C O N T E N T S

EFFECT OF THE EXTIRPATION OF THE THYROID GLAND UPON THE HYPOPHYSIS IN BUFO

By

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INTRODUCTION

A great deal of study has been made and experimental work done upon the glands of internal secretion for the last few years in the Zoology Department at the University of Kansas. In the spring of 1918, Professor B. M. Allen, head of the Zoology Department, taught the writer the technique for removing the anlagen of the thyroid gland in the larvae of Bufo americanus. He suggested the problem here described and some work was done the following fall and winter. A preliminary report appeared in the Anatomical Record, Volume 15.

On account of absence from the University and hence lack of facilities for doing research work, the problem was given up for the time being. Again this year it became the object of study. I wish here to express my thanks to Professor B. M. Allen for much kindly assistance. His interest and help have made this paper possible.

LITERATURE

During the past decade a great amount of literature has appeared which has quite conclusively proven that the removal of the thyroid gland causes the hypertrophy of the hypophysis. Most of the work has been done on mammals and the observations have been more or less limited to a study of the anterior lobe.

That after the removal of the thyroid gland the hypophysis becomes altered and enlarged was first shown in 1886. Degener found that the increase in weight was directly proportional to the time which had elapsed after the removal of the gland and that this removal affected all parts of the hypophysis but more especially so the pars anterior. This worker also observed the increase of hyaline and granular masses in the pars intermedia and their passage in large numbers thru the pars nervosa into the infundibular extension of the third ventricle. Herring makes the statement that this denotes an increased activity on the part of the pars

intermedia.

Rogowitsch (88) removed the thyroid gland in rabbits and dogs and in eight to fourteen days after the operation he found an increase in the size of the hypophysis. Upon a further study of the cells, he found this increase due to an actual increase in the size of the cells, enlargement of the spaces between the nuclei, increase in the number of vacuoles and the amount of colloid in the cell body as well as a marked dilation of the blood vessels.

Hofmeister in 1892 found that the extirpation of the thyroid gland of young rabbits, the external parathyroids left in situ, was followed by a retardation of growth and the development of a condition of chronic cachexia. In the internal organs, Hofmeister found a condition similar to that described by Rogowitsch in the case of adult rabbits, namely, enlargement of the glandular portion of the hypophysis cerebri and the appearance of large vacuoles in the protoplasm of the enlarged principal cells.

Bredl found an increase of colloid and also that the degree of enlargement varied according to the length of life after the operation. In animals which lived for some time after the removal of the thyroid glands, the hypophysis frequently became two or three times as large as the control. Allen, Adler and Smith have all shown that only the anterior lobe of the hypophysis and the thyroid gland are necessary for the phenomenon of metamorphosis. Allen in his recent work on gland implantation has shown this very conclusively. He finds that a pituitaryless tadpole undergoes metamorphose when the anterior lobe alone of the hypophysis of a mature frog is implanted subcutaneously.

Rodgers (18) working in the Kansas University Zoological Laboratory found that the removal of the thyroid gland increases the size of the anterior lobe of the hypophysis in Rana pipiens. To the writer's knowledge, this is all the work of this nature which has been done on amphibian material.

Atwell (18) in describing the development of the hypophysis of the Anura uses the terms which will be used by the writer in this paper. The same

terms are used by Rasmussen (21) in describing the hypophysis of the woodchuck. The pars intermedia and pars nervosa together are often spoken of as the posterior lobe. The latter term seems a bit unsatisfactory because the pars nervosa is not of epithelial origin but of neural origin. According to Atwell, the hypophysis of the anura consists of three epithelial parts and a neural part. The lobes of epithelial origin are the anterior lobe proper, the pars intermedia and the pars tuberalis. The pars intermedia is always conformed to the extent of the neural lobe.

In reptiles, birds and mammals the pars intermedia is a thin epithelial layer applied to the neural lobe and is derived from the superodorsal wall of Rathke's pocket. Later in life the pars intermedia invades the tissue of the neural lobe to a considerable extent. In the frog also the pars intermedia corresponds to the neural lobe in shape but with the exception that it is round and bulging where it protrudes beyond each side of the anterior lobe.

MATERIAL AND TECHNIQUE

The material used in this experiment was collected near Lawrence Kansas during the spring and summer of 1918. The writer performed almost all the operations. A few thyroidless Bufo and a few control Bufo of an advanced age were secured from Professor Allen. The thyroidless specimens used varied from five to twenty millimeters in body length. The control specimens varied from five to almost thirty millimeters in body length. The largest controls had completely metamorphosed.

The specimens used for measuring the hypophysis were chosen in pairs, a control and a thyroidless. The total length and body length were used as the deciding criteria for pairing. Thecriticism might be made that these measurements vary considerably even in a given number of controls of the same age but surely when a large number of specimens are used and the averages taken this will not be a great enough factor to materially change results. The metamorphosed and nearly metamorphosed Bufo were compared with the largest of the thyroidless specimens.

The following measurements were made in all specimens,- total length, body length and leg length. They were measured and fixed at different intervals in order to secure a full series from the youngest to the oldest. Bichromate acetic, Bouin's and Zenker's preserving fluids were used. After running the specimens up to seventy percent alcohol, the brains were carefully dissected out and five measurements made on the hypophysis. These measurements will best be understood by referring to figures A and B.

