A STUDY OF PITUITARY CYSTS IN ANIMALS DEPRIVED OF VITAMIN A

by

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TABLE OF CONTENTS

INTRODUCTION

This investigation was planned to determine if a relationship exists between the deficiency of vitamin A in the diet and the occurrence of cysts in the pituitary gland and also to study the nature of such cysts when they occur. It was observed previously, in connection with another investigation in this laboratory (Monson (8)), that pituitary cysts occurred in young chickens deficient in vitamins A and E.

In reviewing the literature it has been found that cysts have been observed in the pituitaries of a variety of animals including the cow, cat, dog, horse, rabbit, rat, hedgehog, pig, chicken, guinea pig, toad, and man. Similar cysts have also been described by Zahn (16) in 1896 in the esophagus, lungs and liver. However, the description of cysts of the pituitary was only incidental in most of these investigations. In general, their main purpose was to study the morphology and histology of the gland as a whole, and, although various theories were advanced as to the origin of the cysts, the frequency of their occurrence in normal and abnormal animals was not considered.

REVIEW OF LITERATURE

Occurrence of the Cysts

Man. The names of the following men and observations on

their findings were taken from Beck (1):

The earliest description of a pituitary cyst was one by Bonnet (Sepulchretum) in 1679. It seems probable that this description is not typical of the ciliated pituitary cysts with which this investigation is concerned, but rather of a teratoma or tumor, as it was described as containing two pounds of water. Another cyst was described by Abercrombie (Krankheiten des Gehirns und Ruckenmarks) in 1821 in an officer as being as large as a hen's egg, having a purulent content, and connecting with the infundibulum between the optic nerves. Rokitansky (Path. Anat. 3 Aufl., bd. II, s. 476) described a swelling coming from the pituitary filled with haemorrhagic contents which was destroying the sphenoid bone and projecting into the pharyngeal cavity. Zenker (Virchow's Archiv bd. 7, 1857) considered these structures to be simple cysts, but Beck (1) believed them to be teratomata. Weichselbaum (Virchow's Archiv bd. 75, 1879) found, in both the anterior and posterior lobes of an 86 year old man, colloid cysts with ciliated epithelia.

The names of the following men and observations are included in Trautmann (14):

Virchow (Untersuchungen über die Entwicklung des Schädelgrundes. Berlin, 1857) described a follicular structure in the anterior lobe. Luschka (Der Hirnanhang und die Steissdrüse. Berlin, 1860) found round cells with a finely granular protoplasm and one or two nuclei located in round or tubular vesicles. The vesicles were lined by cells bearing cilia. Gentes (Sur les

rapports et la situation dela tige pituitaire. Gas. nebd. Sc. med'. N. 14, Bordeaux, 1903) and Pirrone (Contributo sperimentale allo studio della funzione dell'ipofisi. Riforma Med. 1903) reported nervous elements in the wall of the cysts, but Trautmann (14) was unable to verify this. Henle (Über das Gewebe der Hypophyse und Nebenniere. Zeitschr. f. ration. Med. bd. 24, reihe 3), Müller (Über Entwicklung und Bau der Hypophyse. Jenaische Zeitschr. f. Medizin. bd. 6. 1871), Krause (Mikroskopische Anatomie. Hannover, 1876), and Launois (Recherches sur la glande hypophysaire de l'homme. Th. Sc. Doct. Univ. Paris, 1904) found ciliated colloid cysts occurring in the residual lumen. The significance of the ciliated cells was not clear to Trautmann (14).

Lotheringer (6) found tubules and cysts lined with cylindrical epithelium and filled with colloid contents in both children and adults. In some places the cylindrical epithelium was greatly flattened.

According to Byrant (2), the cells composing the lining of the cysts were sensory elements.

Rasmussen (11) found in the cysts of two human cases that the ciliated cells were irregularly distributed. In places, the basement membrane was increased to a well-developed layer of connective tissue. Sometimes there was a layer of round cells interposed between this connective tissue and the columnar cells, making the epithelium two-layered. Occasionally he also saw long, columnar cells with numerous fine cilia.

