Thus the mixed culture of *Azotobacter* and *Pseudomonas* gave an increase of 35 milligrammes of nitrogen on the limed soil, and an increase of 25 milligrammes of nitrogen on the unlimed soil.

This gain of nitrogen was not due to any material present in the culture solution, for the autoclaved culture solution shows a gain of 6 milligrammes of nitrogen only, derived chiefly from the dead bacteria in the solution.

Taking an acre of soil 4 inches deep as weighing about 1,000,000 lbs., a gain of 35 milligrammes of nitrogen per 100 grammes would represent an increase of nearly 350 lbs. of nitrogen per acre.

That the nitrogen fixed by this mixed culture of bacteria in the soil is readily assimilated by plants is shown by a number of experiments now in progress, full details of which will be described in a future communication.

On the Structure, Development, and Morphological Interpretation of the Pineal Organs and Adjacent Parts of the Brain in the Tuatara (Sphenodon punctatus).

By Arthur Dendy, D.Sc., F.R.S., Sec. L.S., Professor of Zoology in King's College (University of London).

(Received June 22,—Read June 30, 1910.)

(Abstract.)

The memoir of which an abstract is here given contains a detailed account of the pineal organs and associated parts of the brain in Sphenodon, from the morphological, histological, and embryological points of view, accompanied by numerous illustrations, and may be regarded as a continuation and amplification of my earlier work on the subject.

The material upon which my results are based consisted partly of a number of adult living Tuataras presented to me by the New Zealand Government, the cost of transmission of which to England was defrayed by a grant from the Government Grant Committee, and partly of specimens (chiefly embryos) preserved by myself while in New Zealand. I defer the expression of my thanks to the numerous friends who have helped me in the work until the publication of the complete memoir.

As I have already pointed out in my work on the intracranial vascular system,* there is in Sphenodon a very extensive subdural cavity between the brain and the cranial wall, and advantage was taken of this fact to fix the

* 'Phil. Trans.,' B, 1909.

delicate organs of the pineal complex *in situ* by the injection into the cranial cavity of acetic bichromate of potash. After fixation the pineal eye itself, with the parietal plug, can be dissected clean out of the parietal foramen, and the necessity of decalcification before section cutting thus avoided.

The "pineal complex" is formed chiefly by the dorsal sac, the paraphysis and the pineal sac ("epiphysis" or right pineal organ), united in a common pial investment and forming a bilaterally flattened, funnel-shaped structure attached above to the cranial roof by the *dura mater* and below to the optic thalami and habenular ganglia.

Across the subdural space numerous fine threads of connective tissue extend from the surface of the brain to the *dura mater*, and these are concentrated to form imperfectly developed vertical supporting membranes for the pineal complex, one placed transversely on either side of the dorsal sac and one lying in the sagittal plane behind the pineal sac. The very large subdural space arises late in development, which perhaps indicates that the relatively small size of the brain is a cænogenetic character due to arrested growth.

The fundamental relations of the different parts of the fore-brain and its derivates in Sphenodon are already to a large extent familiar to us, but the following points may be noticed:—

Gisi has described the lateral choroid plexuses (*plexus hemisphærium*) as arising from a transverse fold behind the paraphysial opening, while Elliot Smith has described them as arising from a transverse fold in front of the paraphysial opening. The explanation of this discrepancy is that while they really arise one on either side of the paraphysial opening their roots of attachment may extend a little in front of and behind the latter. There is no unpaired *plexus medianus* as described by Gisi.

The paraphysis, as I have previously shown, is part of the same system of folds of the epithelial *lamina supraneuroporica* which gives rise to the *plexus hemisphærium*, but growing outwards instead of inwards. It originally opens into the prosencephalon immediately in front of the *commissura aberrans.* In an advanced stage of development, however, a longitudinal supra-commissural canal is formed above the *commissura aberrans*, and this leads to the formation of a new opening for the paraphysis in the adult, directly into the dorsal sac at some distance above the commissure. The original opening of the paraphysis, in front of the *commissura aberrans*, is blocked up by the growth of the anterior choroidal veins and arteries.

There is no true *commissura mollis* as described by Gisi, though the lateral walls of the third ventricle come into contact with one another over a considerable area.

Both in advanced embryos and in the adult animal three pairs of lateral diverticula, in addition to the cerebral hemispheres, the optic lobes, and the pineal outgrowths, open into the central canal of the fore- and mid-brain. These are, from in front backwards, (1) the *recessus optici laterales*, which appear to be remnants of the cavities of the stalks of the optic vesicles; (2) a pair for which I propose the name *recessus thalami prenucleares*, because they lie in the substance of the optic thalami in front of the *nuclei rotundi*; (3) the *recessus geniculi* (of Gisi), which lie on either side of the entrance to the *iter*, beneath the posterior commissure. It is suggested that these three pairs of diverticula may be serially homologous with one another and with the cerebral hemispheres, the outgrowths which form the pineal sense-organs and the optic lobes, and that each of these pairs of outgrowths indicates an original neuromere. In accordance with this view the cerebral hemispheres would belong to the first neuromere of the fore-brain, the optic vesicles of the lateral eyes to the second, the *recessus thalami prenucleares* to the third, and the pineal outgrowths to the fourth, while the *recessus geniculi* would belong to the first, and the optic lobes to the second neuromere of the mid-brain.