This first phase of the work might be called a gross measurement method. Some slides were made from this material and used in the microscopic study. On account of this work being interrupted, as previously explained, much of the material used for the second phase of the work or the microscopic study was secured from Professor Allen during the summer of 1921. He has also placed at my disposal a number of his prepared slides of the brains of Bufo. The operated specimens he gave me were Rana

pipiens.

In preparing this new material the same technique was used as before but the gross measurements of the hypophysis were not made. The sections were cut in a sagittal plane from five to ten micro thick and stained with Heidenhain's iron alum-haematoxylin and haemalum with an eosin counter stain.

It was my purpose to study the differences brought about in the cells of the different lobes of the hypophysis of the thyroidless tadpoles to determine the result of the enlargement and at the same time to give especial attention to the basophile and acidophile cells in the operated as well as control specimens but it was found that to answer these questions would demand a great deal more material than was at my disposal. This study is reserved for a later paper.

In the microscopic study, the three lobes were differentiated and every fifth or tenth section projected by a camera lucida on paper ruled into square millimeters. These squares were

counted, divided by the magnification and multiplied by the thickness of the section and the number of sections. By this method the volume of each gland was ascertained. This also acts as a check on the gross measurements and shows in a graphic manner the size differences in the different lobes as well as size differences in comparing the controls with the operated specimens.

R E S U L T S

A. Gross Measurements.

Five measurements were made on the hypophysis of eighty-five thyroidectomized Bufo and upon seventy-five control Bufo.

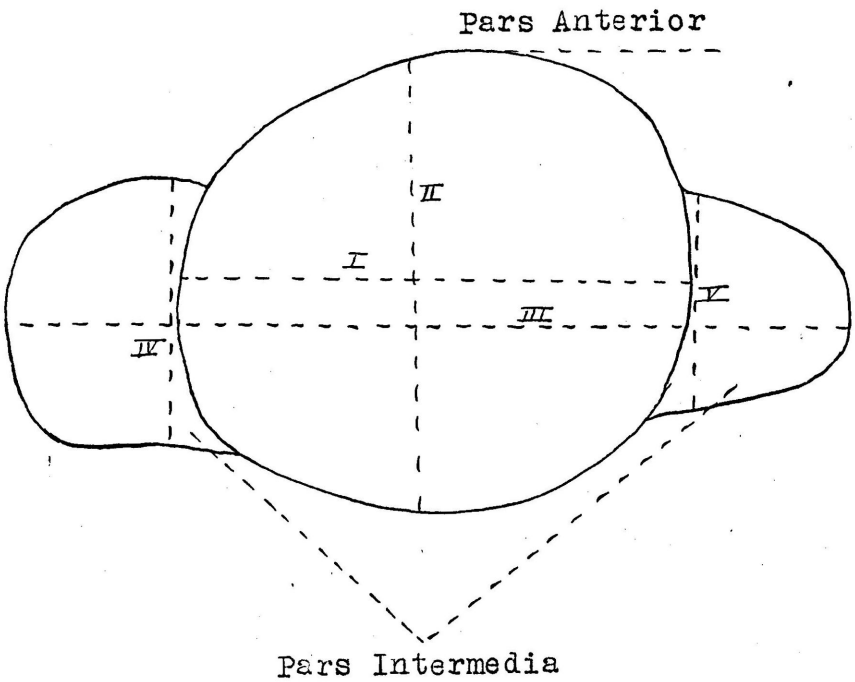
The removal of the thyroid gland prevents metamorphosis and causes the tadpole to retain its larval characters in spite of the fact that it grows in size. The effects of the extirpation can be detected quite early in the incomplete development

of the hind legs as compared with those of a control of a corresponding age. In Bufo this difference is not quite so pronounced as in Rana. In the former the body factors which have an effect on metamorphosis exert their influence for a greater length of time in the absence of the thyroid.(Allen).

In five and six millimeter operated larvae of Bufo the incomplete development of the hind limbs was noted. In many cases they were mere buds. Also the measurements of the hypophysis of the same specimens showed a slight increase over the controls. This increased as size increased.

The accompanying figures A. and B. will serve to illustrate the position of the five measurements made on every hypophysis studied and will at the same time graphically show the immense enlargement that results from thyroidectomy.

A.


Pars Anterior
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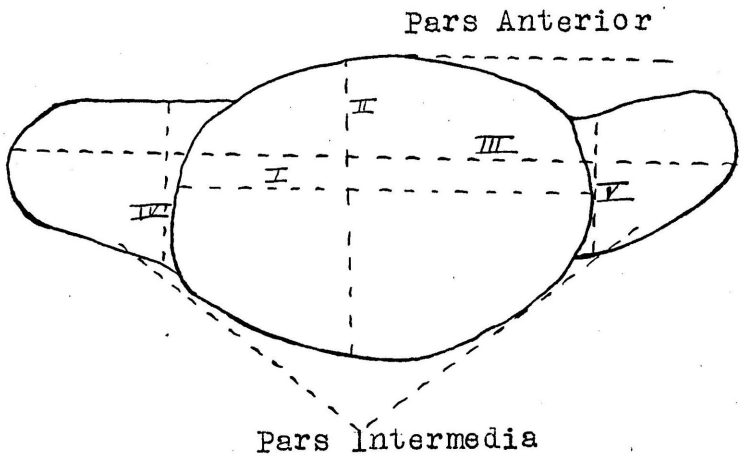
IV
Pars Intermedia

B.