Fulstow (5) described a cystic tumor situated between the anterior and posterior lobes of the pituitary gland. It was unilocular, filled with a soft yellow gelatinous material and lined by stratified squamous epithelium in which there appeared, here and there, ciliated cells and cells containing secretion.

Cattle. Dostoiewsky (4) found, in 1886, cavities lined with ciliated epithelium in the anterior lobe of the hypophysis.

Trautmann (14) found such cavities in older animals but was unable to see any ciliated epithelium.

In more recent times (1942), Madsen, Hall, and Converse (7) saw pituitary glands with cysts in young beef and dairy cattle which were deficient in vitamin A and showed that the later feeding of vitamin A did not cause the repair of the cysts.

Dog. In 1886 Lothringer (6) found many small cysts where the epithelium of the pituitary was at its thickest. He determined their diameter to be 20 to 60 micra. The content of these cysts was a fine grained material composed of rudiments of cells. He did not mention seeing cilia.

Later, in 1913, Stendell (12) found many cysts in the middle lobe of the pituitary of a growing dog. These cysts lay intermingled with cut-off outpocketings of the hypophysis and were filled with colloid or with degenerating cells.

According to Trautmann (14), colloid cysts are never lacking in the dog.

Cat. Lothringer (6) reported, in 1886, that pituitary cysts occur only exceptionally in the cat.

Stendell (12) found, in 1913, numerous cysts filled with clumps of degenerating cells.

In 1909 Trautmann (14) observed them almost without exception in the epithelial layer of the cat's pituitary. These cysts were bordered with cylindrical cells and were filled with colloid.

Rat. Stendell (12) reported colloid cysts in the pituitary of the rat, but he did not see any ciliated lining.

In 1940 Opper (9) studied 139 rat pituitaries and found the incidence of cysts to be 10 per cent in the anterior lobe. All were lined with ciliated epithelium except two. The largest cyst that he found was 160 micra in length.

Rabbit. Lothringer (6) found numerous cysts in the pituitaries of rabbits. The cysts were filled with a colloid mass and lined with very long cilia. The cells of the ciliated epithelium were cuboidal, more flattened in the larger cysts than in the smaller ones. Lothringer (6) considered this to be due to the extension of the larger cysts by their contents. In one cyst the cilia were lacking in one part of the circumference of the cyst wall.

Stieda (13) also observed that many colloid cysts were lined, in part, with ciliated epithelium.

Chicken. In 1926 Collins (3) found cysts of the pituitary gland to be rather common in domestic chickens. He described the cysts as being quite small, yet visible to the naked eye. The cysts were lined by a discontinuous ciliated epithelium. In some places the cells of this epithelium were cylindrical,

ciliated, with oval nuclei and firmly implanted cilia. Among these cells were typical mucous cells with nuclei thrown back toward their base. Between the ciliated cells and the mucous cells were all intermediate stages of development. Some of the mucous cells were very large and Collins (3) considered that they arose from the simultaneous mucous transformation of many neighboring cells. In places the ciliated epithelium was made up of very slender cells separated from each other by intercellular fissures which opened into the interior of the cyst cavity. In other places the epithelium was cuboidal or flattened and without cilia. Occasionally the epithelium had disappeared completely. Collins (3) stated that these cysts were distinct from the colloid vesicles which other authors have considered different forms of the same structure.

In 1939 Rahn (10) found the same types of cysts in chicken pituitaries.

In 1940 Monson (8) observed the cysts lined with a cuboidal or squamous epithelium which was sometimes slightly stratified and usually bordered with cilia. She found these cysts in approximately three-fourths of all the glands which she studied. The smallest diameter of the cysts ranged from a few micra to 70 micra in the larger ones and a few cysts occupied almost one-fourth of the cross-sectional area of the entire anterior lobe.

