Histological and Morphological Studies of the Endocrine Glands of Xenopus laevis.

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HISTOLOGICAL AND MORPHOLOGICAL STUDIES OF THE ENDOCRINE ORGANS OF XENOPUS LAEVIS.

General Introduction.

Xenopus Laevis has provided, during the last ten years, the basis of a growing body of physiological enquiries initiated by Jolly's researches on reflex action. Its viability in the laboratory and amenability to operative procedure in particular, make it a peculiarly suitable object for investigation. It is regrettable, therefore, that existing literature on the anatomy of Xenopus has been directed to elucidating those characteristics which are of especial interest to the Systematists and Morphologists rather than detailed information of a type which is essential to operative procedure. There is in particular no extant account of the endocrine system of Xenopus, although it is evident from superficial inspection that the suprarenal complex differs from that of the more familiar Anura. The present enquiry concerns the Thyroid Gland, Pituitary and Epiphyseal Complexes with some observations concerning the possible occurrence of chromaphil cells in the kidney of Xenopus laevis. The data have been placed on record specifically with a view to facilitating the localization and constitution of these organs in physiological operations.

The Pituitary Gland.

De Beer in the preface of his monograph on the Pituitary Body holds that the Pituitary being a complex organ any attempt to deal with it experimentally, either by preparing extracts or by excision, must be preceded by a knowledge of the morphology of the gland. He adds that "Since the glands of the different classes of vertebrates are dissimilar in form and to a certain extent in function also, its comparative anatomy might be expected to throw light on its comparative physiology" This view has been borne out in recent work on the pituitary of Xenopus Laevis , - the S.A. Clawed Toad. Hogben and Slome in the course of some experiments on the effects of excision and injection of extracts of the pituitary, encountered discrepancies between Rana and Xenopus. The operative methods were identical in both cases, as was the technique of making up the extracts and the process of injection. In these circumstances it was suggested by Professor Hogben that a thorough investigation of the morphology and histology of the organ in Xenopus should be undertaken.

The pituitary gland, although found in every class of vertebrates varies greatly in form. It consists typically of three glandular components, the pars anterior, pars intermedia and the pars tuberalis, derived in ontogeny from the hypophysis, together with the pars nervosa which is formed by a ventral down growth (infundibulum) of the floor of the embryonic forebrain. The pars anterior occurs in Elasmobranch fishes as a single layer of epithelium thrown into loose folds, and only a few cells in thickness. In other vertebrate groups these folds become more and more compressed until in mammals no trace of the epithelial structure is apparent except in the course of the development of the embryo.

The pars intermedia on the other hand seems to lose in importance as far as size is concerned, as the vertebrate series is ascended. It is separated from the pars anterior by the Hypophysial cavity persisting as a permanent cleft. In Selachians or Elasmobranch fishes the pars intermedia is by far the more predominant glandular portion of the pituitary body. It is forked and more conspicuous than the pars anterior. In mammals it is present as a well-defined and still conspicuous structure, but in Birds it is not possible to distinguish it as a separate Morphological structure. The pars nervosa is said to be absent in Selachians. While it is true that the Selachian pituitary lacks a pars nervosa set apart and bounded in a specific manner, there is a definite region composed of neuroglia fibres, which extends from the border of the infundibular cavity and penetrates the pars intermedia.

The pars tuberalis has only been recognised for the last few years as a separate element in the pituitary complex - chiefly due to the work of Tilney. In Selachians and Cyclostomes it has not so far been admitted to be present, although in these classes the part of the pituitary which at present remains under the ambiguous title of Ubergangsteil shows definite traces of its relationship histologically at any rate, to the pars tuberalis of the higher vertebrates. In Amphibia it begins to assume a more prominent individuality, and in mammals it lies like a collar round the Infundibular stalk - which acts as the connecting link between the pars nervosa and the brain. In mammals and birds the pars tuberalis is an important structure arising in development from the pars anterior and eventually becoming separated from it. It comes to lie in the vicinity of the tuber cinereum. In birds the collar-like tuberalis is even more conspicuous than in mammals.

The salient features of the comparative histology of the pituitary gland are as follows:-

The pars anterior is highly vascular and is made up of both oxyphil and basophil cells. In Selachians where the pars anterior consists of a few simple folds of epithelium, the oxyphil cells are few in number, and are regularly arranged along one surface. This surface is bordered by blood vessels, and is furthest removed from the hypophysial cavity. The Basophil cells lie banked up behind the oxyphil cells, and give rise to and replenish the stock of oxyphil cells from time to time. In the higher vertebrate groups where the corrugated nature of the pars anterior has entirely disappeared there is an apparently indiscriminate mixing of the oxyphil and basophil cells, but it may be noted that the matured oxyphil cells usually bordered a blood vessel or vesicle (text figure 15). Any secretion or secretions formed by these cells are provided with an obvious means of egress to the other parts of the body.

The cells of the pars intermedia are considerably smaller than those of the pars anterior, and are homogeneous in appearance. They contain granules which take up a basic stain, and often form ~~in~~ distinct groups, having a small vesicle containing colloid in the centre of the group. Herring first described these vesicles as well as certain other masses or globules of colloid unsurrounded by a definite boundary, and containing traces of a nucleus somewhere in the mass. It was thought that the colloid material probably made its way through the pars nervosa into the cerebrospinal fluid, the feeble vascularity of the pars intermedia offering inadequate portage for the colloid. More recent research, however, tends to show that these cysts and vesicles are found principally in aged animals, and that the cysts show obvious signs of decay, e.g. the 'trace' of nucleus.

When Burn and Dale (1922) tested the material found in these cysts they found it to be inactive and while that in itself cannot be taken as conclusive evidence that the material does not in actual fact represent the active secretion or secretions (if any) of the pars intermedia, still it is an undoubtedly significant indication that the cysts are nothing more than degeneration phenomena, a feature which will be taken into detailed consideration later in this thesis.

In any account of the comparative histology of the pars intermedia mention must be made of the structure known as the Cone of Wulzen. This structure so far has only been recognised in the ox and the pig. It is a cone-like portion of the pars intermedia projecting into the hypophysial cavity. Its length varies considerably in different specimens. In one ox it may be only just visible to the naked eye while in another it may be 7 or 8 mm. in length. Although it arises in the pars intermedia it differs from it greatly in histological structure, and is indeed in many respects more like the pars anterior. Its basophil cells are coarser than those found generally throughout the pars intermedia, and moreover, it contains oxyphil cells. The cone is much better supplied with blood vessels than the rest of the pars intermedia, and a query in this connection immediately presents itself.

The oxyphil cells of the pars anterior are recruited from the basophil cells behind them - the oxyphil cells lie next to blood sinuses and blood vessels; therefore it might be permissible to say that basophil cells coming into contact with richly oxygenated blood undergo a change in chemical composition, rendering them insensitive to basic dyes. In this connection it would be valuable to know whether the Autqcoid or Autqcoids (if any) secreted by the pars intermedia differ in any respect from those of the cone (if any).

Grafting of the anterior lobe, pars intermedia and pars tuberalis has met with but indifferent success. Portions of the lobe have been inplanted in various situations (under the skin, bone marrow, kidney, spleen and brain) but the grafts do not survive long, and soon disappear altogether, a fact which might be correlated with the nature of the basophil cells - an argument which will be discussed later in this paper.

The pars nervosa consists principally of neuroglia cells, glia cells and ependyma cells. The area is more vascular than that of the pars intermedia, and extracts prepared from it give more positive results than extracts prepared from the pars intermedia (Hogben and de Beer, 1925). There is a concentration of blood vessels in the pars nervosa on the border line of the pars intermedia. These vessels may form the channel by which the secretion of the pars intermedia is carried off, and in such circumstances it is obvious why extracts prepared from the pars nervosa are more powerful than those prepared from the pars intermedia.

The difficulty of separating the pars intermedia from the pars nervosa in the majority of animals is great in the extreme, since the boundaries of the two are irregular in outline and the cells of the pars intermedia frequently penetrate into the pars nervosa, where they can be recognized in various stages of activity and degeneration. The pars tuberalis is composed entirely of coarse basophilar cells arranged in definite pattern around spaces of appreciable dimensions. The region is vascular.

The existing experimental data on the localisation of active substances in the pituitary gland may be summarised as follows:-

In the anterior lobe (pars anterior) there are stated to be:

1. A specific Autacoid which evokes metamorphosis in Amphibia (Smith 1921 and Hogben 1921), this Autacoid being present in the pituitary gland of Amphibia and Mammals.

2. A growth promoting Autacoid (Smith, Evans & Long 1925) present in the Amphibian and Mammalian pituitary gland.

3. A specific Autacoid which induces ripening of the Graafian follicles (Zondek and Ascheim 1927; Parkes 1929 and Bellarby 1929). present in the Mammalian Pituitary gland - also in the Amphibian (Hogben 1930).

In the 'posterior lobe' (The pars intermedia plus the pars nervosa) of the Mammalian pituitary gland at least three Autacoids are present:-

1. A Pressor Autacoid (Schäfer 1896)
2. An Oxytocic Autacoid (Dale 1909)
3. A Melanophore-stimulant Autacoid (Hogben 1921)

2 and 3 are present in all vertebrates, and 1 in all land vertebrata and bony fishes, although it is doubtful if it is present in cartilaginous fishes.

In the Mammalian pituitary gland from which most extracts have been prepared, the anterior lobe is separate from the posterior lobe. Both are histologically complex and how far the diversity of specific autacoids in the pituitary gland can be associated with specific histological elements, is still largely a matter of conjecture.

The pars anterior, pars intermedia, pars nervosa and pars tuberalis, are now all recognised as separate entities in Anura, Chelonia and Mammala. It is tempting to say in birds also, but there the presence of the pars intermedia is still open to doubt. There is no pars tuberalis in Squamata. It's condition in Urodeles will be referred to at a later stage.

Anatomy of the Pituitary Gland in Xenopus Laevis

In Xenopus laevis, the S.A. Clawed Toad - the condition of the pituitary gland is not identical with that which has been described in frogs and toads.

The pars anterior is conspicuous and well developed. It is pinkish white in colour, and roughly ovoid in contour (text fig 1 macroscopic drawing of the pituitary in situ) as in the frog or toad. There is, however, a quite evident macroscopic difference between the anterior lobe of Xenopus and that of other Anura which have been described up to date. The anterior lobe of the Frog can be easily detached from the rest of the complex by a fine jet of fluid or very gentle lateral pressure with a needle in operative manipulations. When the attempt is made to detach the anterior lobe alone in Xenopus, it is found that it obstinately clings to the stalk by a tapering process of the anterior margin, and this process is different in hue and texture from the remainder of the anterior lobe. This process is a grayish trilobed structure (see text figure 1) the out-curving parts of which are let into complémentary grooves on the ventral and posterior portion of the tuber cinereum. It will be seen at a later stage, when the significance of the pars tuberalis will be taken into consideration, that this portion of the anterior process of the anterior lobe is histologically different from the remainder, and its topographical relations with the tuber cinereum as well as its characteristic histological peculiarities are brought into prominence in sections which are cut in the plane tangential to the ventral surface of the gland. (See the accompanying diagrams of the Tangential series of sections).

Behind the pars anterior and towards its anterior end, the pars intermedia and pars nervosa together form a compact dumbbell shaped posterior lobe with the constriction in the median plane. The lateral margin of the posterior lobe extends considerably beyond that of the anterior lobe. In shape the posterior lobe of Xenopus is exactly like that of other Anura. On this account it appears to be much smaller than the anterior lobe in median longitudinal sections, and much larger than the anterior lobe in transverse sections. One noteworthy difference which is not insignificant in operative technique distinguishes it from that of Rana or Bufo. The cleft between the posterior and anterior lobes is much less sharply defined. So that the separation into constituent lobes offers some difficulty. Macroscopically it was not found possible to identify anything corresponding to the two plaques of tissue lying on either side of the tuber cinereum in Anura.

Histology.

The pituitary glands of Xenopus which were sectioned were fixed in Bouin, Carnoy or acetic alcohol. The latter proved the most satisfactory fixative of the three. It was found that fifteen minutes fixation in acetic alcohol was sufficient, and that the best results were obtained if the whole process of fixation, dehydration, clearing, embedding etc. was done in one day. The stains used for most of the sections were Alkaline Methylene Blue and Orange G. Three minutes suffice in a saturated solution of Alkaline Methylene Blue. The sections are then washed in running water and carefully differentiated under the microscope with Orange G.

The major portion of the pars anterior of Xenopus is made up of oxyphil cells, arranged to a large extent around blood vessels and sinuses. Basophil cells are also present but are much less numerous. They are found, as is the usual condition, intercalated among the oxyphil cells. The nuclei of the two types of cells do not differ very markedly and the size and contour of the cells is very similar. The cells of the pars intermedia are principally deeply staining basophil in type with a few oxyphil cells scattered among them. The pars nervosa shows cells of the usual neuroglia and glia type. The area appears to be almost non-vascular.

In none of the points which have been mentioned does the histological structure of the gland differ from that of Rana and Bufo. The most noteworthy feature in the histology of the gland and one which can be readily seen in a complete series of median longitudinal, transverse and tangential sections is the definite histological differentiation of the process continued forward on to the stalk from the anterior margin of the anterior lobe. The histological differentiation of this region can be quite clearly discerned in the accompanying photomicrographs, one of which is a median longitudinal section through the gland. The other ~~being~~ is a tangential section through the region over the grooved distal extremity of the tuber cinereum.