Pars Anterior
II
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I
V
IV
Pars Intermedia

Both of these figures were drawn by means of a camera lucida and at the same magnification. Figure A. is the hypophysis of a thyroidectomized Bufo and B. of a metamorphosed control Bufo. The length of the body of the operated specimen was only 16.2 millimeters as compared with the metamorphosed control whose body length was 28.2 millimeters. In spite of the difference in body size, the hypophysis of the thyroidless Bufo was considerably larger in all of the measurements.

Table I gives the average body and hypophysis measurements of all of the specimens studied.

TABLE I.

Pairs of Specimens	Body measurements in mm			Hypophysis measurements in mm.				
	Total Length	Body Length	Hind leg length	Horizontal Measurement of Anterior Lobe 1	Vertical measurement of anterior lobe 2	Horizontal measurement of Par Intermedia 3	Vertical measurement of Pars Intermedia 4	5
Control	12.1	5.5	.2132	.0792	.0847	.1968	.0382	.0382
Thyroidless	11.0	5.5	.0710	.0874	.0902	.1612	.0464	.0464
Control	16.4	6.8	.7579	.1180	.1204	.2861	.0475	.0475
Thyroidless	13.4	6.8	.2640	.1173	.1246	.2261	.0638	.0656
Control	17.1	7.6	.9713	.1371	.1379	.3045	.0519	.0515
Thyroidless	18.2	7.6	.5774	.1678	.1692	.3087	.0745	.0753
Control	18.9	8.2	5.3020	.1625	.1617	.3564	.0627	.0685
Thyroidless	17.7	8.3	.6835	.1968	.1944	.3481	.0756	.0857
Control	19.9	9.4	5.9329	.1618	.1752	.3578	.0738	.0688
Thyroidless	19.8	9.4	1.5370	.1922	.1916	.3429	.0828	.0879
Control	19.3	10.0	4.568	.1581	.1667	.3365	.0686	.0916
Thyroidless	22.1	10.4	1.885	.1863	.1901	.3280	.1048	.0879
Control	22.3	11.2	6.7258	.1822	.1550	.3660	.0775	.0730
Thyroidless	24.4	11.3	1.3390	.2012	.1960	.3369	.0961	.0984
Control	21.0	12.0	9.2820	.1541	.1755	.4182	.0803	.0803
Thyroidless	25.8	12.4	1.5450	.2230	.2676	.3552	.1049	.0918

As suggested before, the increase of the hypophysis is noticeable in the very youngest specimens and it becomes more pronounced as older and older Bufo are studied. These measurements were all made by means of a micrometer eyepiece at the same magnification. The average lengths of the fore legs were omitted because a very few of even the eleven and twelve millimeter specimens showed the first appearance of a fore limb.

Several of the operated specimens lived a few months past the normal time for metamorphosis. In comparing the hypophysis of these with the hypophysis of metamorphosed and nearly metamorphosed controls, the size differences were so marked that the comparison could readily be made without the aid of a microscope. Table II. contains a few representative pairs out of the dozen or more pairs of such large specimens measured.

TABLE II.

Pairs of Specimens	Body measurements in mm.				Hypophysis measurements in mm.				
	Total Length	Body Length	Fore leg length	Hind leg length	Horizontal measurements of anterior lobe 1	Vertical measurements of anterior lobe 2	Horizontal measurement of pars Intermedia 3	Vertical measurements of Pars Intermedia 4	5
Control 88	14.1	14.1	5.9231	10.684	.1558	.1394	.2870	.0656	.0656
Thyroidless 41	37.0	14.0	-----	9.328	.3280	.4100	.0984	.1312	.1312
Control 44	14.0	14.1	5.684	10.1320	.1476	.1394	.2788	.0574	.0574
Thyroidless 2	30.1	14.2	----	6.0000	.2870	.2788	.4100	.3608	.3608
Control 81	22.0	22.0	15.000	30.200	.3526	.4592	.9348	.1722	.1722
Thyroidless 107	41.8	16.2	----	6.400	.5740	.4920	.6704	.1640	.1640
Control 80	19.0	19.6	12.5000	25.500	.3198	.4428	.9020	.1640	.1640
Thyroidless 101	41.8	19.0	----	4.510	.7954	.8528	1.1024	.3362	.3562
Control 91	26.3	26.3	18.200	33.900	.1344	.3690	.6642	.0984	.0984
Thyroidless 98	43.8	20.0	----	3.280	.8282	.7872	1.2628	.4592	.4920
Control 86	23.0	meta	13.500	25.800	.3280	.3690	.7134	.1640	.1804
Thyroidless 96	42.0	16.4	-----	5.000	.5248	.6150	.9666	.2050	.2050

In these gross measurements the neural lobe could not be measured except as associated with the pars intermedia. The only successful way to measure the neural lobe is by means of microscopic sections. As previously explained the neural and intermediate lobes are very closely associated. In Bufo, especially, the neural lobe protrudes slightly beyond the intermediate but it can only be detected upon exceedingly careful differentiation. This lobe is also comparatively late in development. It does not make its appearance in Rana until the larva is about eighteen millimeters in body length. (Atwell) It probably develops some earlier in Bufo.

The two measurements on the anterior lobe are fairly simple to make but the measurements on the intermediate lobe offer a more difficult problem. In the averages of the third measurement, namely, across the length of this lobe, a discrepancy often appears. This measurement in the control is sometimes as long as the same measurement in the normal. This is true only in younger

specimens and is probably due to the fact that the pars intermedia bulges out beyond the anterior lobe rather irregularly. This is especially true in the operated specimens and probably at first the growth increase is more in depth than length.