Horse. Lothringer (6) observed cysts in the horse similar to those which he saw in the dog. The cysts were filled with colloid and, although they were very small, were sometimes vis-

ible to the naked eye.

Guinea Pig. Vanderburgh (15), in 1917, observed cysts in the pituitaries of guinea pigs but denied the presence of colloid within the vesicles. He described the contents, rather, as a granular mucoid substance. He found the vesicles usually lined with ciliated epithelium.

Hedgehog and Toad. Stendell (12), in 1913, reported that colloid cysts were numerous in both the hedgehog and the toad.

Origin of the Cysts

Beck (1) considered the origin of the pituitary cysts to be a fetal malformation. Lothringer (6) thought the cysts were outpocketings from the hypophysis cavity and that the colloid content was the product of transformation of chromophil cells. Stendell (12) also believed the cysts to be the result of the cutting-off of outpocketings from the hypophysis cavity. Rasmussen (11) stated that, though their origin was uncertain, he considered them to be from hypophseal tissue or naso-pharyngeal elements. Fulstow (5) believed the origin of the cysts to be traceable to remnants of the hypophyseal duct. Gentes and Pirrone (as quoted in Trautmann (14)) considered that the cysts originated from sensory elements in the pituitary. Opper (9) regarded the cysts as arising from Rathke's pouch and as not uniformly inheritable.

Other Ciliated Cysts

In 1896 Zahn (16) found that cysts which were entirely or partly lined with ciliated epithelium occurred in different regions of the head and trunk. He found the cysts in the brain, in the wall of the naso-pharyngeal cavity, in the upper jaw, along the middle and sides of the neck, in the region of the foramen caecum, at the bottom of the oral cavity, in the wall of the esophagus, in the mediastinum, mesentery, liver, ovary, ligamentum latum, uterus, naval, and lungs. He believed that the cysts of the esophagus, lungs, and liver were congenital structures, and that this assumption might be correct for all other cysts lined with ciliated epithelium except for those of the upper pharyngeal wall which he stated were probably retention cysts developed during later life.

MATERIAL AND METHODS

Young chickens and rats were the experimental animals used for this investigation.

Chickens

The chickens were Single Comb White Leghorns and were obtained at an age of two days from the Missouri State Hatchery. They were divided into two groups. One group, consisting of 21

chickens, was used as a control group. The other group of 22 chickens was the experimental group. The two groups were placed on diets simultaneously and kept on the diets for a period of two months. By the end of this time, the difference in the appearance of the two groups was marked enough to show that a deficiency of vitamin A had resulted in a difference in the development of the experimental group.

The basic diet used was the same for both groups except that the control group received vitamin A in addition, and the experimental group received no vitamin A. The basic diet was as follows:

	Per cent
Casein, dry	18
Osborne-Mendel salt mixture	4
Corn starch	36
Rice, whole grain	20
Dried brewers' yeast	10
"Delsterol" (vitamin D)	2
Lard	5
Oat hulls, ground	5

At the end of the two month period the chickens of both groups were killed by cervical dislocation and their pituitaries were removed. The pituitaries were immediately dropped into the fixing agent, Susa, and the following procedure was used to prepare them for sectioning:

1. Susa — overnight

2.	Dioxan 50%, water 50%	2 hours
3.	Dioxan (a few drops of iodine were added until no more decolorization occurred)	2 hours
4.	Dioxan (1 part), warm paraffin (2 parts), incubated at 45°-50° C.	2-4 hours
5.	Paraffin (45°-50° C.), 5-7 changes	20 minutes for each change
6.	Paraffin (55°-58° C.), imbedded	

The imbedded pituitaries were then placed on blocks and sectioned at six micra. The entire pituitaries in serial sections were then stretched on slides with a thin layer of egg albumin and stained.