It has been mentioned that this region seems to be peculiar to Xenopus and that in Xenopus it was not possible to identify by macroscopic examination anything corresponding precisely to the paired plaques of glandular tissue which are identified as the pars tuberalis of Rana and Bufo. On the other hand it has been shown by Atwell that the latter are absent in Urodela and further that in Urodeles the anterior lobe is continued forward over the tuber cinereum as a pair of processes composed of basophil cells which are not separated from the pars anterior sensu stricto. This continuation of the anterior lobe over the tuber cinereum in Urodeles has been identified as the pars tuberalis by Atwell and de Beer. A study of transverse sections failed to reveal the characteristic tuberalis elements of other Anura in the gland of Xenopus. Several complete sets of series of transverse sections were studied. On the other hand it reinforced the suspicion that the condition of the pars tuberalis in Xenopus is essentially analogous to that which exists in the Salamanders. The process by which the anterior lobe extends into the grooved distal extremity of the tuber cinereum is composed of deeply staining basophil cells exclusively . It is richly supplied with blood vessels and contains no oxyphil cells. It has a lobulated structure which can be distinctly seen in the photomicrograph in the longtudinal sections of Xenopus. The deeply staining basophil cells are arranged around vesicles of appreciable dimensions, the colonies of basophil cells are surrounded by connective tissue through which blood vessels run.

It thus seems justifiable to conclude that this portion represents the pars tuberalis and that the pituitary gland of Xenopus laevis while in its superficial anatomy it is closely similar to that of other Anura, essentially conforms to that of the Salamandrine type in the incomplete separation of the pars tuberalis from the pars anterior. If this conclusion is justified its importance in operative procedure, and in the analysis of the physiological activity of the component lobes of the pituitary gland is of some significance. The extirpation of the pituitary gland in other Anura involves the removal of the pars intermedia, pars nervosa and pars anterior alone, hitherto carried out on adult animals. In Xenopus laevis the operation of hypophysectomy would appear to involve in addition the removal of the pars tuberalis, since this is still part of the anterior lobe according to the view stated above. It might, however, be expected that any discrepancies in the post operative consequences of hypophysectomy in Xenopus, Rana or Bufo, would throw light on the fundamental significance of the pars tuberalis. The characteristic features of the pituitary gland in Xenopus laevis are shewn ~~on~~ IN the accompanying Figures, the major portion of which are drawings of lantern projections from the slides. Text figure 1 is a macroscopic diagram drawn to scale and representing the pituitary gland of Xenopus laevis in situ. O.C. represents the optic chiasma; T.C. the tuber cinereum and P.A. the pars anterior. Figure 2 is a diagrammatic longitudinal reconstruction of Figure 1.

Text Figure 3 indicates the levels at which sections A.B. and C. were cut (A.B. &. C.) being represented by text figures 4, 5, and 6.

Figure 7 is drawn from a tangential section while 8 represents a reconstruction of 7.

Text figures 9 and 10 are drawn from a complete longitudinal series of sections which were fixed in Carnoy and stained with Alkaline Methylene Blue and Orange G. The photomicrograph of No. 9 is found in text figure 13. Figures 11 and 12 are drawn from complete series of transverse and tangential sections respectively. Figure 14 is the photomicrograph of tangential section No. 2 (in text figure 12).

Series 11 and 12 are self-explanatory. Section 1 being the most ventral in position and gradually proceeding backwards until in section 6 the maximal dimensions of the pars anterior are attained. No. 9 is the last section containing any trace of the pituitary gland.

In the transverse series the order is from the posterior to the anterior. Section 10 representing the most anterior section. In this section the region of the pars anterior, pars intermedia, pars nervosa and pars tuberalis have already been passed through and only the tuber cinereum remains.

The difference between the staining power of the cells of the pars tuberalis, and pars anterior is sharply brought out in the accompanying photomicrograph of tangential section No. 2 while in text figure 13 the lobulate nature of the pars tuberalis is represented.

In considering the essential nature of the basophil and oxyphil cells, mention must be made of the "functional difference theory" discussed in de Beer's monograph on the pituitary body (1926). The theory maintains that the basophil and eosinophil (oxyphil) appearance of the cells found in the pars anterior and pars intermedia, is not due to the cells being of two different types and structure but is to be accounted for on the ground that one type of cell exists in two functional stages. It is held that the oxyphil cells are responsible for the active secretions of the pars anterior and further that after the oxyphil cells have poured out their secretions they become basophil, a stage which is interpreted as a "rejuvenation phase", during which the cell elaborates more secretion and returns to the oxyphil condition. In which case, of course, intermediate stages ought to be demonstrable in almost every vertebrate. In actual fact they have only been found in the pars anterior of the Rabbit. Here an otherwise basophil cell has a red border, which may be quite pronounced. This border is known as the eosinophil ring of Stewart (1922).

Against the functional difference theory the condition existing in the pars anterior of the ox is cited. There is in the pars anterior of this animal an area entirely composed of basophil cells, oxyphils being absent. However, it is not essential to assume that every basophil cell inevitably gives rise to an oxyphil cell, and moreover, in support of an alternative view (mentioned on page 9) of the essential nature of the basophil and oxyphil cells, the condition found in the pars anterior of the ox may be cited since the basophil area is almost entirely devoid of blood vessels.

An objection to the functional difference theory mentioned in the paragraph describing the eosinophil ring of Stewart, was the failure to discover intermediate stages between basophil and oxyphil cells. In the ox and cat which have been examined in great detail for such phenomena, no transitional stages were found, and if such a transition does take place it must be extremely rapid, but this objection arises from what is probably a wrong conception of the functional nature of the cells.

If the assumption advanced in this paper is made, the difficulties elaborated above tend to disappear. Firstly, the basophil cells are considered as the original or parent cells and certainly they arise first in the development of the pituitary gland. Since ~~the~~ in Cyclostomes and

Selachians the pars anterior consists almost entirely of basophil cells, and oxyphil cells occur only on the surface bordering the blood vessels.

In Selachians no oxyphil cells are found scattered throughout the general expanse of the pars anterior. As the vertebrate series is ascended and the folds of the pars anterior with the blood vessels lying on their surface sink inwards - oxyphil cells become scattered throughout the region, although invariably arranged around a blood vessel or sinus. In the basophil cell can be seen numerous deeply stained granules. When the basophil cell comes to lie near a blood vessel or richly oxygenated area it is easy to conceive that the chemical compounds forming these granules are oxidized, and render the cell incapable of taking on a basic stain. They present then the typical appearance of oxyphil cells. The products of these cells are taken up by the blood corpuscles, and carried away. The oxyphil cells immediately bordering the vessel then "die" making room for fresh recruits from the basophils lying behind.

It is perhaps worthy of note that during oestrus in animals basophil cells become more abundant in the pars anterior and pars tuberalis.

The fact that the pars tuberalis is highly vascular and that it contains only basophil cells might at first sight seem to present an insuperable obstacle to the argument, but it is first necessary to consider the nature of the Golgi apparatus of these cells and their peculiar arrangement. It is held (Reiss 1922) that the Golgi apparatus is always found on the side of the nucleus which corresponds with the pole of discharge of a secreting cell. In the basophil cell the Golgi apparatus is said to be on the side directed towards the blood vessels, but according to de Beer (Dev. of Pit. Body page 18) the Golgi apparatus appears to differ according to the position of the cell. Those cells which are situate round a small vesicle with little or no connective tissue lining have small separate dot-like elements at the edge of the cell where it borders on the vesicle. The other cells have compact dense masses close to the nucleus. Around the aggregations of basophil cells lies the connective tissue and through which the blood vessels run. The spaces separating one colony of basophil cells from another are in some instances very pronounced - this is more often the case in the region of a blood vessel. So that even although the pars tuberalis is highly vascular and the cells are basophil in type the cells are in reality protected from direct contact with blood vessels by an adequate amount of connective tissue. It has been stated (Reiss 1922) that the Golgi apparatus of basophil cells was on the side directed towards blood vessels, and in oxyphil cells it was on the opposite side. That this is not a feature of essential difference between the cells seems to be indicated by the fact that both conditions exist in the basophil cells of the pars tuberalis.

In 1922 Hogben and Winton found that removal of the whole pituitary, i.e. pars anterior, pars intermedia and pars nervosa in Rana, produces permanent maximal pallor. This has since been shown to be true of Xenopus by Slome and Hogben. Hogben and Winton did not find that removal of the anterior lobe alone had any effect on the chromatic function of Rana. More recently Slome and Hogben have proved that two endocrine factors located in different parts of the pituitary gland are involved in the pigmentary effector activity of Xenopus laevis. They have also shown that removal of the anterior lobe produces permanent darkening. But previous work by Hogben and Winton could not substantiate the latter conclusion in Rana. If it is correct to conclude that the pituitary of Xenopus is of the Salamandrine type the difference between the behaviour of Rana and Xenopus is easily explicable on the assumption that the "W" or second Melanophore principle of Slome and Hogben is located in the pars tuberalis.

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XENOPUS LAEVIS.

PITUITARY GLAND.

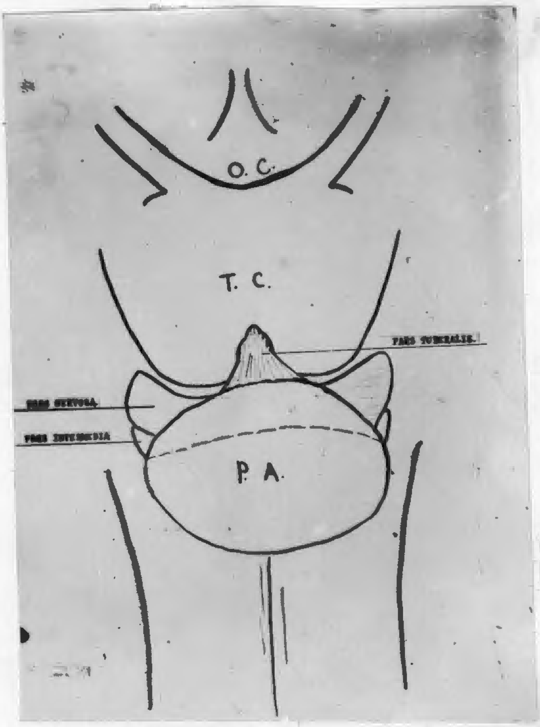


FIG. 1.

MACROSCOPIC DRAWING OF PITUITARY IN SITU.

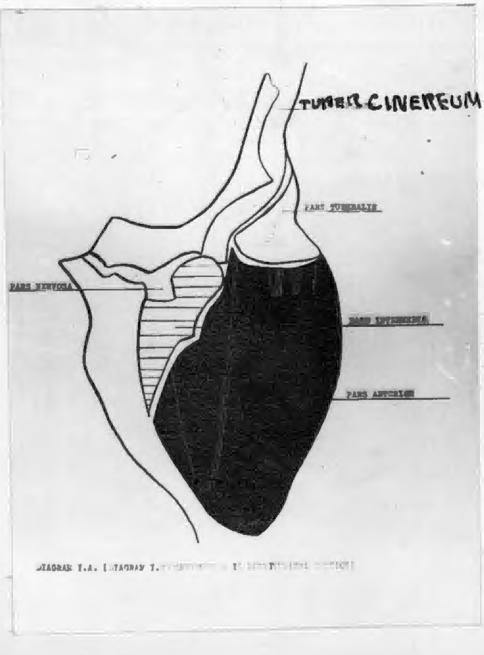


FIG. 2.

LONGITUDINAL RECONSTRUCTION OF FIG. 1.

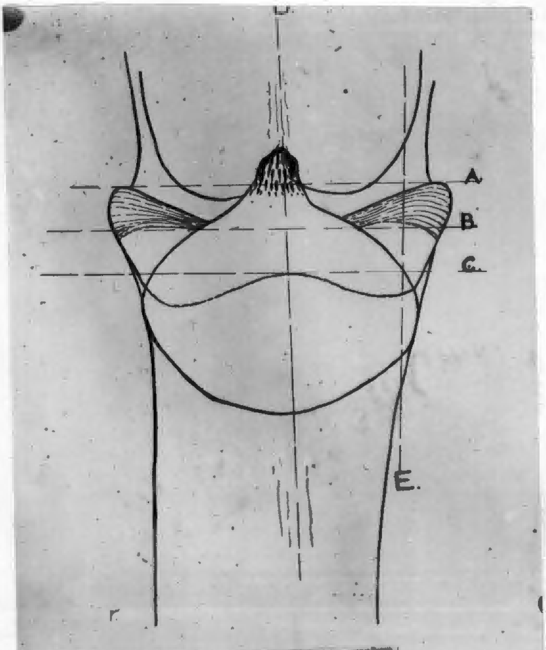


DIAGRAM SHOWING LEVELS AT WHICH A.B.C.D.AND E. WERE CUT.

FIG. 3.

LEVELS AT WHICH A. B. + C. WERE CUT.

DIAGRAM 3. TRANSVERSE SECTION THROUGH LEVEL A.