The middle portion of the pineal complex is formed by the thin-walled dorsal sac, the roof of which gives rise to a well-developed choroid plexus supplied by branches of the saccular arteries. The folds of this choroid plexus are covered with an epithelium composed of polygonal cells with well-defined boundaries and conspicuous nuclei. Attached to this epithelial layer, and lying in the cavity of the dorsal sac between the folds of the choroid plexus, is a cytoplasmic network containing numerous nuclei and apparently composed of extrusive connective-tissue cells. The choroid plexuses of the fourth and lateral ventricles are practically identical in histological structure with that of the dorsal sac.

The paraphysis grows upwards immediately in front of the dorsal sac, and its upper end turns backwards over the roof of the latter. It must be regarded as a compound tubular gland. Its walls become greatly folded, and in the adult we find a central lumen surrounded by numerous crypts and opening into the dorsal sac. Between the crypts numerous blood spaces develop, which sometimes form a regular network of thin-walled sinuses or capillaries. These are supplied with blood by paraphysial branches of the saccular and anterior choroidal arteries, and drain into the *sinus longitudinalis* beneath the pineal sac.

The paraphysis is invested by *pia mater*, which attaches it firmly to the dorsal sac. Its epithelial lining has a very characteristic histological structure, consisting of a single layer of cells without distinct boundaries, and connected

together by radiating threads of cytoplasm to form a syncytium. In connection with this epithelium there is a very conspicuous but irregular network of nucleated cytoplasm lying in the paraphysial lumina. The nuclei in this network are very poor in chromatin and undergo amitotic division. Sometimes little rounded knobs, covered with the syncytial epithelium, project from the wall of the paraphysis into its various cavities.

I have already described the origin of the two pineal organs from the brain-roof, and how, from its first appearance, the one which is destined to give rise to the pineal eye usually lies a little to the left of the other, which will give rise to the pineal sac. I am now able to confirm Schauinsland's subsequent observation that these two vesicles are at first in open communication with one another, but I do not consider that this need prevent us from regarding them as members of an originally symmetrical pair, and fresh evidence in favour of this view is put forward in the present memoir.

The opening of the pineal sac into the third ventricle, between the superior and posterior commissures, closes up at a comparatively early date, but vestiges of the connection remain in the "infra-pineal recess" and in the "pineal tract" by which the pineal sac of the adult remains connected with the brain-roof. The pineal sac grows upwards in close contact with the posterior wall of the dorsal sac, to which it is firmly attached by the *pia mater*, and its upper end turns forwards over the roof of the dorsal sac and over the upper part of the paraphysis. In the adult it is a relatively large organ and takes an important part in the formation of the pineal complex. It remains tubular, but its walls become greatly folded and much thickened. They are supplied with blood by the anterior and posterior pineal arteries and drain into the *sinus longitudinalis*. There is little or no evidence that the pineal sac is a glandular body, but, on the contrary, its histological structure points to a sensory function. Its thick wall is made up of nucleated radial supporting fibres, numerous ganglion-cells and nerve-fibres, and numerous sense-cells whose inner ends project slightly into the lumen of the organ. These constituents are identical with those which occur in the retina of the pineal eye, and their arrangement is essentially the same. In one case, in which the tip of the pineal sac projected unusually far forwards, so as to come under the influence of the light passing through the transparent parietal plug, a pigmented evagination of the wall of the pineal sac was formed, and the resemblance to the retina of the pineal eye became still more obvious. These observations, confirming and extending earlier observations by Hoffmann, Gisi, and myself, greatly strengthen the view that the pineal sac and pineal eye are bilaterally homologous structures.

The pineal sac is provided with a well-developed nerve, composed of non-

medullated fibres, which runs in the "pineal tract" and joins the brain-roof in the middle line between the superior and posterior commissures, which remain perfectly distinct throughout life.

The histological structure of the pineal eye itself has been investigated with especial care, and various methods of fixation and staining have been employed for the purpose. The sharp distinction between lens and retina appears at a very early date, and though they remain in contact with one another throughout life, the actual connection between the two is henceforth very slight, and the transition from the one to the other is perfectly abrupt.