Two kinds of stains were used, Delafield's haematoxylin with eosin as a counter stain and Mallory's triple stain. The procedure followed for haematoxylin-eosin was as follows:

1.	Remove paraffin with xylol	time varies
2.	Place in xylol 50%, absolute alcohol 50%	5 minutes
3.	Place in absolute alcohol	)
4.	Place in 95% alcohol	)
5.	Place in 85% alcohol	)
6.	Place in 70% alcohol	) each 1 minute
7.	Place in 50% alcohol	)
8.	Place in 35% alcohol	)
9.	Place in distilled water	5 minutes
10.	Stain in haematoxylin	2 minutes
11.	Rinse in tap water until dark blue	)
12.	Destain in 2% solution of HCl in water	) time varies

13.	Rinse in tap water until blue again	)
14.	Rinse in distilled water	)
15.	Rinse in 35% alcohol	)
16.	Rinse in 50% alcohol	) each 1 minute
17.	Rinse in 70% alcohol	)
18.	Rinse in 85% alcohol	)
19.	Counter stain in eosin until cytoplasm is faintly pink	time varies
20.	Rinse in 90% alcohol	)
21.	Rinse in 95% alcohol	) each 1 minute
22.	Rinse in absolute alcohol	)
23.	Rinse in xylol 50%, absolute alcohol 50%	2 minutes
24.	Rinse in xylol	5 minutes
25.	Cover with a number one cover slip using clarite and allow to dry	

The following procedure was used in staining with Mallory's triple stain:

1-9.	Follow the same steps as for haematoxylin-eosin	
10.	Stain in Mallory's number one	2 minutes
11.	Stain in Mallory's number two	2 minutes
12.	Destain and differentiate in 90% alcohol	time varies
13.	Rinse in 90% alcohol	)
14.	Rinse in 95% alcohol	)
15.	Rinse in absolute alcohol	) each 1 minute
16.	Rinse in xylol 50%, absolute alcohol 50%	)
17.	Rinse in xylol	5 minutes
18.	Cover with a number one cover slip using clarite and allow to dry	

The slides were then ready for examination under a binocular, compound microscope. They were examined a first time under low power merely to locate and count the cysts and later under oil immersion to study the structure of the individual cysts. Measurements of the cysts were made under high power with a calibrated ocular micrometer.

Rats

The rats used were an inbred strain of Wistar Institute white Swiss rats obtained originally from the H. L. Snyder Memorial Research Foundation, Winfield, Kansas. They were studied in two major groups, each one containing a control and an experimental group of animals. The reason for studying the rats in this fashion was that it was difficult to obtain them in large enough numbers at one time at the right stage of development.

For the first experiment, recently weaned young rats were divided into a control group and an experimental group of 12 rats each. The control group received the basic diet (see page 9) with vitamin A added and the experimental group received the basic diet alone. They were kept on the diet until it seemed advisable to kill them before some of the experimental rats died. These first 24 rats were killed on November 2, 1949, after having been on the diet a little over one month. An indication as to the effectiveness of the vitamin A in the control rats is

shown by the difference in their average weights at the time of killing them.

Control group	Experimental group
165.6 gms	131.7 gms

In the second experiment rats were selected in the same way as previously and divided into a control group of eight and an experimental group of six. They were fed the same respective diets and at the time of killing a comparison of their weights was as follows:

Control group	Experimental group
204.1 gms	101.7 gms

Both groups of rats, those sacrificed on November 2 and those sacrificed on November 30, were killed with lethal doses of chloroform.

The rat pituitaries were dissected out, fixed, sectioned, stained, and examined as were the chicken pituitaries.