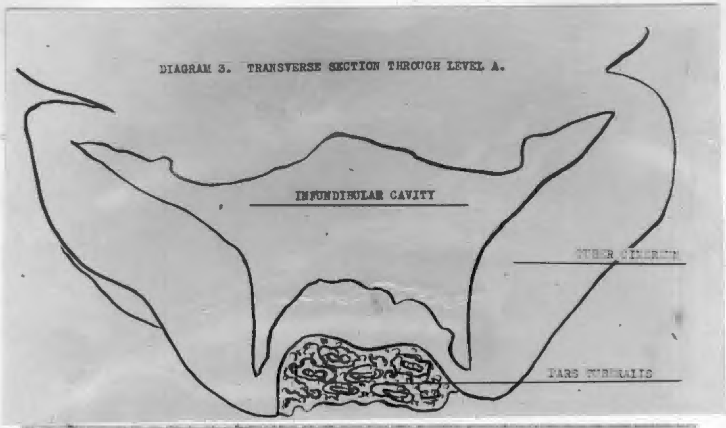
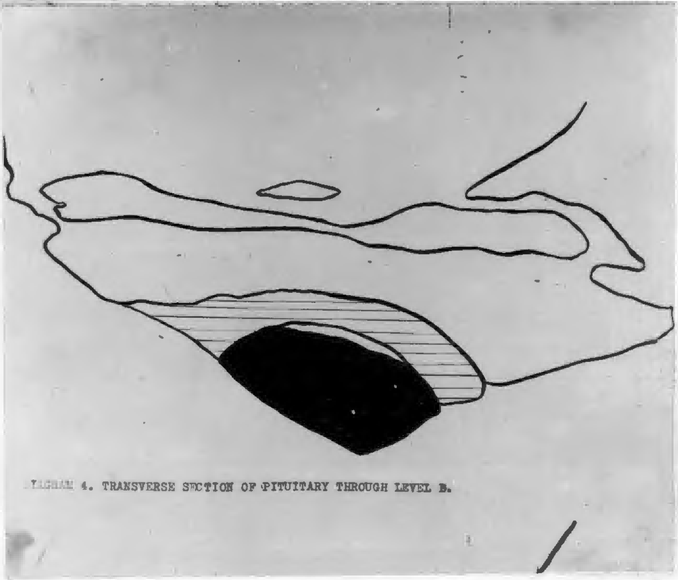


FIG.4.

LEVEL A.

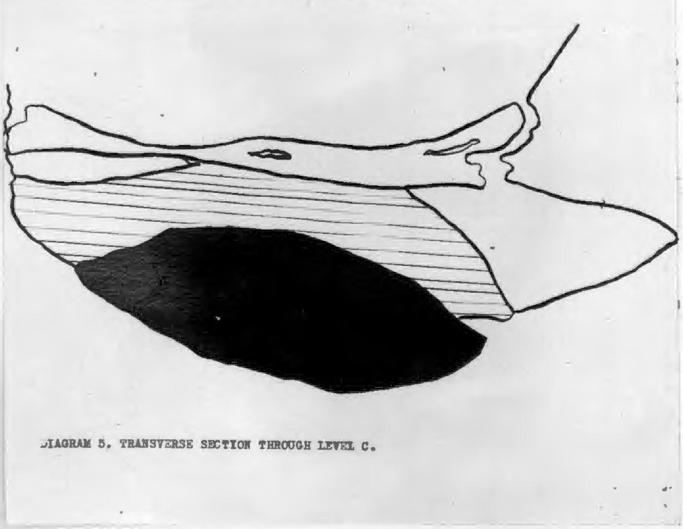


IAGRAM 4. TRANSVERSE SECTION OF PITUITARY THROUGH LEVEL B.

FIG.5.

LEVEL.B.

IAGRAM 4. TRANSVERSE SECTION OF PITUITARY THROUGH LEVEL B.



IAGRAM 5. TRANSVERSE SECTION THROUGH LEVEL C.

FIG.6.

LEVEL,C.

TANGENTIAL SECTION.

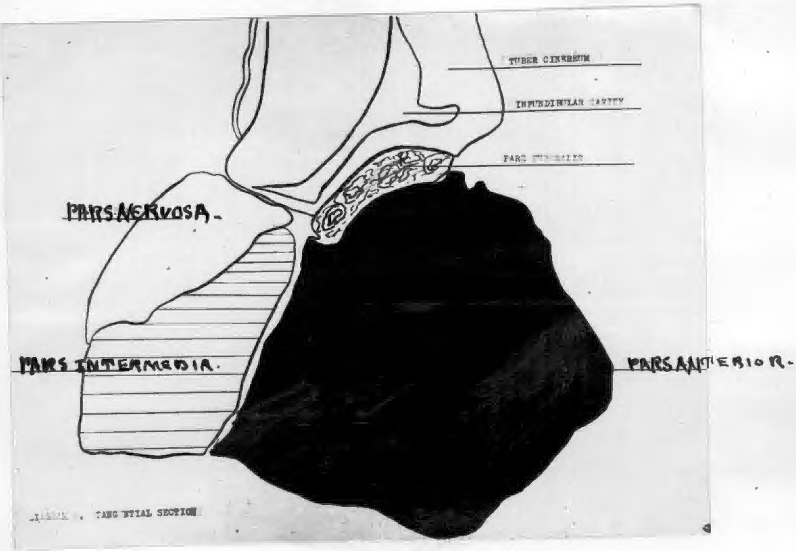


FIG. 7.

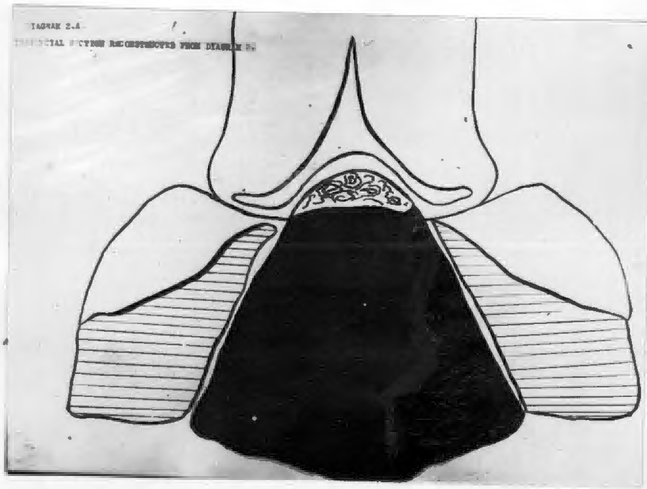


FIG. 8.

RECONSTRUCTED FROM
FIG. 7.

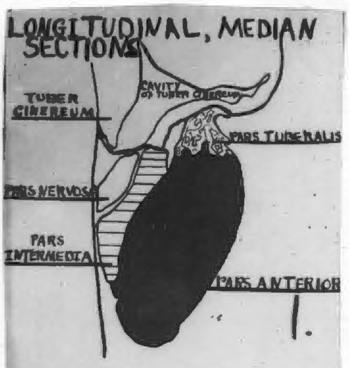

LONGITUDINAL, MEDIAN SECTIONS
CAVITY OF TUBER CINEREUM
TUBER CINEREUM
PARS TUBERALIS
PARS NERVOSA
PARS INTERMEDIA
PARS ANTERIOR
1.

FIG. 9.


TUBER CINEREUM.
2.

FIG. 10.

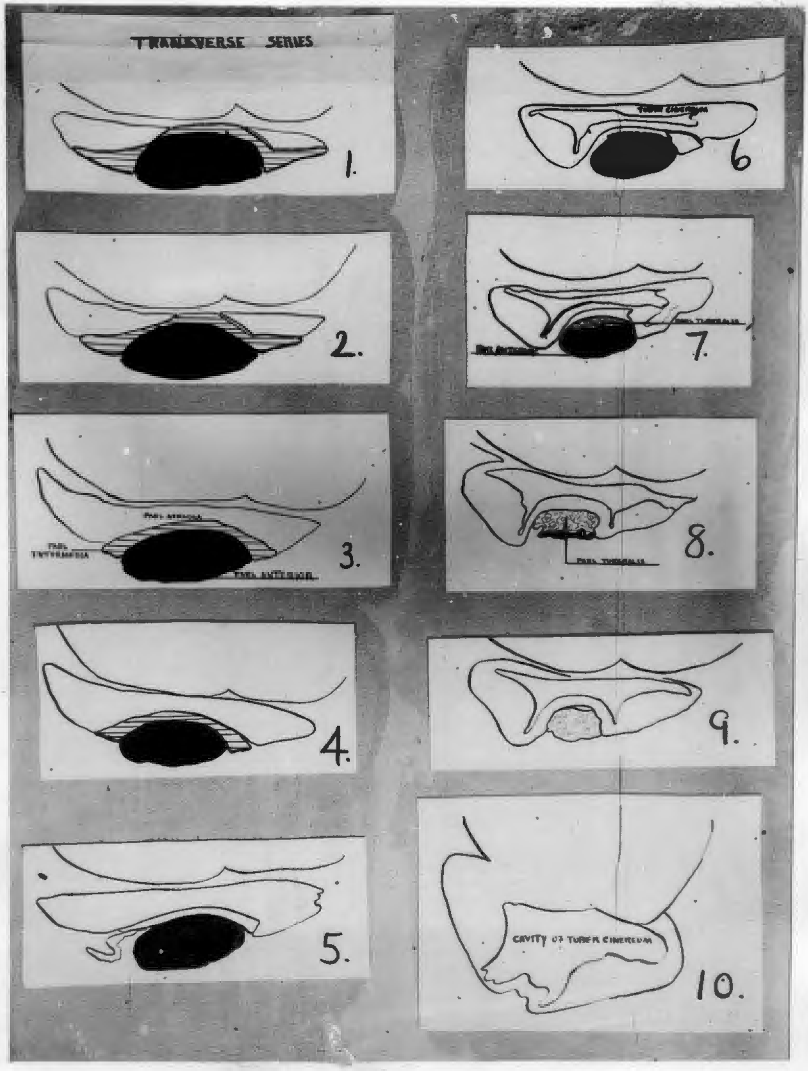

TRANSVERSE SERIES
1.
2.
PARS NERVOSA
PARS INTERMEDIA
PARS ANTERIOR
3.
4.
5.
TUBER CINEREUM
6
PARS TUBERALIS
PARS ANTERIOR
7.
PARS TUBERALIS
8.
9.
CAVITY OF TUBER CINEREUM
10.

FIG. 11.

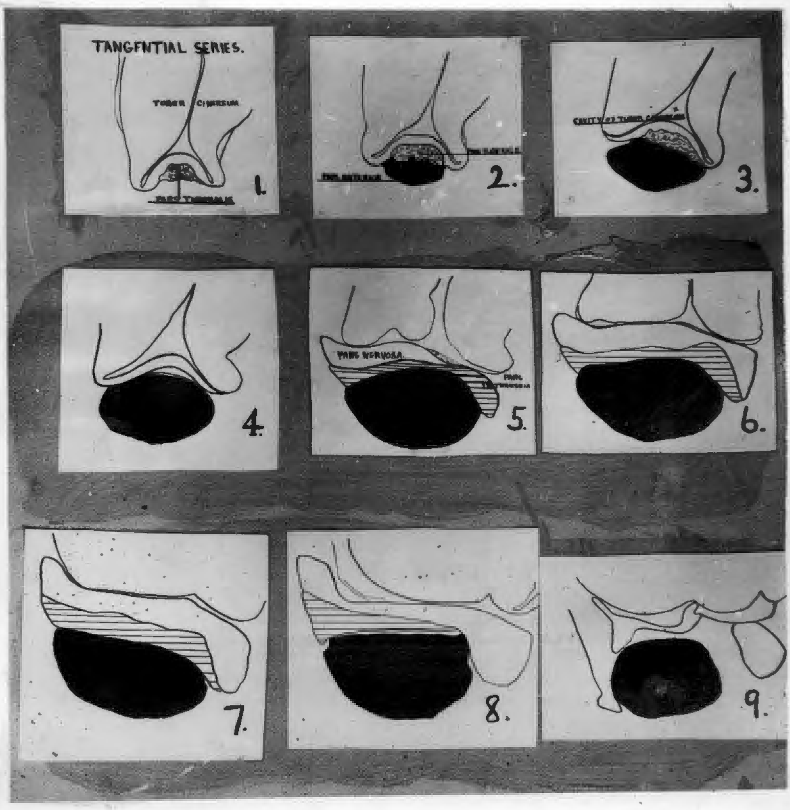

TANGENTIAL SERIES.
TUBER CINEREUM
PARS TUBERALIS
1.
2.
CAVITY OF TUBER CINEREUM
3.
4.
PARS NERVOSA.
PARS INTERMEDIA
5.
6.
7.
8.
9.

FIG. 12.

PHOTOMICROGRAPHS.

PITUITARY GLAND OF XENOPUS LAEVIS.

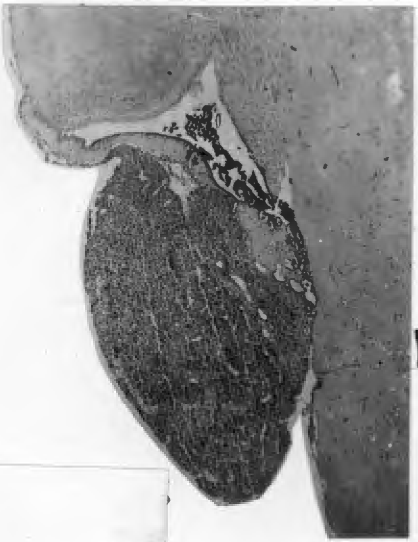


FIG. 13

MEDIAN LONGITUDINAL SECTION.

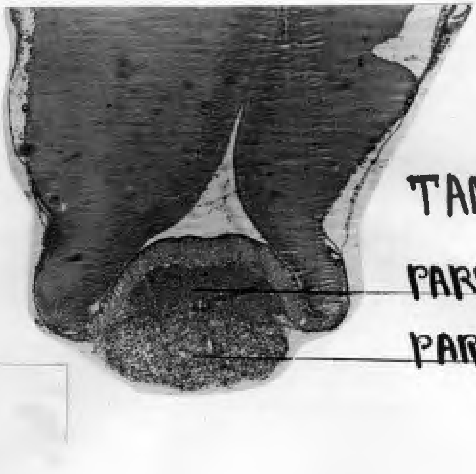


FIG. 14.
TANGENTIAL SECTION

PARS TUBERALIS.