At a very early stage the development of the nerve-fibres divides the retina into a thick inner and a thin outer layer, with the nerve-fibre layer between them. The inner layer contains many nuclei belonging to sense-cells, and also nuclei which belong to ganglion-cells. The outer layer contains only a single layer of nuclei, belonging to the radial supporting fibres. Later on the ganglion-cells come to lie more to the outside of the nerve-fibre layer, next to the nuclei of the radial fibres.

In the adult retina, omitting for the moment the pigment, we find only three kinds of histological elements: (1) radial supporting fibres, (2) ganglion-cells and nerve-fibres, (3) sense-cells.

The radial supporting fibres are comparable to the Müller's fibres in the lateral eyes, and probably extend right through from surface to surface of the retina, their inner ends forming the well-developed internal limiting membrane, and their outer ends abutting against the inner capsule of the eye. Their nuclei appear to be all lodged in their outer portions, which have the misleading appearance of a layer of short conical cells.

The ganglion-cells are numerous, and are readily distinguished by their large spherical nuclei, finely granular cytoplasm (with usually one large projection), and the shrinkage cavity which surrounds them.

The sense-cells are slender, elongatedly spindle-shaped, with large oval nuclei. Their outer ends run into the layer of nerve-fibres. Their inner ends project slightly into the cavity of the eye, but are covered with little conical caps, formed apparently by extension of the internal limiting membrane.

In most respects the structure of the retina agrees closely with that of Anguis and Lacerta as recently described by Nowikoff. That author, however, gives a somewhat different account of the projecting ends of the sense-cells and of the distribution of the pigment.

Baldwin Spencer considered that in Sphenodon the pigment was especially associated with the sense-cells; Nowikoff, on the other hand, maintains that

in Anguis and Lacerta the pigment is lodged in the radial supporting fibres. According to my own observations on Sphenodon, the pigment granules lie between the various constituents of the retina, and are brought in from outside the eye by wandering pigment cells. Such cells are abundant in the connective tissue around the eye, between the inner and outer capsules, and sometimes they also occur in the form of pigment-balls in the cavity of the eye itself, having apparently passed through the retina without breaking up and discharging their contents. Usually, however, they appear to break up in the outer part of the retina, and the granules which they contain stream in in radial lines and streaks between the radial fibres and sense-cells, to such an extent as greatly to obscure the histological structure of the retina. The wandering pigment-cells may possibly obtain their pigment granules from the very large stellately branched pigment cells which lie in the *dura mater* outside the capsule of the pineal eye.

The pigment is especially abundant towards the margins of the retinal cup, near its junction with the lens, and here accessory cavities are not infrequently developed in the retina, each surrounded by radiating streaks of pigment granules. The lens contains only occasionally a very few pigment granules. The vitreous body also usually contains very little if any pigment, but occasionally a good deal.

At stage R, when the pigment first appears, it is found only in very minute granules, chiefly, if not entirely, in the inner part of the retina. In the adult much coarser granules appear, though the small ones can still be recognised in the innermost part of the retina.

Perhaps the most novel results obtained are those which concern the lens of the pineal eye, which is shown to be a glandular organ, secreting part, at any rate, of the vitreous body. At a very early stage in development we can recognise two zones in the lens, an outer or marginal zone, in which the cells remain undifferentiated and continue to divide actively by mitosis; and a central portion in which the cells become greatly elongated at right angles to the two surfaces of the lens, which thereby becomes greatly thickened in the middle. Growth of the lens is probably effected mainly by the marginal zone of actively dividing cells, but it is not impossible that the cells may continue to divide after elongation. The distinction between the central and marginal zones of cells persists to a very late stage in development, though possibly not in the adult.

In the adult the arrangement of the elongated cells becomes far less uniform, and they are irregularly curved so as to appear cut through in various directions in vertical sections. They probably extend right through from surface to surface of the lens, but their inner ends are somewhat

specially differentiated, and project as small rounded protuberances into the cavity of the eye. The nuclei are situated at various levels, and the cytoplasm of the inner portions of the cells is very distinctly fibrillated in a longitudinal direction, while darkly staining bodies resembling centrosomes can sometimes be seen close to the inner extremities of the cells.

In the adult lens, about the middle, one usually, if not always, finds one or more irregular masses of a finely granular, deeply staining substance. It was the observation of a large mass of this kind, with a centrally placed nucleus, which led to my description of a "central cell" in the lens, and it was chiefly with a view to further investigation of this remarkable structure that this research was undertaken. I now find that such central masses are very constant features of the adult lens, and their true nature was indicated by the fortunate occurrence of an adult specimen in which such a mass was actually being extruded in the form of "mucus" into the cavity of the eye to take part in the formation of the vitreous body. I have observed this extrusion of "mucus" into the cavity of the eye in several cases, and as early as stage R. With the "mucus," nuclei may pass out from the lens, and there can be no doubt that the secretion is formed by degeneration of cells in the middle of the lens. The extrusion always appears to take place from the middle of the lower surface of the lens at a very definite spot, but an actual aperture is probably present only at the time when the secretion is being poured out.