RESULTS

Comparison of the Cysts

Chicken. Out of a large number of cysts taken at random in equal numbers from both the experimental and control groups, the following averages were made from measurements of three dimensions, the greatest and smallest diameter of the cross section of the cyst (called height and width respectively) and the

duration of the cyst through a number of sections (length):

	Height (micra)	Width (micra)	Length (micra)
Control group	78.8	59.9	101.2
Experimental group	82.2	66.0	110.1

The number of cysts per chicken was also determined on the basis of the entire number found in both the experimental and control groups. The averages are as follows:

Control group 2.48 cysts per chicken

Experimental group 3.50 cysts per chicken

Rat. Although, in the rats, 17 pituitaries out of the 38 studied contained one or more cysts, only 5 of these cysts were the typical ciliated type, the others being "colloid cysts". This means that, although the occurrence of cysts in the rats was 42.1 per cent, only 3.1 per cent of these were ciliated. As these 5 ciliated cysts were too small a number to make highly significant averages of their measurements, the measurements of each are given as follows:

	Height (micra)	Width (micra)	Length (micra)
Control group	127.5	90.0	157.5
	84.0	82.5	87.5
	42.5	40.0	66.0
Experimental group	35.0	27.5	42.5
	200.0	126.0	237.5

The number of cysts (both ciliated and "colloid") per rat

was determined on the basis of the combined experiments as follows:

Control group	4.05 cysts per rat
Experimental group	4.16 cysts per rat

Description of the Cysts

Chicken. The cysts observed in the anterior lobe of the pituitary gland were well defined structures. Their walls were composed of cells arranged in a more or less characteristic fashion. The cells were cuboidal or columnar and sometimes slightly stratified. They were arranged, if cuboidal, in neat rows tightly fitted together so that the wall of the lumen presented a continuous surface; if columnar, they were arranged with their long axes perpendicular to the lumen, their short sides bordering it, and their nuclei back toward the base of the cell; i.e., on the side away from the lumen. The nuclei of the cuboidal cells were placed centrally.

From the lumenal border of these cells, cilia projected. These cilia appeared to be firmly implanted in the surfaces of the cells. In places, the cilia from a single cell seemed to adhere to each other, forming tufts. In other places, the cilia from a row of cells appeared to be continuous and were all leaning in the same direction. In some sections of the cysts, the cilia lined the entire lumen. In others, the ciliated portions of the wall were very discontinuous and perhaps lacking alto-

gether.

Among the cells which composed the wall of the cyst, occasionally a few were outstanding from the others. These cells differentiated themselves by taking a much heavier stain (whether stained by haematoxylin-eosin or Mallory's triple stain) similar to that taken by the basophilic cells elsewhere in the gland.

The contents of these cysts was a mass of somewhat particulate matter, resembling cellular debris, staining only lightly, and sometimes containing dense clumps of heavily staining matter. In some cysts the contents had the appearance of oily droplets or seemed to stick to the walls of the lumen.

Such cysts, with the cells composing the walls arranged in a definite pattern throughout the cysts, were the rule in the chicken and were found usually at the edges of the anterior lobe. Thirty-five chicken pituitaries out of the 43 studied contained one or more of them. This is an occurrence of 81.5 per cent.

Rat. Although the ciliated cysts described above were the only kind of cyst found in the chickens, only an approximate 3.1 per cent of all the cysts studied in the rats had the characteristic architecture of wall or possessed cilia. The remainder of the cysts were scarcely to be recognized as such and were often very numerous. As many as 36 occurred in one pituitary. These cysts (if they indeed were cysts) had no well defined cellular architecture making up their walls but seemed to be random spaces in the cells of the anterior lobe. Cysts of this type were very small, being only a few micra in any diameter.

Their contents were also different from the ciliated cysts by being a smoother, more compact and homogeneous mass, sometimes adhering to the edge of the lumen by many threadlike connections.

These "colloid" cysts were sometimes very abundant in a single pituitary, sometimes mixed with the ciliated type. The ciliated type might be present alone, or no cysts might be present at all. This last situation was found to be the case more often than not, as can be seen by the fact that, out of 38 rat pituitaries studied, only 17 contained cysts of any kind. Seventy-five cysts were found in the experimental group and 81 in the control group. The ciliated and "colloid" cysts were approximately equally divided between the two groups.