PARS ANTERIOR.

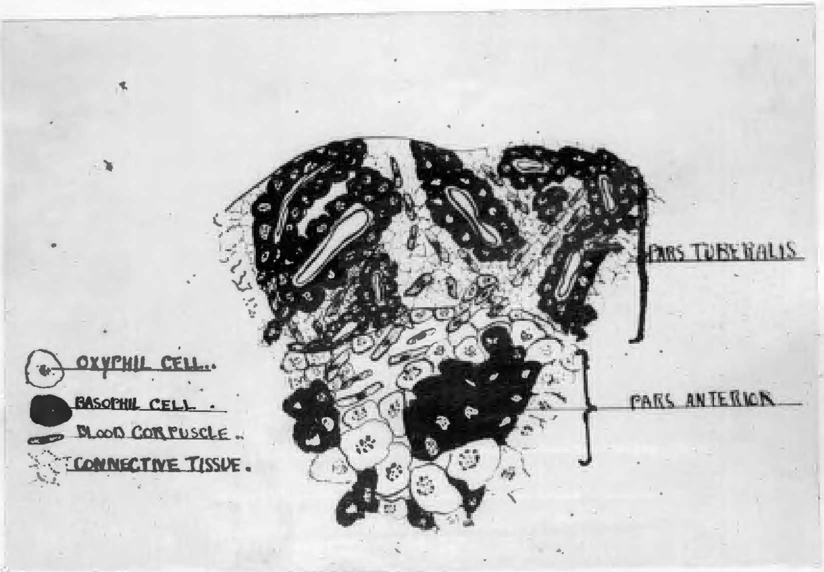

OXYPHIL CELL.
BASOPHIL CELL.
BLOOD CORPUSCLE.
CONNECTIVE TISSUE.
PARS TUBERALIS
PARS ANTERIOR

FIG.15.

THE THYROID GLAND IN XENOPUS LAEVIS.

The identification of the Thyroid Gland in Xenopus is somewhat difficult to determine by reference to its position in Rana owing to differences in the musculature and vascular supply of the Sternohyoid region. To locate it, therefore, with precision it will be necessary to give an account of the muscular anatomy of the surrounding parts.

It will be found convenient to describe the structures encountered in exposing the gland in the order in which they emerge in the course of dissection.

After deflecting the skin of the belly forwards to the head, the muscles in text Fig. 2. were exposed. These are the Sub-maxillaris (1) and its hyoid origin (1A)., The four pectoralis muscles and a portion of the longitudinal fibres of the rectus abdominis muscle. Muscle 1A receives the title of Sub-hyoideus from Grobbelaar (1923) while muscle 1 + 1A is styled the Mylohyoid by C. von Bonde. Ecker, Wiedershem and other writers in general refer to muscle 1 + 1A as the muscle submaxillaris. It is undesirable to adopt the same title for this muscle as for the muscle which forms the floor of the mouth in man, and which goes by the name of Mylohyoideus.

The submaxillaris has two sources of origin (1) from the whole inner surface of the upper border of the lower jaw (except for a short distance near the angle), and (2) by a small portion from the anterior cornu of the hyoid cartilege. The two portions of the muscle unite to form a thin layer, the fibres of which run transversely and meet in a band of connective tissue along the mid-line. Slightly in front of its posterior border the muscle is attached along its whole breadth to the skin of the throat by a delicate layer of connective tissue. A fascia attached to the posterior border of the muscle passes downwards to the deeper muscles, and is connected to them.

In text figure 3, the submaxillaris muscle has been cut through along the midline from the anterior boundary of the head to the anterior boundary of the sternum (or pectoral girdle) and each half deflected. The sternum has likewise been cut through and one half deflected. The muscles so exposed are the Geniohyoid and Sternohyoid. There is in addition a small stout muscle underlying the Submaxillaris and labelled Muscle "Y" in text fig. 3.

The Geniohyoid is a paired muscle arising from the lower jaw on either side of the midline. In Rana the places of origin of this muscle are covered by the Submentalis Muscle of which no trace could be discovered in Xenopus. (The Submentalis muscle is described as being present in Xenopus by Grobbelaar, but not indicated as being present by Von Bonde). The Geniohyoid muscle is a long flat muscle divided posteriorly into two portions. The median portion runs down over the place of origin of the Sternohyoideus muscle, and then keeping on the inner margin of the Sternohyoideus passes down the surface of the hyoid plate, and is inserted just above where the lungs enter the cavity of the hyoid plate. In Rana the median portion of the Geniohyoid is inserted into the inner border of the posterior cornu of the hyoi plate, a position considerably anterior to the situation of the glottis (Text Fig. 8). In Xenopus the insertion of the median portion of the Geniohyoid is posterior to the position of the glottis. (Text. Fig. 6).

The lateral portion of the Geniohyoid runs to the outer margin of the Sternohyoid and passing beneath it is inserted on the rounded latero-posterior portion of the hyoid plate. There being no secondary posterior cornu present in Xenopus as there is in Rana, and in which, in the latter, the muscle is inserted.

The sternohyoideus muscle is the cervical portion of the rectus abdominis muscle.

It is divided up into three main portions, the uppermost consists of a thin layer of muscle fibres passing forwards from the rectus abdominis muscle over the upper surface of the Metasternum to the upper surfaces of the coracoid and epicoracoid.

The lower layer is divided into two portions the median one running forward to be inserted into the anterior margin of the hyoid plate near the midline, and the lateral portion, consisting of oblique fibres is attached at its point of origin from the rectus abdominis muscle to the under-surface of the epicoracoid cartilage. The oblique fibres are inserted along the sides of the hyoid plate for some distance.

In Rana there is a prominent Hyoglossus muscle running up the midline of the Hyoid plate between the Sternohyoid muscles. In Xenopus this muscle is absent.

When the Sternohyoid is removed and the insertions of the Geniohyoid muscle exposed, a transverse muscle is noticed (Text. Fig. 5) lying on top of the Geniohyoid near the posterior margin of the hyoid plate. This muscle may be traced back to its point of origin on the lateral extremity of the pro-otic bone, and it is inserted into the posterior part of the hyoid plate in the median line, below the position of the glottis. It would seem that this muscle corresponds to the posterior Petrohyoideus muscle of Rana, but in the latter it is inserted into the outer part of the ventral surface of the hyoid plate far <u>above</u> the position of the glottis. The three anterior petrohyoideus muscles found in Rana are absent in Xenopus. There appears to be in Xenopus no muscle corresponding to the Omohyoideus muscle of Rana.

The foregoing resumé includes all the muscles of special significance to be encountered in the region of the Thyroid glands. Once the muscles are recognised it is a fairly simple matter to uncover the glands. It is best to work as far as text fig. 3, in the manner noted in the foregoing account. Once this stage has been reached the branches of the Geniohyoid may be pulled gently up towards their point of origin, snipped through and deflected. Then by carefully turning aside the oblique fibres of the Sternohyoid the thyroid glands will be found lying on the longitudinal fibres, which run below the oblique fibres. The glands lie one on either side of the midline of the hyoid plate, very slightly below the level of the glottis. They are avoid in shape and similar in size to those of Rana.

Vascular Supply.

Three arteries are given off from the region of the carotid gland - the outermost is the carotid artery - the median the lingual and from the inner side of the carotid gland the hyothyroid artery runs forward. In the region of the thyroid gland it sends off an artery to the gland which enters it on the outer lateral surface, and halfway along the length of the gland. The hyothyroid having given off this artery continues forward unbranched for some distance.

The vein coming from the thyroid leaves the gland at its posterior extremity and a short distance below the gland joins a small transverse vein which passes back until it unites with a vein running near the mid-line of the hyoid plate. These two unite to give rise to the external jugular (as in text fig. 11) which then passes back receiving only one more vein before it passes into the precaval vein.

The diagram (text figure 11) represents a standard condition of the veins, but most of the animals dissected proved to have a greater or lesser development of one side or the other, so that the symmetry of the external jugular veins often proved difficult to see clearly. Apart from the variability of equal development of the two sides, which may at first seem confusing, it is always possible to identify the thyroid gland of Xenopus by reference to it's vein and artery, a proceeding which might otherwise be tedious since nodules of fatty tissue are common in Xenopus in the region of the thyroid and might easily be mistaken for it.

The histology of the gland is so uniform throughout the Vertebrate series that it calls for no detailed description here. There is one noteworthy feature, however, in the Thyroid of Xenopus. That is the extremely large size of the vesicles containing colloid material, and the absence of any small vesicles. A foam-like appearance is usually seen round the margin of the vesicles. Physiologically such a foam-like appearance is usually associated with a great activity of the gland. As the toads used were of all sizes and ages the intense activity of the gland cannot be accounted for by taking the stage of growth into consideration.

XENOPUS LAEVIS

THYROID GLAND.

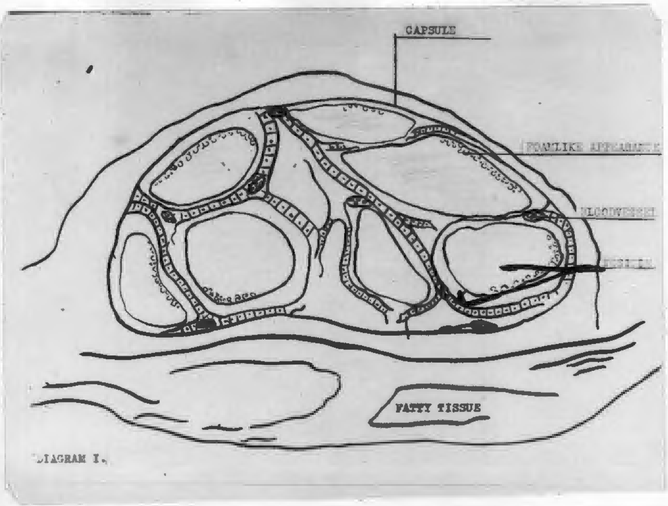


DIAGRAM I.

FIG. 1.

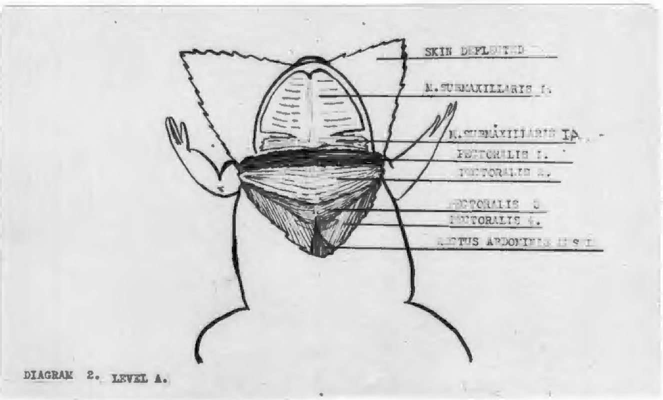


DIAGRAM 2. LEVEL A.

FIG. 2. LEVEL A.

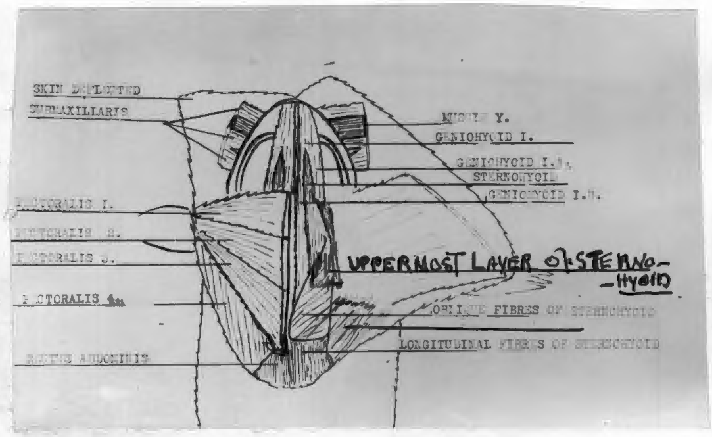


FIG. 3. LEVEL B

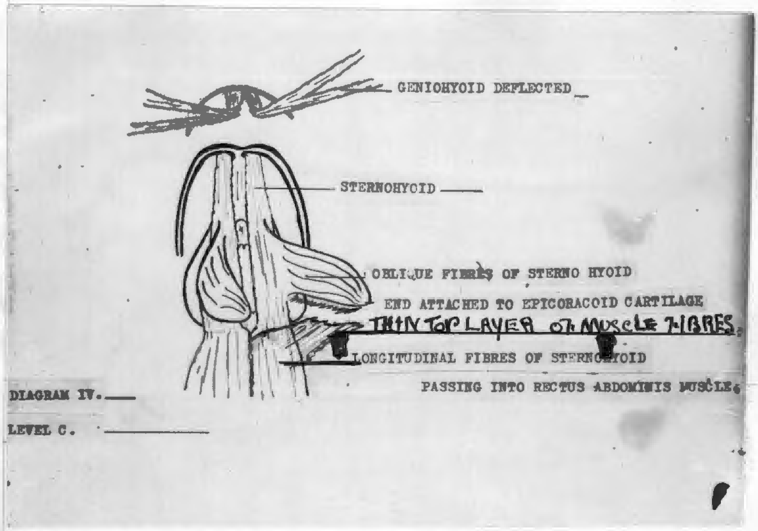


FIG. 4. LEVEL C

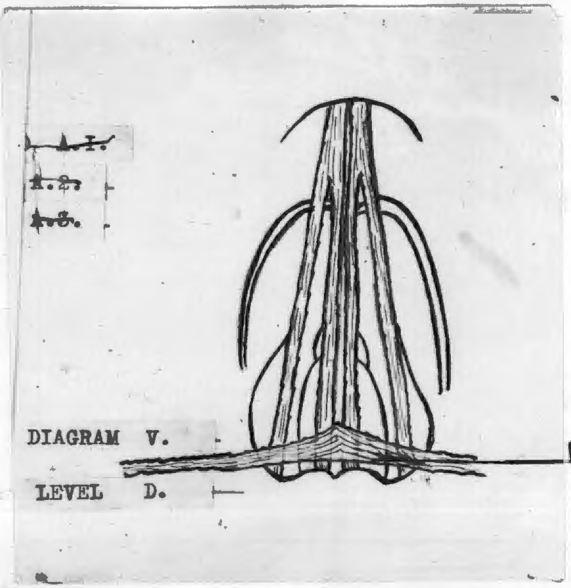
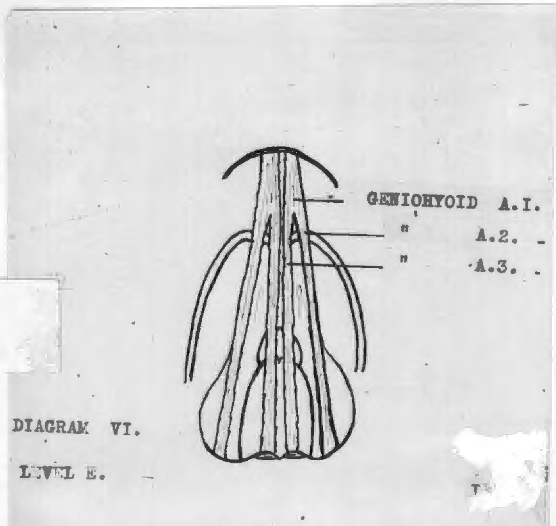


FIG.5.LEVEL,D.