B. MICROSCOPIC STUDY

Now that it has been shown by gross measurements that the pars anterior and pars intermedia enlarge because of the removal of the thyroid gland it becomes of interest to test the same point by a study of serial sections of normal and thyroidectomized specimens. As suggested previously, successful gross measurements of the neural lobe cannot be made and therefore especial attention was given to the study of this lobe in the microscopic sections.

The serial sections of twenty-one hypophyses were studied. Of these, nine were controls and

twelve operated Bufo and Rana. For the sake of convenient comparisons, these specimens were divided into three groups, large, medium and small.

The Hammar paper method was used for determining the volumes of the different lobes. This method is explained earlier in this paper.

TABLE III

	Body Measurements	Volume of anterior lobe	Volume of intermediate lobe	Volume of neural lobe	Number of sections in series
	LARGE				
Control Bufo 17.15	Body length 11.9 Total length 11.9	.004250	.001420	.002285	36
Control Bufo 17.17	Body length 11.7 Total length 11.7	.001510	.000988	.0008639	50
Control Bufo 17.16	Body length 11.0 Total length 11.0	.003400	.002092	.001164	37
Thyroidless Bufo 19.76	Body length 21.6 Total length 43.4 Hind leg length 5.3	.198585	.08675	.021180	127
	MEDIUM				
Control Bufo 17.19	Body length 10.1 Total length 20.8	.002412	.000954	.001085	33
Control Bufo 17.18	Body length 11.6 Total length 23.5	.002800	.001600	.001185	30
Thyroidless Bufo 19.86	Body length 18.2 Total length 23.5	.018000	.014607	.003772	63
Thyroidless Bufo 19.71	Body length 19.0 Total length 43.9	.043510	.019025	.002890	71
Thyroidless Bufo 19.72	Body length 19.1 Total length 43.0	.030451	.015839	.006693	86
	SMALL				
Control Bufo XVIII	Body length 9.2 Total length 23.0	.001520	.000885	.001015	45
Control Bufo II	Body length 27.0 Total length 11.0	.002085	.001255	.001285	45
Thyroidless Bufo XXXI	Body length 9.5 Total length 22.0	.008135	.007985	.001055	65
Thyroidless Bufo VI	Body length 11.2 Total length 27.9	.016340	.006185	.000756	61

TABLE IV

	Body Measurements	Volume of anterior lobe	Volume of intermediate lobe	Volume of neural lobe	Number of sections in series
Control Rana XI	Body length 14.0 Total length 10.0	.001599	.001326	.001525	45
Control Rana B	Body length 21.8 Total length 24.3	.003950	.003775	.003795	57
Thyroidless Rana XI	Body length 25.0 Total length 11.2	.0115695	.008269	.0011329	106
Thyroidless Rana #3	Body length 20.1 Total length 40.3	.016160	.018656	.003275	77

In a comparison of Control XVIII and thyroidless XXXI specimens, the difference in volume of the anterior lobe and pars intermedia is already quite noticeable. These were the youngest specimens studied. In the older specimens the enlargement of the two lobes was simply enormous. Plates I and II are the camera lucida drawings of sections of the hypophysis of a metamorphosed Bufa and a thyroidectomized Bufo. The control is specimen Number 17.15 in Table III and the thyroidectomized specimen is Number 19.71 in the same table. The body length of the control was 11.9 millimeters and the same measurement for the operated specimen was 19.9 millimeters. These were all drawn at the same magnification and bring out very clearly the size comparisons of the different lobes and especially the enlargement of the lobes in the thyroidectomized Bufo.

Every fifth section in the series was drawn. The hypophysis of the control consisted of thirty-six sections and of the operated there were seventy-three sections each cut 10 μ thick.

In all sections of the hypophysis of the controls the three lobes were decidedly easy to differentiate. The anterior lobe was the largest in every case. In the majority of sections, the pars intermedia is as large as or slightly larger than the neural lobe. There is probably very little actual size difference in the two.

In all of the serial sections of the operated specimens there were several more sections than in the controls of corresponding age. This shows an actual size increase. In all but one of the thyroidectomized specimens (Rana #3), the anterior lobe has a greater volume than the intermediate lobe but the intermediate lobe is especially large when compared with the same lobe of a control specimen.

The neural lobe is very difficult to differentiate in the operated tadpoles. In many sections it was almost impossible to distinguish a trace of neural material. In many sections if there was

such material it was loose and scattered along the margin of the infundibulum and not collected into a mass which could be differentiated as a lobe. The pars intermedia apparently occupies the position of the combined intermediate and neural lobe of the control. Undoubtedly the neural lobe, because of its normally late development and because of the interference of this normal development by thyroidectomy, does not develop to any appreciable extent. Conditions comparable to this are noticed in the study of other structures in thyroidless tadpoles.

CONCLUSIONS

I. The extirpation of the thyroid gland causes the hypertrophy of the anterior lobe proper and the pars intermedia of the hypophysis in Bufo americanus and Rana pipiens.

II. In younger larvae the neural lobe is quite distinct but it has lost its sharp outline in Bufo of nineteen millimeters in body length while in older specimens, if such a lobe can be differentiated, it is very small.