DISCUSSION

The description of pituitary cysts in chickens will be seen to be similar to the one given by Collins (3) in which he stated:

> The interior surface of the collagen capsule is lined, not be a continuous epithelium, but by some patches of ciliated epithelial cells of various importance. In certain of these patches the cells are cylindrical, ciliated, with oval nuclei, and the flagella are implanted on a thick plateau, irresolvable by the methods employed. Among these cells, one could distinguish, at the first glance, some typical mucous cells, calciformed, with nucleus thrown back towards the base....Elsewhere, also, the epithelium was cuboidal or flattened, deprived of flagella. Finally, in places, the epithelium had disappeared by desquamation....

and also the one given by Monson (8) in which she stated:

> Nearly all cysts were lined with a simple cuboidal or squamous epithelium; slight stratification appeared in some cases. The majority of the cells bordering the lumina possessed long cilia.

References to both kinds of cysts in the rat were found in the literature. Stendell (12) stated:

> There arise cystic cavities which are filled with colloidal, condensed secretion....The 'colloid cysts', as the spaces arising on account of the secretion were named, are not to be confused with the above mentioned remnants of the hypophysis cavity outpocketings. These latter show a clear epithelium which, in the rabbit, is trimmed with cilia.

Opper (9) stated:

> One-hundred and thirty-nine rat pituitaries were studied in serial section. Fourteen (ten per cent) were found to contain epithelial cysts of the anterior lobe. With the exception of two, all were lined with ciliated epithelium....The largest cyst in these rats was 160 micra long. The material in the lumen was mucinous.

Both the "colloid cysts" and the ciliated cysts have been found commonly in a variety of animals. This is evidenced by the following descriptions:

Dostoiewsky (4),

> ....I found in cattle in different sections of the anterior lobe, different large cavities, covered with cells with cilia. One can find similar cavities in different sections of the anterior lobe of the hypophysis.

Lothringer (6),

> In the hypophysis of the rabbit cysts are found abundantly which often reach a considerable size.... The content is partly the familiar colloid mass, partly, in some of them, a granulated cell-containing material like that found in the branchings of the hypophysis cavity of the dog. The lining of the cysts is a ciliated epithelium with very long and remarkably strong cilia. The cells of this ciliated epithelium are cuboidal; in the larger cysts they are

> expanded by much secretion and appear shorter than in the smaller. In one cyst the cilia were lacking in one part of the circumference....

Fulstow (5), (man),

> The cystic tumor described was situated between the anterior and posterior lobes of the pituitary. It was unilocular and filled with soft yellow gelatinous material and lined by stratified squamous epithelium which formed papillary projections and which showed a tendency to variation by the presence of ciliated cells and cells containing secretion.

Similar descriptions can be found concerning other animals as has been mentioned in the "Review of Literature".

These authors agree with the findings of this investigation on the following points:

1. The cells of the epithelial lining were columnar or cuboidal and sometimes slightly stratified.

2. The ciliated portions of the cyst wall were discontinuous or lacking occasionally.

3. The contents of some cysts resembled cellular debris.

4. Some cysts contained a colloidal condensed mass and showed no ciliated lining.

CONCLUSIONS

Ciliated cysts of the anterior lobe of the pituitary gland occurred commonly in young chickens and rats. The ciliated type had a definite wall and was more common in the chicken than it was in the rat. In the chicken the ciliated cysts occurred as 100 per cent of the cysts found; in the rat the ciliated cysts

occurred as about 3.1 per cent of the cysts found. Of the chicken pituitaries studied, 81.4 per cent contained cysts. Of the rat pituitaries studied, nearly half that many, or 42.1 per cent, contained cysts. Whether or not the animal was deficient in vitamin A seems to have had no bearing on the occurrence of the cysts or their size.

Whether or not the occurrence of ciliated cysts in the pituitary is due to other dietary deficiencies, inheritance, or abnormalities in embryonic development are left for further investigation.