GENIOHYOID MUSCLE AND IT'S ATTACHMENTS TO HYOID PLATE AND LOWER JAW.

FIG.6.LEVEL,E.

XENOPUS.
HYOID PLATE

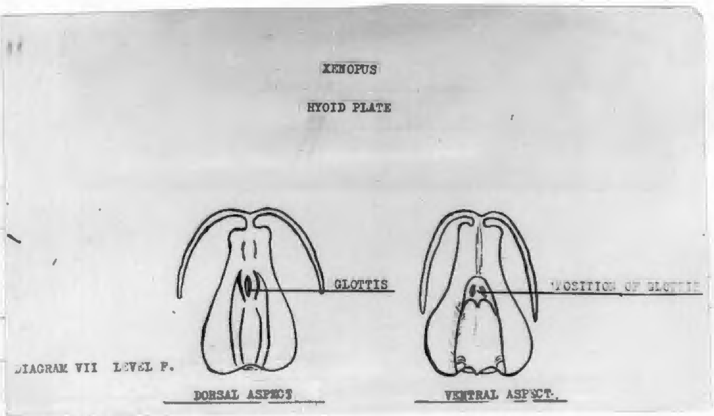


FIG 7 LEVEL F.

RANA.
HYOID PLATE AND MUSCULATURE OF SAME.

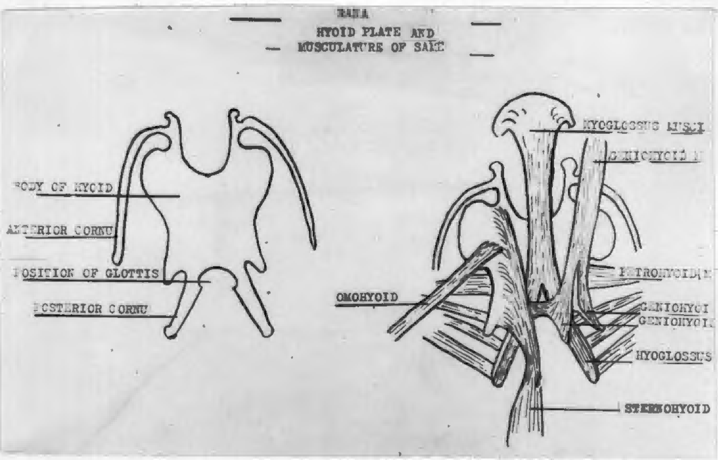


FIG.8.

POSITION OF THYROID GLAND.

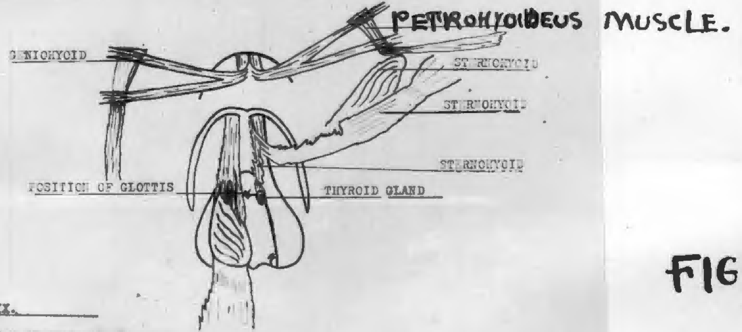


DIAGRAM IX.

POSITION OF THYROID GLAND.

FIG. 9.

ARTERIAL SUPPLY OF THYROID

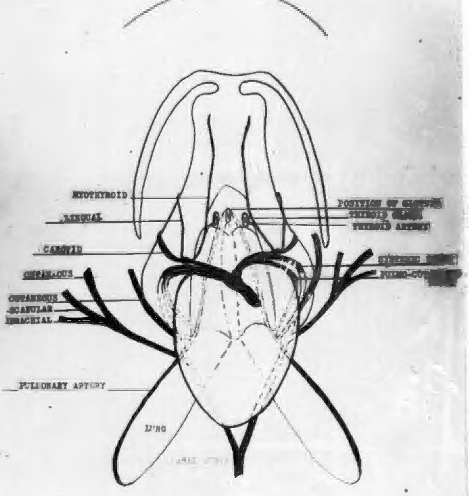


DIAGRAM X.

FIG. 10.

VENOUS SUPPLY OF THYROID GLAND.

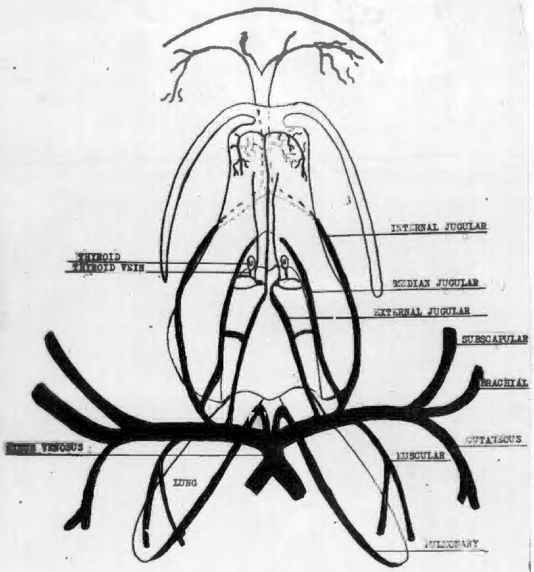


DIAGRAM XI.

FIG. 11.

THE EPIPHYSEAL COMPLEX AND PINEAL REGION IN XENOPUS LAEVIS

Introduction.

The epiphyseal complex and pineal region have now been identified in every group of Vertebrates. Some workers consider the structure functionless and vestigial, others suggest for it an important role in the economy of the internal secretions. Apart from the significance of the glandular function of the epiphyseal complex, the phylogenesis of the organ, in particular in its relation to the parietal eye, is of great interest. In the following account an attempt will be made to make clear the morphology and histology of the pineal region in Xenopus laevis.

Before proceeding to that task however, a brief resumé of what is known about the comparative morphology of the gland will be given. The separate constituents of the epiphyseal complex are usually recognized to be:

1. The pineal organ consisting of (a) an end vesicle (b) a stalk (c) a proximal portion (d) a peduncle.
2. The parapineal organ consisting of (a) an end vesicle (b) a stalk and (c) a proximal portion.

The foregoing enumeration of the constituent parts applies only to the epiphyseal complex proper but it is misleading to consider the epiphyseal complex apart from the adjacent parts of the brain. The roof of the third ventricle gives rise to several structures. Some of these are to be identified throughout the Vertebrate series while others are less constant appearing in a few classes only but accumulated evidence shows that all have an important bearing on the epiphyseal complex in its relation to the history of the phylum. As suggested by Minot the structures forming the roof of the interbrain are termed collectively (by the majority of workers) the pineal region.

Minot defined the limits of the pineal region as beginning at the lamina terminalis and ending at the commencement of the midbrain. The region is comprised of (1) the paraphyseal arch. (2) the velum transversum, (3) the dorsal sac (4) the epiphysis (pineal 'body'), (5) the posterior commissure.

The paraphysis. is an evagination following upon the lamina terminalis and continuing backwards to meet the velum transversum. The glandular appearance of the paraphysis indicates a secretory significance. The paraphysis is more highly specialized and developed in Amphibia than in any other class of Vertebrates, being but poorly represented in the highest and lowest members of the phylum. It has been identified in man but so far only in the embryo.

The velum transversum is a transverse groove immediately behind or following on the paraphysis. It may be single in form or assume a folded and highly vascular condition or again it may be entirely absent.

The dorsal sac. is a sacculated balloon like evagination which is usually most conspicuous, like most of the evaginated structures of this region there is a tendency to high vascularity. In some forms the dorsal sac comes to resemble a choroid plexus. The same observation holds true for the nature of the velum transversum.

The pars intercalaris anterior. appears as the thickened posterior end of the dorsal sac and usually consists of neuroglia cells. It has only been recognized in a few types.

The commissura habenularis includes the two ganglia habenulae in its general mass and in those forms in which the parapineal organ is present the nerve from the organ proceeds downwards, ending finally in this region.

The epiphysial complex is made up of two elements, the pineal and parapineal organs. As already stated the pineal organ consists of an endvesicle, a stalk, a proximal portion and a peduncle; the proximal portion together with the peduncle are usually termed the epiphysis. The epiphysis is supposed to give rise to the endvesicle. The stalk connecting the two is often termed the pineal nerve.

The parapineal organ when present consists of an endvesicle, a stalk and a proximal portion and is usually well developed in the simpler types of Vertebrates e.g. Cyclostomes, Selachians, Etc., in fact, in those forms in which the parietal eye reaches its highest development, a circumstance which will be taken into account later in this paper.

The pars intercalaris posterior. The posterior portion of the epiphysis is often considerably thickened, forming a region with recognizable features between the epiphysis proper and the commissura posterior. Fibres of the pineal stalk have been described as entering this region and ending in the mass described as the commissura posterior. This latter structure is the final element involved in the pineal region. The midbrain follows immediately behind it.

Comparative Morphology: A detailed study of the comparative morphology of the pineal region is outside the scope of the present paper. The following table will present at least a rough idea of the degree of development of the various constituents of the pineal region in the main classes of vertebrates. For the purpose of clarity plus and minus symbols have been adopted to denote the degree of development. + representing an average amount of development in the adult and ++ a maximal. – indicates a minimal amount of development and – – a transitory appearance of the structure in the embryo only, while 0 indicates entire absence both in embryo and adult.

Constituents of Pineal Region	Cyclos-tomes	Sela-chians	Teleosts (including Dipnoi)	Amphibians	Reptiles	Birds	Mamma
Paraphysis	-	+	+	++	+	+	- 0
Velum Transversum	-	+	+	+ +	+	+	-
Dorsal sac	-	+	+	+ +	++	++	+
Commissura Habenulares	+	-	-	-	+	-	-
Pars Intercalaris Anterior	-	-	-	-	-	-	-
Epiphyseal Complex							
1. (a) end-vesicle	++	+	+	+	--	o	o
(b) Stalk	+ +	+	+	+	--	o	o
(c) Proximal portion	+	+	+	+	+	+	++

Comparative Histology.

In Cyclostomes the end-vesicles of both the pineal + parapineal evaginations develop visual organs. Each has a more or less well developed retina showing rod-like cells and various other elements of a distinctly neuro-mechanical nature. Nerve fibres are present in the stalks of both, those coming from the pineal end-vesicle terminating in the posterior commissure while those of the parapineal end-vesicle terminate in the commissura habenulare. These facts taken in conjunction with a mass of additional histological detail indicate a strong tendency to develop or renounce a second pair of eyes. So far no glandular organisation has been identified in the epiphyseal complex of cyclostomes. As between Selachians and Cyclostomes there is a noticeable absence of any tendency towards a development of the end vesicles as visual organs and indeed the parapineal makes a transitory appearance in these animals. The evidence at present available points to the presence of glandular elements in the end-vesicle of the pineal evagination. In teleostians there is generally a considerable ~~great~~ development of the epiphyseal complex. The end-vesicle of the pineal evagination presents the appearance of a tubular gland and its lumen is in communication with the third ventricle by means of the long and in this case hollow stalk.

In Amphibia the parapineal is absent or at best developed as a vestige is the embryo. The end-vesicle of the pineal evagination in the young animal presents the primitive elements of retina and lens but these tend to degenerate rapidly as age advances. The stalk is made up very largely of nerve fibres terminating in the posterior commissure. The proximal portion of the complex is well developed and distinctly glandular. The other constituents of the pineal region in Amphibia are also of a very pronounced glandular nature and there is a noteworthy development of the paraphyseal arch. There are therefore some indications in favour of the view that the epiphyseal complex has an internal secretory significance.