III. Thyroidectomy causes a continuation of larval characteristics in the amphibia and hence the neural lobe is hindered in its growth because of its normally late development. Probably in the oldest thyroidectomized specimens material from the enlarged intermediate lobe has migrated into the small amount of neural tissue present and for that reason the two appear inseparable in many sections.

IV. In normal controls, the neural lobe and pars intermedia are of about the same size. The intermediate lobe is probably somewhat larger. The anterior lobe is considerably larger than either of these two.

V. The anterior lobe of thyroidectomized Bufo which have lived past the normal time for metamorphosis is usually at least twice as large as the pars intermedia, and the pars intermedia several times larger than the neural lobe.

PLATE I.

Drawings of every fifth section of the hypophysis of a metamorphosed Bufo. All figures were drawn with the aid of a camera lucida. A Bausch and Lomb 3 mm. objective and ocular 10 were used.

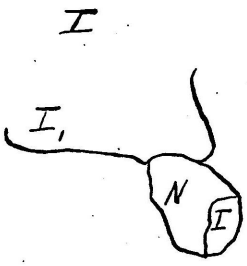
Abbreviations:

A. Anterior lobe

I. Intermediate lobe

N. Neural lobe

I_i Infundibulum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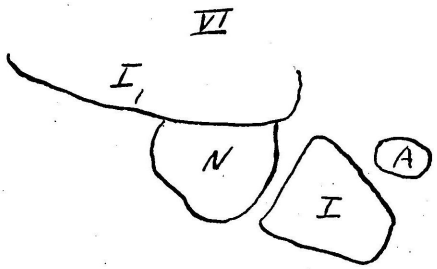
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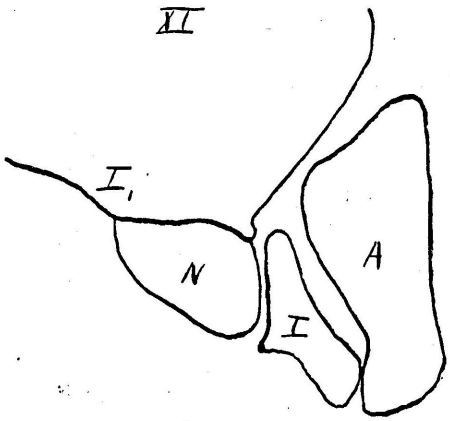
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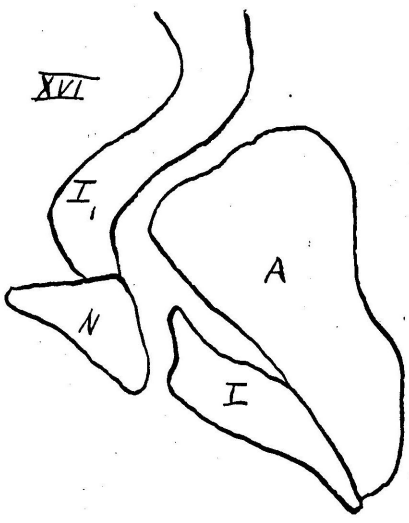
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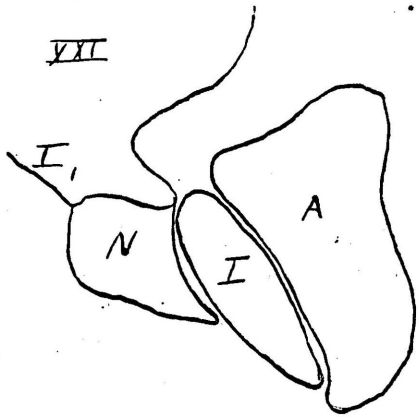
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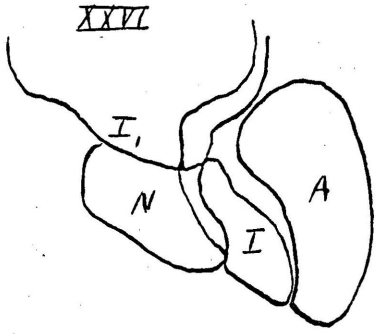
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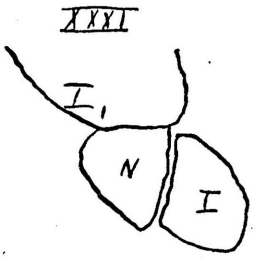
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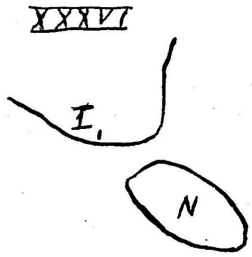
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PLATE II.

Drawings of every fifth section of the hypophysis of a thyroidectomized Bufo. All figures were drawn with the aid of a camera lucida. A Bausch and Lomb 3 mm. objective and ocular 10 were used.