The vitreous body always contains, in preparations, a reticulum of slender fibres or thin lamellæ, and some of these are attached, on the one hand, to the inner surface of the lens, and, on the other, to the inner surface of the retina, apparently in many cases to the projecting ends of the sense-cells, but probably really to the caps which cover these. Whether the presence of this reticulum is due to *post-mortem* changes or not remains an open question.

A large amount of time has been devoted to following out the course of the nerve of the pineal eye, and I have been able to demonstrate very clearly that it is not a median structure, but belongs to the left side of the body—another striking piece of evidence in favour of the paired origin of the pineal organs.

In the adult animal the anterior end of the nerve, like the eye itself, has been shifted into the middle line. For the greater part of its course, however, it lies between the wall of the pineal sac and the wall of the dorsal sac, and considerably to the left side of the middle line. It is very easy to follow it from the eye towards the brain up to a certain point, where it breaks up into a number of separate strands. This point lies between the posterior

wall of the dorsal sac and the anterior wall of the pineal sac, not far from the lower extremity of the latter. Up to this point it consists of a well-defined bundle of non-medullated nerve-fibres, with a definite sheath of connective tissue in its more anterior portion, and with numerous elongated nuclei lying between the nerve-fibres. It exactly resembles an ordinary non-medullated nerve, and I can see no reason for regarding it as exhibiting degeneration. The separate strands into which it breaks up at the point mentioned, however, do not contain the characteristic elongated nuclei, which doubtless really belong to associated connective-tissue or nutrient cells, and owing to the slenderness of these strands, and the difficulty of distinguishing them from the connective-tissue fibres of the *pia mater*, I have not succeeded in following them continuously to the brain in the adult animal.

In several series of sections of embryos of different ages, however, the nerve has been traced to the brain as one continuous bundle of fibres without difficulty, and it is quite clear that it enters the left habenular ganglion. It becomes closely attached to the roof of the dorsal sac, however, before it reaches the habenular ganglion—or the spot where this will be developed—and this fact probably explains why the lower part of the nerve is broken up into separate strands in the adult, for the rapid growth of the thin wall of the dorsal sac may be supposed to cause the spreading out of the nerve-fibres over its surface.

Nerve-fibres first appear in the retina of the pineal eye while the latter is still resting upon the brain-roof, and I have come to the conclusion that they grow from the retina to the brain as in the case of the lateral eye.

A curious feature of the nerve of the pineal eye in the adult animal is that it receives bundles of nerve-fibres from the wall of the pineal sac as well as from the eye itself. This point has already been noted by Gisi.

The left habenular ganglion in the adult is produced upwards to meet the wall of the dorsal sac in a characteristic manner at a point where it receives nerve-fibres from the latter in special abundance. The right habenular ganglion also receives fibres from the wall of the dorsal sac, but is not produced upwards to the same extent as the left one. This asymmetrical development of the habenular ganglia further supports the conclusion that the left habenular ganglion is especially associated with the pineal eye.

It is extremely difficult to form any conclusion as to how far the pineal eye of Sphenodon still functions as a light-percipient organ. Such experiments as have hitherto been made have yielded entirely negative results. The concentration of a bright light upon the skin above the pineal eye

elicits, so far as I have been able to make out, no response; but then it must be remembered that the animals are extremely sluggish, and a similar experiment with the lateral eye may be continued for some time without producing any visible effect beyond the contraction of the iris.

Structurally, the only sign of degeneration which the pineal eye exhibits is to be found in the very large amount of pigment present in it in the adult, for I do not think we need regard the degeneration of the central lens-cells into the mucus which helps to form the vitreous body as of any significance in this respect.

Eigenmann has shown that a great deal of pigment is developed in association with the degenerating lateral eyes of the blind fishes, Lucifuga and Amblyopsis, but the degeneration of the pineal eye of Sphenodon does not approach in degree that of the lateral eyes of these types, and there seems no reason why it should not still function as a light-percipient organ. The formation of images by the lens is, of course, out of the question, on account of the irregular arrangement of the small scales which overlie the parietal foramen. I find from direct experiment, however, that light can pass through the integument at this point, and also through the more or less transparent parietal plug which covers the pineal eye in the foramen.

Reissner's fibre and the sub-commissural organ ("ependymal groove") are well developed in Sphenodon, and appear to have the usual relations. The latter has the form of a deep groove, lined by the characteristic greatly elongated columnar epithelial cells, and extending forwards from near the hinder end of the posterior commissure, beneath the latter, to the infra-pineal recess. Reissner's fibre is already conspicuous at stage S.