In Reptiles the parapineal attains it's maximal development as a visual structure. The end vesicle of the pineal on the other hand does not exhibit differentiation as a visual organ and the proximal portion is definitely glandular.

The histology of the region in Ophidia and Chelonia is especially worthy of note since the proximal portion of the pineal evagination has the appearance of a much branched and highly vascular gland. In Sphenodon the parietal eye reaches its highest development. The more modern reptiles evince no tendency to develop visual structures in connection with the epiphyseal complex nor in fact to the development of any neural mechanism whatever in this connection.

In Birds the parapineal is absent and no tendency is exhibited by the epiphyseal complex to develop visual organs, all differentiation so far observed being in favour of glandular formation. The cells are granular in character and are arranged round alveoli . The region is highly vascular.

The development of the proximal portion of the epiphyseal complex to form a gland may result in the formation of any one of three main types of gland (a) the tubular type in intimate relation to the ventricular system (b) a vascularised type (c) a type combining the characteristics of (a) and (b). Tilney and Warren consider the foregoing evidence afforded by birds to be in favour of the glandular nature of the epiphyseal complex.

In Mammals the accounts of the histology of the gland are conflicting as likewise are the conclusions drawn by the various observers. According to Tilney and Warren, who seem to offer the best evidence for their conclusions, the main features in the early stages of differentiation are that the nuclei of the ependymal cells are so large and the cytoplasm so scanty that they give the impression of lymphoid tissue (hence probably the lymphoid nature attributed to the gland by Schwalbe, Henle, Lord and others) but later on the cytoplasm increases so considerably in amount that the cells can no longer be thought lymphoid in character. They soon come to have all the appearances ~~as~~ usually associated with glandular cells. That the epiphyseal complex may contain nerve cells and fibres is admitted as probable, but there is no evidence in mammals of the existence of any neural mechanism in the pineal body.

Tilney and Warren conclude that the epiphyseal complex possesses a pluripotentiality whose inherent tendency is towards glandular differentiation and that in a few instances... "As in cyclostomes, amphibia and in primitive reptiles, the parapineal or pineal organ may become further differentiated in the interest of a highly specialized sensory mechanism which has or has had visual function"

THE MORPHOLOGY OF THE PINEAL REGION IN XENOPUS LAEVIS CORRELATED WITH OBSERVATIONS MADE IN RANA FUSCIGULA.

So far as is known to the writer no account has yet been rendered of the pineal region in Xenopus.

A number of accounts have been made of the region in Rana but such confusion in terms exists in re the epiphyseal complex that many of the existing accounts are exceedingly difficult to follow. For instance when it is stated that degenerative changes occur in the pineal gland after the adult condition is reached, the reader is at a loss to know whether the end-vesicle is referred to or the proximal portion of the epiphyseal evagination or both. Schafer (Essentials of Histology) states that in Amphibia the 'Pineal Gland' lies beneath the skin and outside the skull and that its position is marked by a clear spot in the skin on a line and midway between the eyes. He further states that degenerative changes occur in the gland in the adult. It is obvious that the end-vesicle is referred to. In the embryo of Rana fuscigula (¼ in. stage not including the tail) the end-vesicle is well developed, spherical in shape and made up of deeply staining basophil cells with spherical nuclei.

The end-vesicle is attached by a long stalk passing through the roof of the skull to the proximal portion of the gland which is highly vascular and composed of two main types of cells, namely cells with oval nuclei and fine oxyphil granules and cells with spherical nuclei and coarse basophile staining granules. The oxyphil cells are arranged round alveoli and the basophils occur in clumps. Nerve cells appear to be present in the stalk. In the adult specimens examined macroscopically the skin in the region of the clear spot (very conspicuous in Rana fuscigula) was deflected and on its undersurface was discovered a pinkish white, gelatinous looking body, ovoid in shape. Its immediate environment was richly vascular. A long delicate stalk attached to the body could be traced back to a small outlet in the skull just above the region of the 3rd ventricle of the brain. On cutting sections through the skin in the region of this body the writer was only able to discover a mass of tissue showing obvious signs of degeneration. e.g. nuclei were scattered throughout the mass and the majority of the cells lacked any definite boundaries in the way of cell walls or marginal demarcations. Sections through this region were made in at least seven adult frogs (Rana fuscigula) of various sizes. The results were similar in each case. The characteristics of the pineal comple in Xenopus are essentially similar to those of Rana except that the end-vesicle in the adult animal is much smaller and more flattened than in Rana.

Longitudinal sections through the entire brain of Xenopus reveal the morphology of the pineal region very clearly. When removing the brain it is necessary to exercise great care in order not to injure the proximal portion of the epiphyseal complex. This part is attached by means of the stalk, which passes through the skull, to the end vesicle and there is a strong tendency for the proximal portion to remain attached to the stalk rather than to the peduncle with the result that only the torn end of the proximal portion will be visible in sections.

The paraphysis is observed to be a well developed structure but as a rule not so conspicuously vascular as in Rana. The velum transversum takes the form of a single fold and is followed by the well developed dorsal sac. The walls of the dorsal sac are much folded and the whole structure is highly vascular. The mass representing the corpus habenulare is also exceptionally vascular and is followed by the elongate pars intercalaris anterior certain portions of which (in some of the specimens examined) have an almost plexiform appearance owing to their exceeding vascularity. The proximal portion of the epiphyseal complex takes the form of a sac lying close to the interbrain. Its lumen is divided into a number of branches which project downwards into the third ventricle. The vascular system of the proximal portion appears to be in part sinusoidal. This condition closely resembles that described by de Graaf as found in Urodeles. Following the epiphysis can be seen the thick pars intercalaris posterior and the commissura posterior. A more detailed study of the histology of the end vesicle reveals the cells to be somewhat elongate and the appearance of one or two nerve fibres may be detected (in the young animal) The cells of the end vesicle constitute the structure described by several observers as the frontal subcutaneous gland. Its real relationship to the epiphyseal complex was elucidated by Goette who studied the epiphysis developmentally and demonstrated that the subcutaneous frontal gland was the detached distal extremity or end-vesicle of the epiphyseal complex.

The stalk contains traces of nerve fibres and these appear to terminate in the commissura posterior passing down the posterior wall of the epiphyseal complex to reach it.

The proximal portion is a sac like structure with its walls much branched and its lumen in communication with the third ventricle. The cells of which it is comprised are of two main types oxyphils with oval nuclei and basophils with spherical nuclei. The oxyphil cells are arranged round alveoli and the basophils occur in colonies or clumps. The entire structure is highly vascular. In connection with the basophil and oxyphil cells of this region it is of interest to consider the basophil and oxyphil cells comprised in the pituitary complex which lies immediately below this region on the undersurface of the brain. In his Monograph on the pituitary de Beer gives an account of the 'Functional difference Theory' put forward to elucidate the nature and origin of the basophil and oxyphil cells. According to this theory the two types are but two conditions or phases of one cell. That this is not true at any rate of the basophil and oxyphil cells of the pineal seems to be indicated by the definite types of nuclei which are characteristic of the two types of cells (see above). There is of course no present justification for assuming that the oxyphil and basophil cells of the epiphyseal complex are in anyway similar in nature to those of the pituitary or indeed that they are referrable to the same laws or factors of determination. It is tempting however to suggest the comparison although it must be mentioned that in certain pathological conditions of the epiphyseal complex in which cells from this region are observed to migrate down into the pituitary region they can at once be distinguished, both as regards size and appearance, from those of the pituitary.

CONCLUSIONS.

I. In certain respects the Pineal region in Xenopus laevis resembles that found in Urodeles. e.g the proximal portion of the epiphyseal complex is a sac like outgrowth with much branched walls and the paraphysis is not as a rule so well developed as in other Anura. Further the flattened and degenerate end-vesicle is more characteristic of Urodeles (eg. the Salamander) than of most Anura.

II. There is in the embryo a well defined end-vesicle which later shows obvious signs of degeneration histologically.

III. The proximal portion is highly developed and of a definitely glandular nature.

IV. The proximal portion continues in a functional and active state indefinitely i.e. it shows no visible signs of degeneration as age advances. The same appears to be true of Rana fuscigula.

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- GENERAL DISCUSSION-

Several points have to be taken into consideration in any discussion on the nature of the pineal region and epiphyseal complex. The first is whether the epiphyseal complex is to be considered as a vestigial structure or as an organ having an important glandular function. Setting aside for the moment the comparative morphological evidences in favour of significance of the glandular functions of the complex, some interesting data have been desdribed by Tilney and Warren and put forward by them as corroborative evidence. They analysed a series of observations made by Cutore on the ontogenesis of the epiphyseal complex in man. In these observations the weights of the epiphysis, hypophysis and brain were recorded for five more or less well defined epochs of life. They found that of the three structures studied the average weight of the epiphyseal complex alone tends to increase constantly as age advances (up to 70 years). The brain and hypophysis show a constant increment in weight up to 60 years and after that a marked decrease.

Tilney and Warren hold that an organ in the process of regression would not be likely to keep pace so constantly in weight increment with that of the hypophysis, an organ proved to be concerned with internal secretions, and further that observations, indicate that the activity of the epiphyseal complex is not arrested at puberty.

Since although physiological and clinical evidence tends to show that the gland manifests its greatest activity between the ages of 3 and 15 (in man) there is yet evidence indicating that the activity of the gland does not cease even in extreme age.

A factor against the view of the vestigial nature of the epiphyseal complex is that in those animals which develop a corpus callosum the epiphyseal complex resists in a marked manner the encroachment of this structure and adapts itself in a number of ways to the encroaching commissural fibres of the callosum. The paraphysis and dorsal sac may become flattened but the pineal region continues to exist in its entirety and the epiphyseal complex accommodates itself in a number of ways to meet the new conditions imposed upon it. It is reasonably thought that if the complex were vestigial far from accommodating itself to new and decidedly adverse conditions it would tend to regress even further.

Histological evidence also tends to show that the epiphyseal complex is not vestigial since it shows an increasing degree of specialization towards glandular formation as the vertebrate-series is ascended. It's development has been studied in both man and Felis domestica and it has been shown that the complex follows the general procedure characteristic of glandular formation. Further the histology of the complex gives evidence that the structure possesses a pluripo~~n~~tentiality, with a bias towards gland formation and only in one or two instances (eg. cyclostomes and the older reptiles) giving rise to structures with a neuro-mechanical function.

The secretions (if any) to which it gives rise are in all probability in some cases passed into the cerebro spinal fluid but in every group after the Amphibia the histological evidence tends to show that the organization of the complex is endocrine in type and it's secretions ~~being~~ presumably taken up by the blood stream.

The nature of the secretion or secretions (if any) formed by the complex is not yet known since it has not yet been found possible to isolate from the gland any substance giving rise to the conditions which are recognized, by a number of workers, to be associated with the gland. The evidence tends to show that the secretion of the epiphyseal complex bears a particular relation to the development of the gonads and exerts considerable influence on the time of appearance of the secondary sexual characters. A great deal of clinical data is now available which tends to demonstrate that certain well defined pathological conditions are caused by abnormal conditions of the complex (eg. pineal tumour, injury etc).

McCord and Allen in 1917 found that by feeding tadpoles with 'pineal' (which part of the pineal region or epiphyseal complex not clear) both growth and differentiation were hastened. They recorded that a melanophore controlling factor was associated with the gland since tadpoles fed with pineal showed distinct contraction of the melanophores, rendering them paler than normal animals. McCord also found that Paramoecia fed with 'pineal' divide twice as rapidly as controls, and Feather (1919) recorded that "Pineal" extracts increase the pulsation frequency of the contractile vacuole in the same organism.. In this connection the action of the pineal extracts would seem to behave very like extracts of adrenaline or epinephrine and certain preparations derived from the pituitary complex.

SUMMARY

No definite conclusions as to the vestigial or glandular nature of the epiphyseal complex can yet be formed but the evidence at present available tends to be in favour of the complex having a glandular significance.