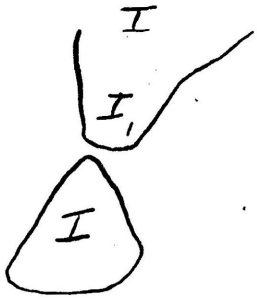
Abbreviations:

A. Anterior lobe

I. Intermediate lobe

N. Neural lobe

I_1. Infundibulum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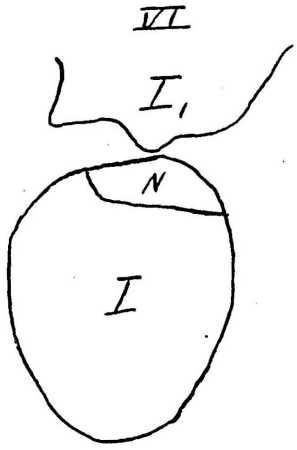
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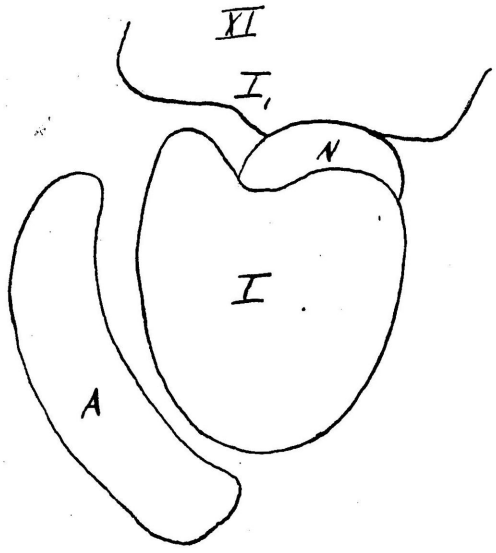
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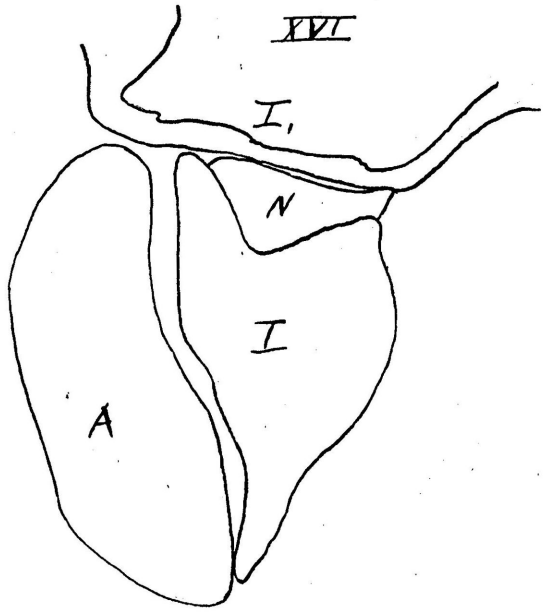
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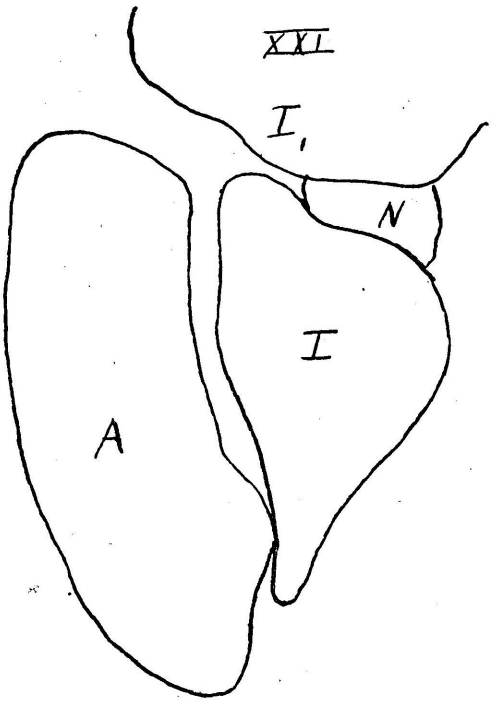
