineal organ, perform loops of 180^0 returning to the commissural region in the epiphyseal stalk. In one case an ending of a commissuro-epiphyseal fibre was seen. This, however, was most probably not a functional terminal. Very rarely, single commissuro-epiphyseal fibres were seen running as far as the caudal part of the organ.

11. The epiphysis shows an extensive autonomic innervation which is principally supplied by two nervi conarii, bilaterally present. These nerve fascicles course in the tentorium cerebelli penetrating into the epiphysis underneath the floor of the confluens sinuum.

12. After removal of both superior cervical ganglia the fibres of the nervi conarii degenerate evidently being postganglionic fibres originating in these ganglia.

13. The terminal autonomic pineal innervation happens by means of interfollicular strands of fibres from which thin single fibres or very small fibre bundles were seen branching and penetrating the follicles or cellnests of pinealocytes, thus running intrafollicularly.

14. Structures being most probably autonomic motor terminals have been observed. They are exclusively related to the pinealocytes, not to the vascular walls.

15. Among the interfollicular fibre strands interstitial cells have been observed. The present author is inclined to share the opinion of investigators holding that these cells are not of a nervous nature but belong to the same category of cells as the lemnocytes do.

16. After removal of both superior cervical ganglia the terminal intrapineal autonomic innervation was seen to disappear completely. In very few preparations only some rare fibres were left. Evidently, pineal innervation is mainly, if not exclusively, orthosympathetic.

17. On the ground of the observations mentioned as well as of the results of recent electronmicroscopical investigations it has been concluded that the inter- as well as the intrafollicular fibres and fibre strands consist of the thin terminal ramifications of postganglionic nerve fibres originating in the superior cervical ganglia. Proof of the existence of a sensory innervation of the epiphysis has not been found so far. The opinion of some authors, holding that, in general, the terminal autonomic "neurofibrillar network" would be formed by the interstitial

cells presumably being synaptically connected with postganglionic fibres is discussed and considered improbable.

18. In the rat, so far, neither nervous nor vascular relations have been observed pointing to the existence of an „epithalamo-epiphyseal-" or „habenulo-epiphyseal complex" that, in any way, could be compared with the hypothalamo-hypophyseal complex.

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