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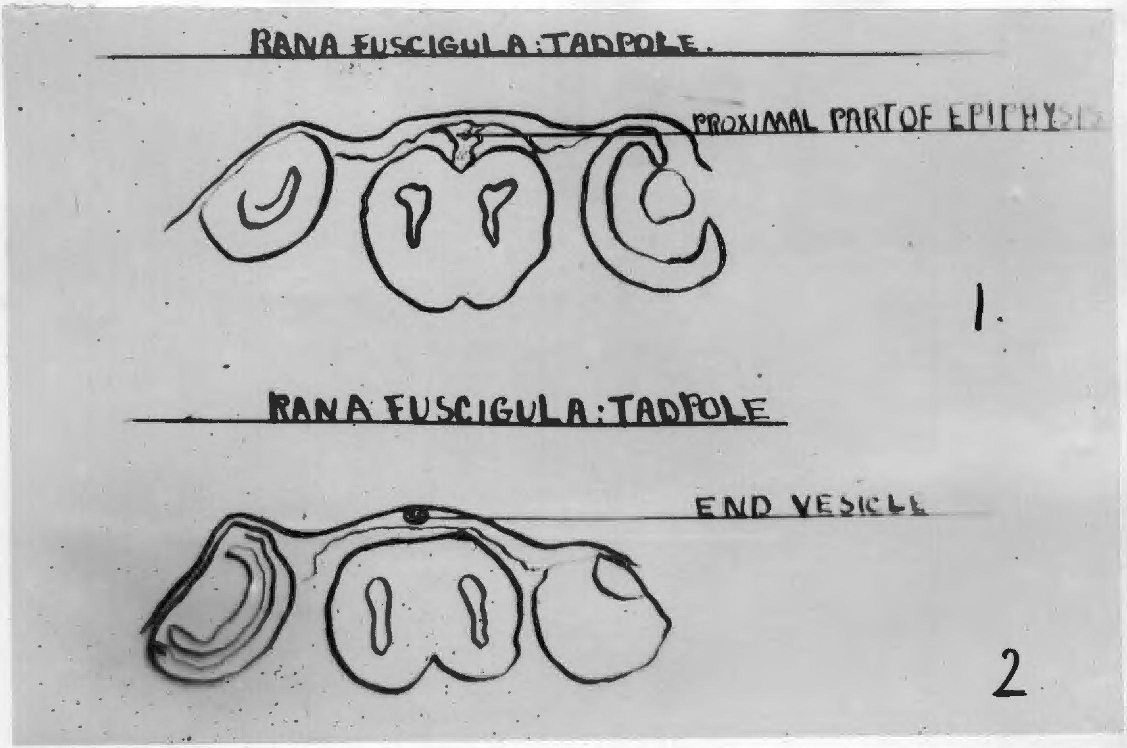
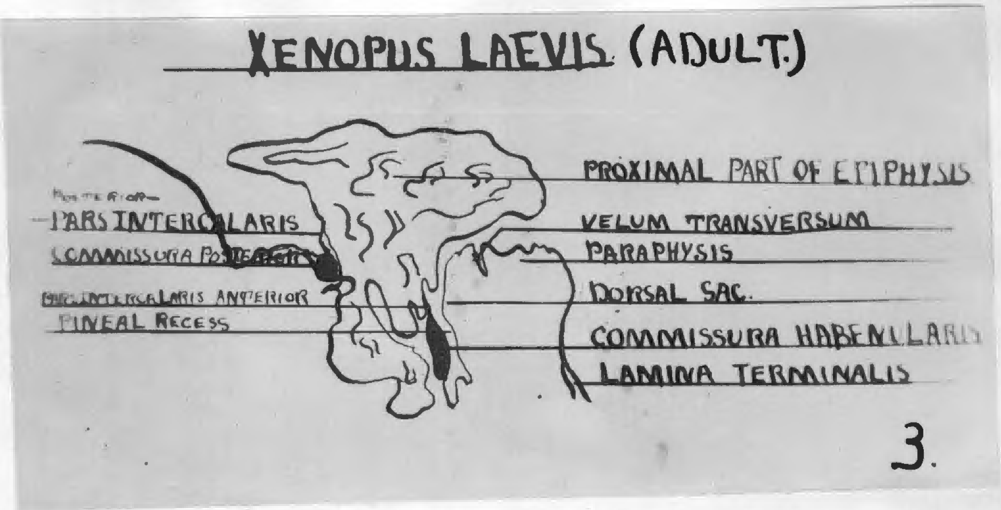
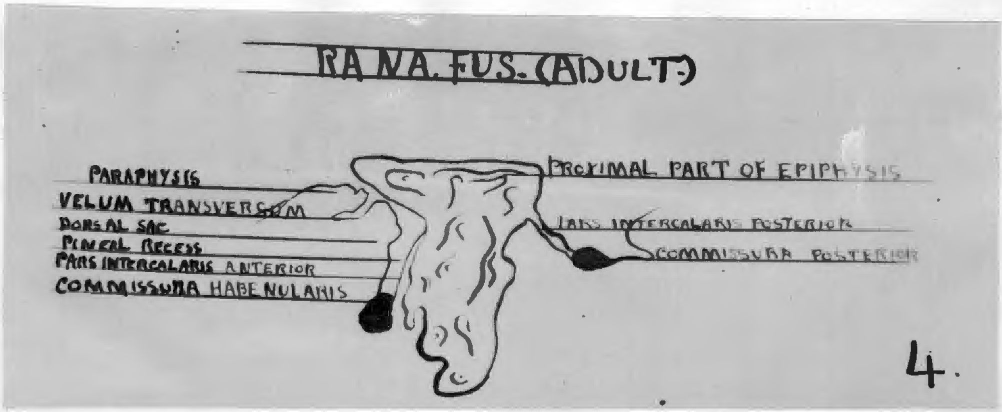


Transverse Sections.
(Section 2, showing the end vesicle, is anterior to section 1.)

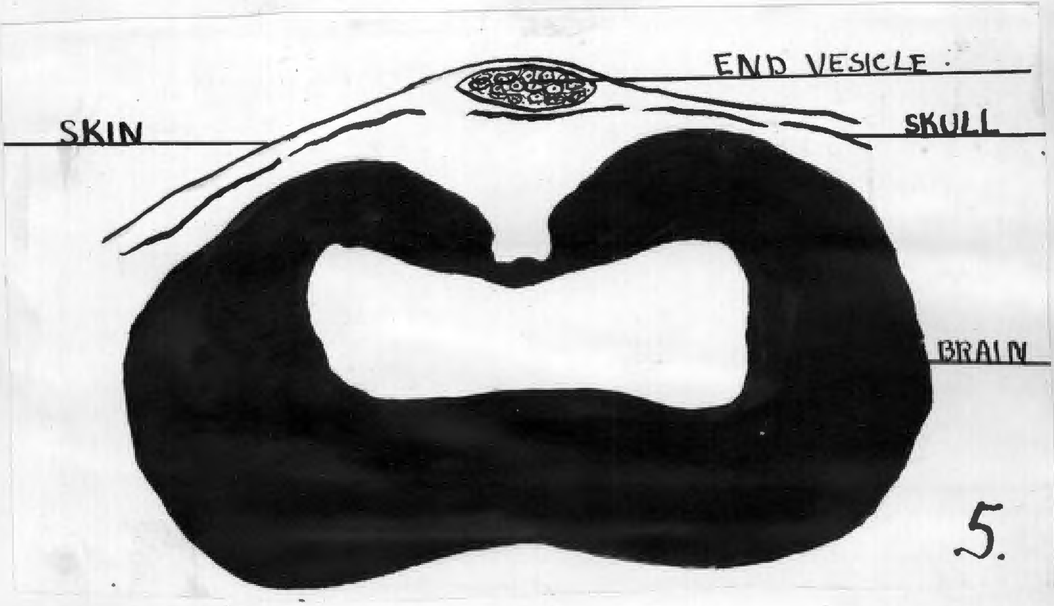


Longitudinal Section.



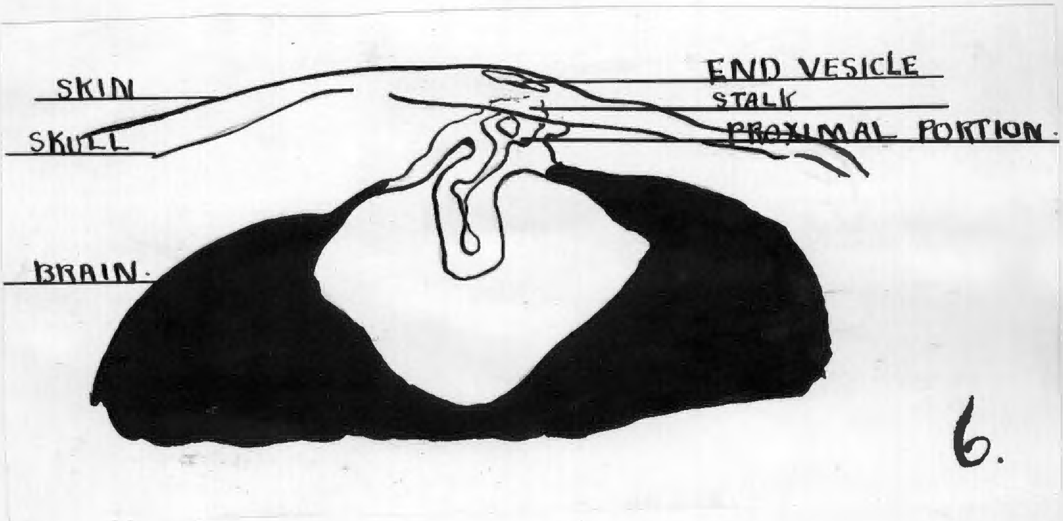
Longitudinal section

XENOPUS LAEVIS.



TADPOLE 10mm STAGE (TRANSVERSE SECTION.)

XENOPUS LAEVIS.



6.

TADPOLE 10 mm STAGE (TRANSVERSE SECTION.)

CONCERNING THE POSSIBLE OCCURRENCE OF CHROMAPHIL CELLS IN THE KIDNEY OF XENOPUS LAEVIS.

It has been stated by von Bonde and others that there is no suprarenal complex present in Xenopus laevis. They are silent as regards the occurrence of chromaffin tissue in the animal and no description has yet been made (so far as known to the writer) of suprarenal cortex tissue occurring in Xenopus. If adrenaline forming cells and cortical cells were absent in the animal it would be exceedingly strange since chromaffin tissue occurs universally in vertebrates.

COMPARATIVE MORPHOLOGY OF THE ADRENAL COMPLEX

There appears to be good evidence of the presence of adrenaline forming tissue in some invertebrates as for instance in the leech as demonstrated by Biedl and by Gaskell.

In Cyclostomes the adrenal complex is

represented by two series of bodies - cortical and chromaphil. The cortical is associated with the posterior cardinal veins, renal arteries and arteries of the dorsal region. The chromaphil by thin strips of tissue running along the large arteries and their branches from the region of the second gill cleft to the tail.

In Elasmobranchs the paired chromaphil bodies are in close relation to the ganglia of the sympathetic chain. The suprarenal cortex is a rod-like structure paired or unpaired lying near the posterior margin of the kidney.

In Teleosts the cortical adrenal bodies are usually paired and lie embedded in the ventral surface of the kidney. In addition, cortical adrenal tissue is found associated with the head kidney, so that in teleostian fishes there are cranial and caudal cortical bodies. The chromaphil tissue is distributed in groups of cells along the lymphoid tissue of the head kidney.

In ganoids the cortical representatives are small yellowish bodies scattered throughout the substance of the kidney, chromaphil elements are found in the walls of the cardinal veins.

The condition of the complex in Dipnoi is not clear as yet. The peculiar nature of the perirenal tissue has still to be thoroughly investigated but an epithelial and glandular appearance has already been ascribed to it.

In Amphibia the adrenal complex is intermediate in many respects between that of the lower and higher vertebrates.

In Urodeles the adrenal is broken up into a series of islets which extend the whole length of the kidney and often as far forward as the subclavian artery.

In Anura the complex is represented by a broad yellow band running down the ventral surface of the kidney. In the majority of Amphibia this band on section proves to consist of two elements - the cortical and chromaphil. The cortical cells are elongate and arranged in columns and at the ends of the columns masses or clumps of chromaphil cells occur. The latter are however not confined to the region of the cortical cells. Colonies of chromaphil cells often occurring anterior to the kidney (especially noticeable in certain Urodeles). This condition is analogous to the paired suprarenal bodies of Elasmobranch fishes. Again in other Amphibia chromaphil cells may occur scattered on the outer surface of the kidney or disposed throughout the entire substance of the kidney in 'nests'.

In every class after the Amphibia the adrenal complex is built up of cortical and medullary elements which are always found associated in the one structure. Accessory chromaphil elements may however occur wherever the sympathetic nervous system penetrates. Extracapsular cortical elements are also found not infrequently but these usually occur in the region of the chief adrenal or in the genital region. In addition to the extracapsular cortical and medullary elements found separately in Amniota true accessory adrenal bodies composed of both elements may also occur. These are said to be found in the neighbourhood of the abdominal sympathetic nervous system and in the region of the body where the cortical elements first arise.

From the foregoing data it may be seen that the adrenals are far from constant in arrangement and position throughout the phylum but are more so in the Amniota than in the Anamnia.

Xenopus Laevis:

In a paper published in the Quarterly Journal of Experimental Physiology (Vol XX No. 1) on the skin secretion of Xenopus, Gunn described a series of experiments undertaken to elucidate the nature of the mucus like substance which Xenopus exudes in such profusion from it's skin. Gunn dried the substance which he collected from the skin and subsequently made solutions of it, noting its effect on blood pressure, respiration, the excised uterus (pregnant and non pregnant) and intestine. As a result he came to the conclusion that there was present in the skin secretion of Xenopus a substance very similar if not identical with Adrenaline or epinephrine. It would be of interest to know if an animal having such an abundant source of adrenaline would be in need of a further supply from a suprarenal source. This consideration may in part account for the condition of the kidney about to be described by the writer but in any event the question of the elements usually associated with the suprarenal cortex would still remain unanswered.

As is well known, there is on the ventral surface of the kidney in Rana a conspicuous yellow streak running down the middle line of each kidney. This yellow streak represents the suprarenal complex. On section it proves to consist of the two chief elements associated with that gland, namely the characteristic cortex cells and the cells of the medulla. On treating with potassium bichromate the cells of the medulla give evidence of their chromaphil nature by staining from yellow to dark brown.

In Xenopus far different conditions obtain. In some individuals a well defined yellow streak is to be seen running down the ventral surface of the kidneys but in many it is either very faint or so far as the naked eye is able to determine, completely absent. The writer has found that in large females well distended with eggs, the streak tends to be most conspicuous and considerably broader than in small females. In males the streak is usually but feebly developed and in young males often appears to be absent. Sections through the kidney reveal the fact that the cells of this region approximate very closely in appearance to the typical cells of the suprarenal cortex. They do not stain with potassium bichromate. They stain light blue with safranin and water, blue and blue to blue violet with Giemsas stain. These are the correct colour reactions for the suprarenal cortex. The cells of the streak differ in appearance from those covering the surface of the rest of the kidney and they are definitely localized to the area of the streak. It must be said that the appearances are in favour of them being the representatives in Xenopus of the suprarenal cortex.