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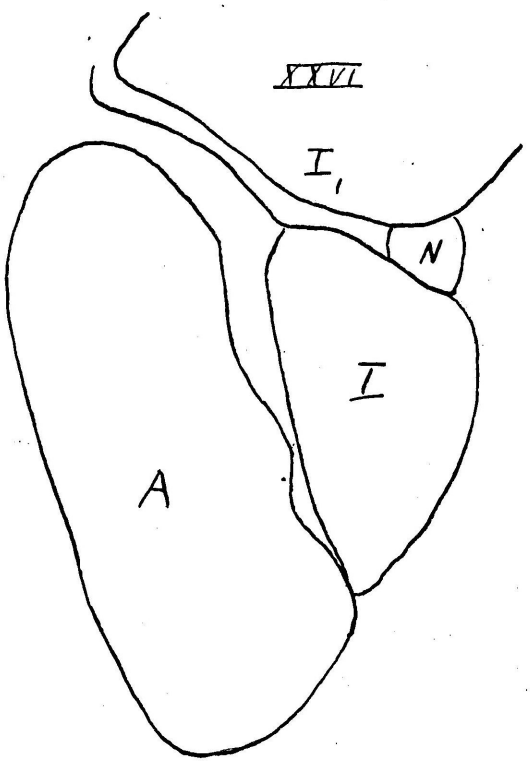
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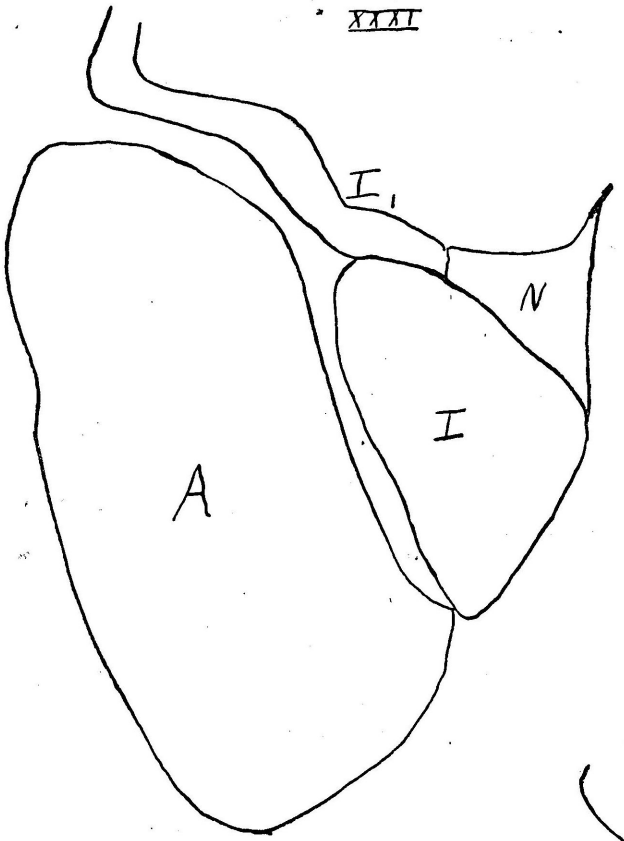
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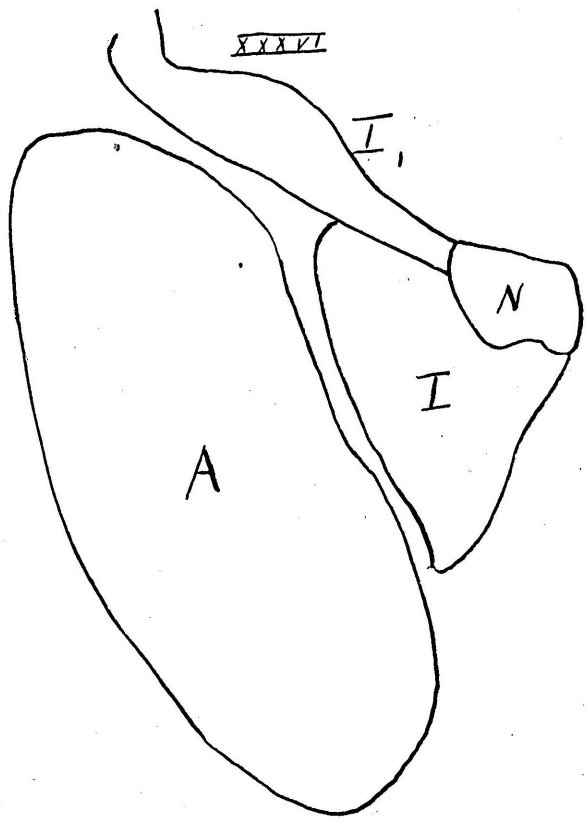
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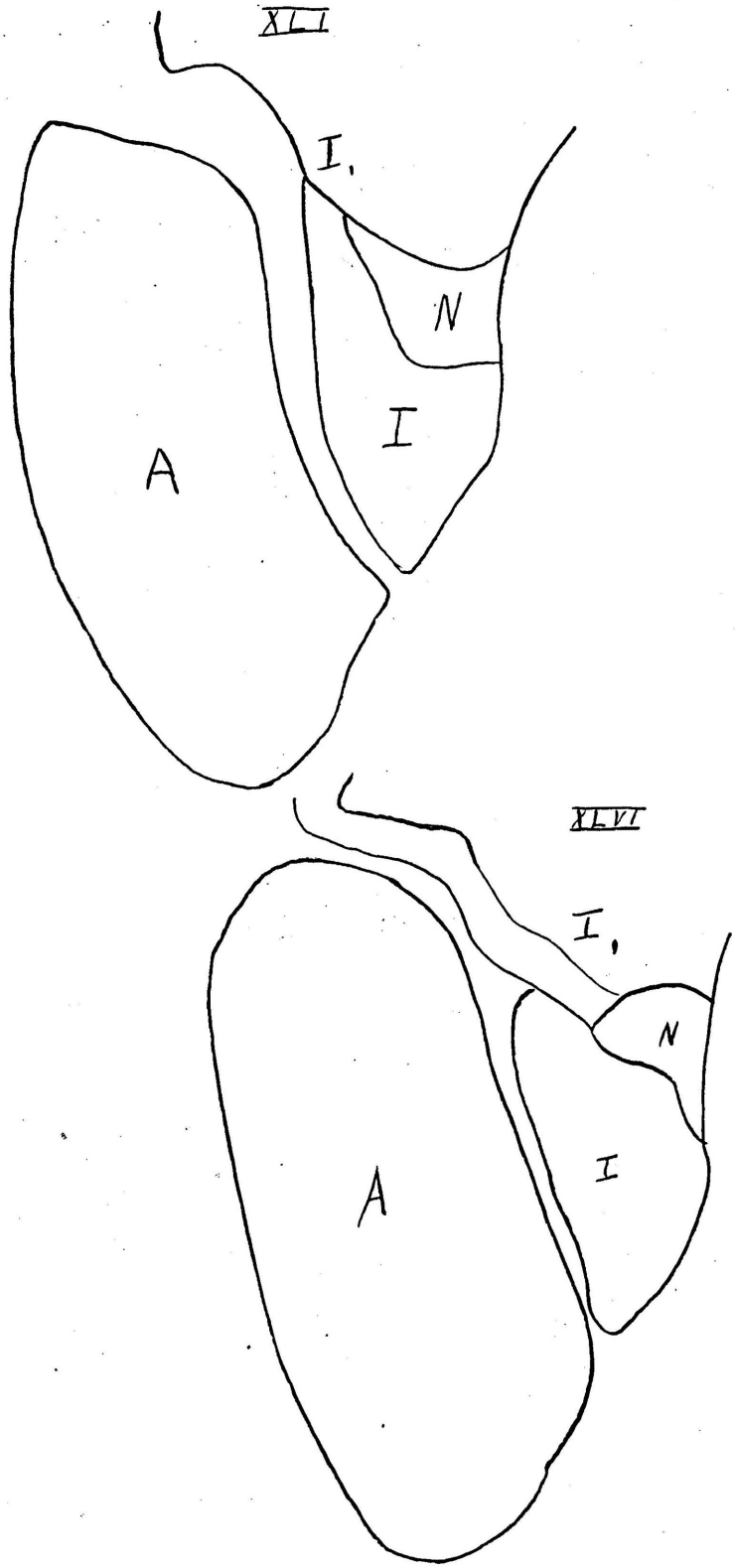
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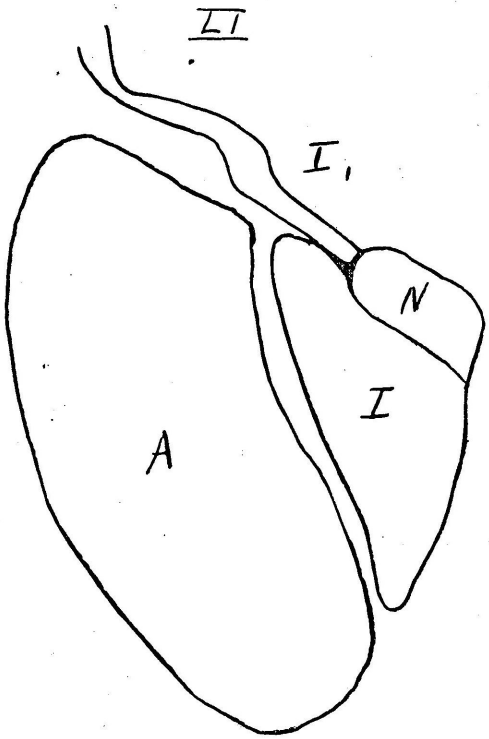
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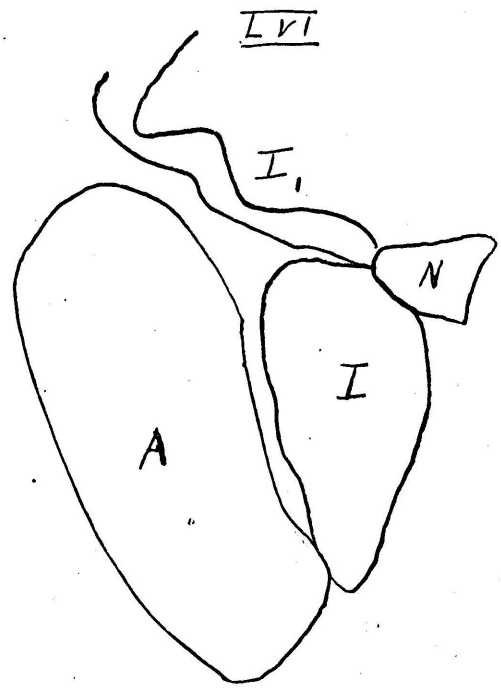
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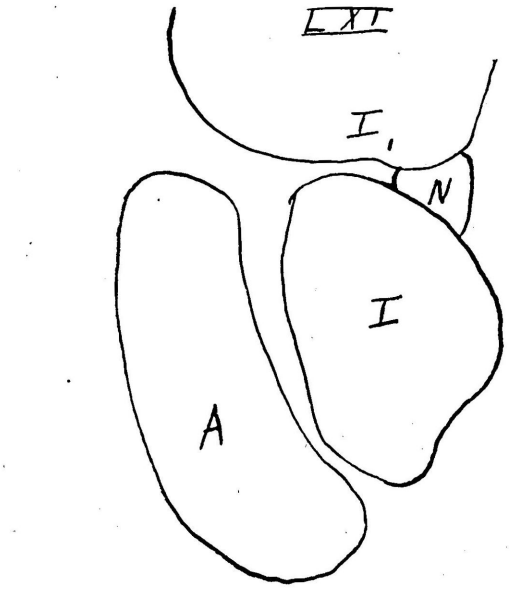
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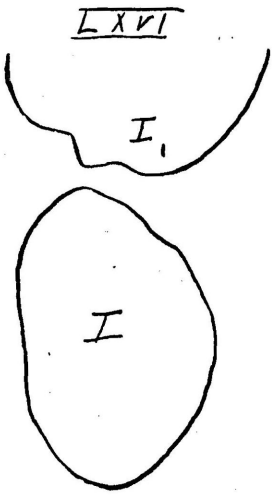
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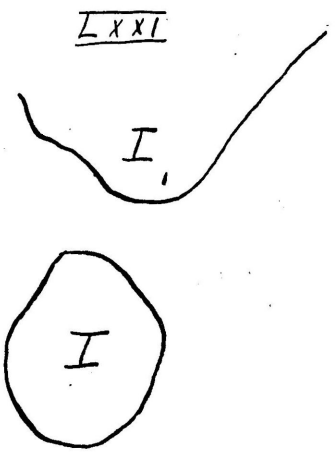
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