Sections through the kidney of Xenopus treated with bichromate reveal small yellowish brown cells scattered on the outer surface of the kidney. These cells are indistinguishable in appearance from the chromaffin cells associated with the suprarenal complex in other Vertebrates and moreover they agree with the description of the condition found in certain other amphibians where the chromaffin cells occur scattered over the outer margins of the kidneys. As further histological work proved unavailing in definitely deciding the question of the identity of these cells - an attempt was made to investigate the matter biochemically.

Through the courtesy of Professor Gunn it was possible for the necessary tests to be made in the Pharmacology Department in this University. The kidneys were taken from ten animals (Xenopus) and freed of urine by washing in normal saline solution, they were then put into acetone and cut up into pieces. These pieces were allowed to dry for a short time (15 to 20 minutes) in the acetone and were then removed and placed in a des~~s~~iccator to be dried in vacuo. They were removed after two days (still smelling strongly of acetone) and ground up in a mortar with a few ccs. of ordinary Ringer solution to which a drop of 1/N HCL had been added. This solution when injected into a large cat at first produced a decided slowing of the cardiac and respiratory rhythm followed by a very slight rise above the former normal level. A rise which was almost equalled when an equal quantity of ringer was injected. The great drop produced in the first instance was attributed to the presence of acetone in the injected solution. Numerous other tests conducted on the same lines were made with other extracts from the kidney but all with very indefinite results until with one preparation (made up from the tissue of 72 kidneys and freed as much as possible from acetone) the results recorded in the accompanying tracings were obtained. These, although far from being undeniably conclusive,favour the theory of adrenaline being present in the kidney.It will be noticed in Tracing I. that perfusion of the isolaated heart of the cat with a solution extracted from Xenopus kidney (freshly made)resulted in a great increase in the cardiac rythm.The break in the tracing occurred when the ~~pointer~~ lever rose so high it went above the paper.The rise of pressure was followed by heart block.This tracing is characteristic of adrenaline tracings but similar tracings are obtainable for a number of other substances.

Tracing 2 is a record of the effect of a solution of the extract on the isolated pregnant uterus of the cat.It will be observed that it has an effect typical of adrenaline but again other substances might produce a like effect.

Tracings 3 and 4 made with a solution at least six hours old show a slight inhibitary effect on a strip of rabbit intestine. This effect is far from being as definite as the presence of adrenaline might be expected to render it, but still the fact remains, slight inhibition was induced.

Tracings 5, 6 and 7 show the effect of a concentrated solution of kidney extract (1½ kidneys per 0.1 cc of solution) on the isolated rabbit intestine Again the effect is slight but is nevertheless of the adrenaline type.

In a fresh preparation made from 36 kidneys by grinding up with powdered glass and extracted with 10 ccs. of warm distilled water to which a drop of glacial acetic acid was added a pink colouration was obtained on treating a portion of the solution with weak iodine and a green colouration was obtained with a few drops of weak ferric chloride. These colour reactions faint though they were, are characteristic of adrenaline. Colour reactions being infinitely more crude than biochemical tests it seems justifiable to conclude that adrenaline is present in the kidney of Xenopus but it would appear to be so in exceedingly small quantities since for the experiments which yielded the accompanying tracings the kidneys of large numbers of animals were used to make a few ccs. of solution.

Taking into consideration Professor Gunn's findings re the nature of the skin secretion in Xenopus the small quantity of adrenaline obtained from the internal organs is less surprising than it might otherwise be. Whether the chromaphil complex of the kidney in Xenopus laevis is to be considered as rudimentary or vestigial further investigation must be left to determine.

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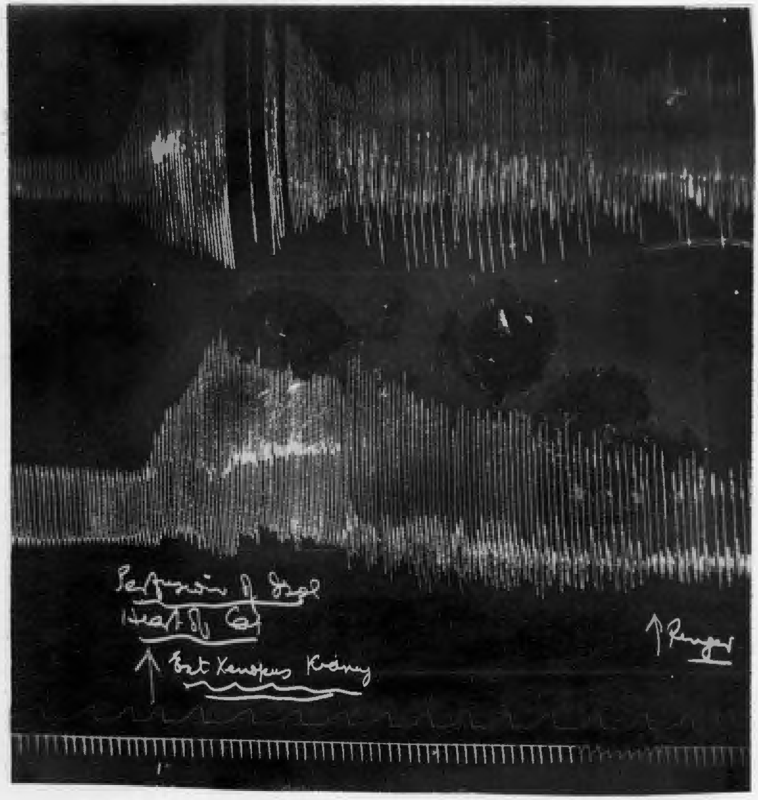
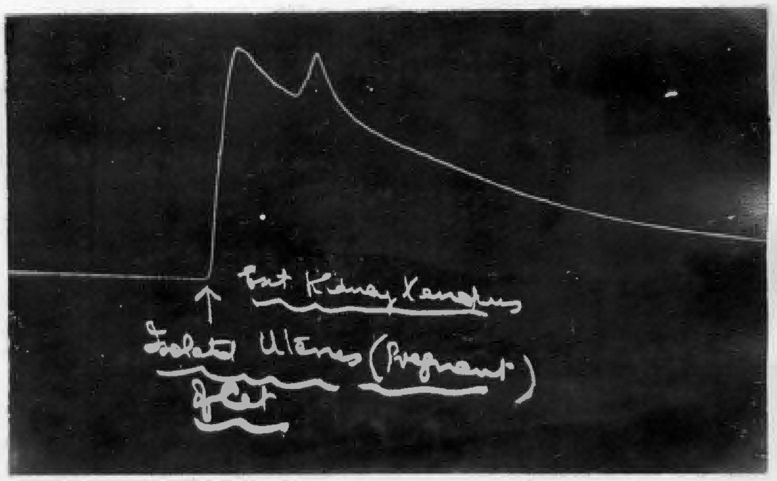
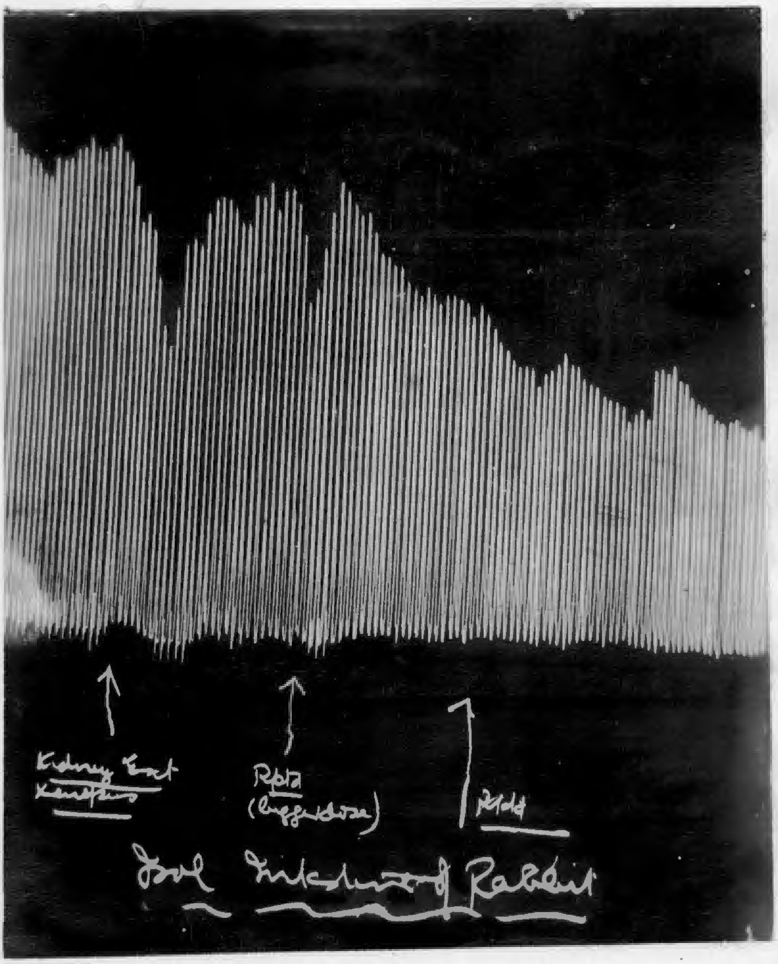


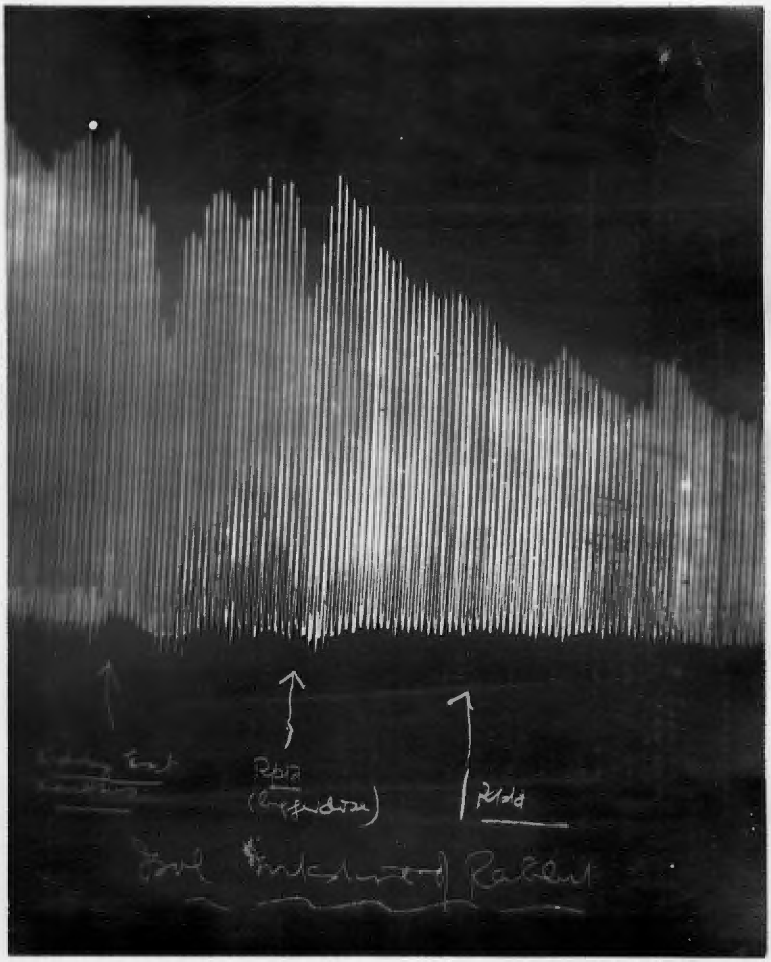
Tracing I.



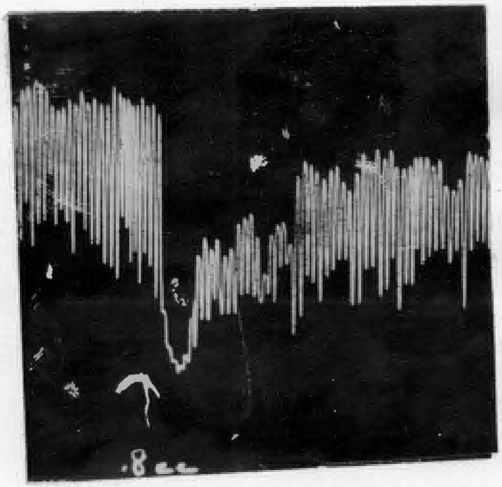
Tracing 2.



Tracing 3.

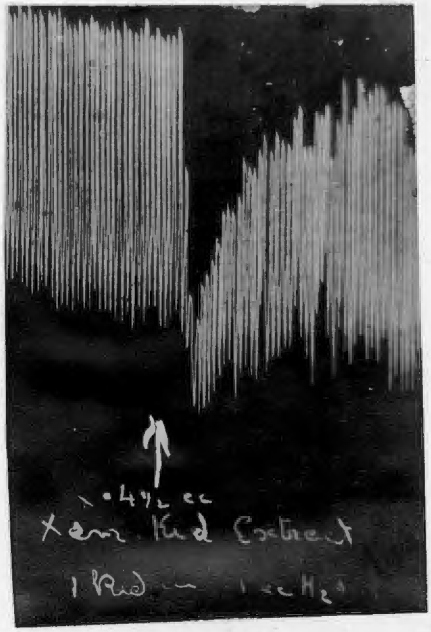


Tracing B.4.



Tracing 5
Xenopus Kidney Extract
(1½ kidneys in 0.1cc sol.)
Isol. Intest. of Rabbit.

Tracing 6.
Isol. Rabbit Intestine



Tracing 7.
Isolated Rabbit Intestine