ACKNOWLEDGMENTS

The author is indebted to Dr. E. H. Herrick for his suggestions and assistance throughout the course of this investigation, to Mrs. Milton Snow for her translation of Collins (3) which was in French, to Assoc. Prof. D. F. Munro for his kindness in helping the author translate the articles in German, and to the N. L. Snyder Memorial Research Foundation of Winfield, Kansas, for supplying the rats.

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APPENDIX

EXPLANATION OF PLATE I

Fig. 1. Chicken pituitary, slide 18 B, experimental.

Fig. 2. Chicken pituitary, slide 14 A, control.

PLATE I

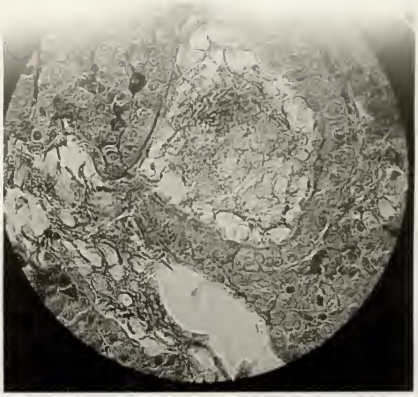

Fig. 1

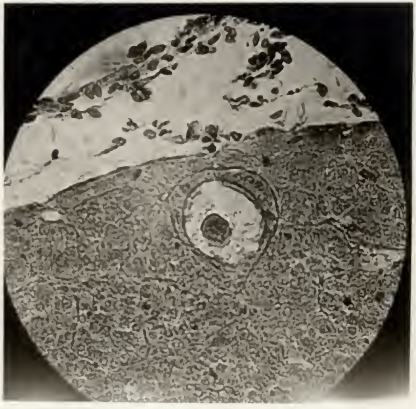

Fig. 2

EXPLANATION OF PLATE II

Fig. 1. Chicken pituitary, slide 22 B, experimental.

Fig. 2. Chicken pituitary, slide 19 A, control.

PLATE II

Fig. 1

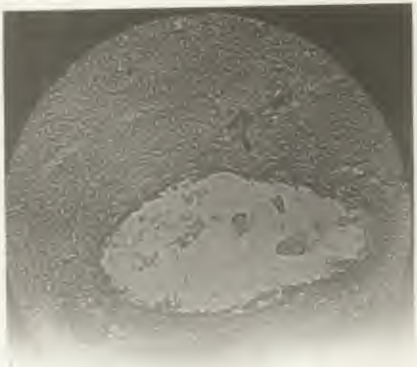

Fig. 2

EXPLANATION OF PLATE III

Fig. 1. Chicken pituitary, slide 12 A, control.

PLATE III

Fig. 1

EXPLANATION OF PLATE IV

Fig. 1. Rat pituitary, slide 34 A, experimental.

Fig. 2. Rat pituitary, slide 32 A, control.

PLATE IV

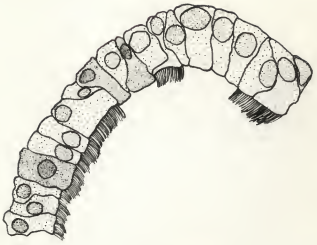

Fig. 1

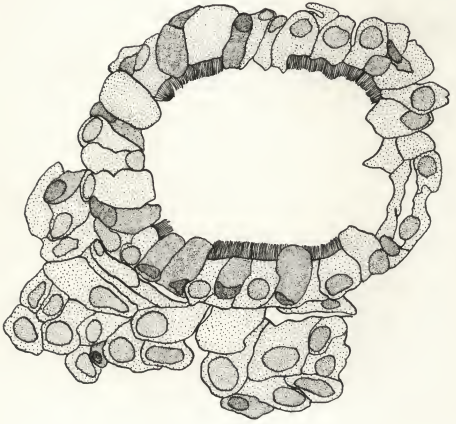

Fig. 2

EXPLANATION OF PLATE V

Fig. 1. Chicken pituitary, slide 14 A, control.

Fig. 2. Chicken pituitary, slide 5 A, experimental.

PLATE V

Fig. 1

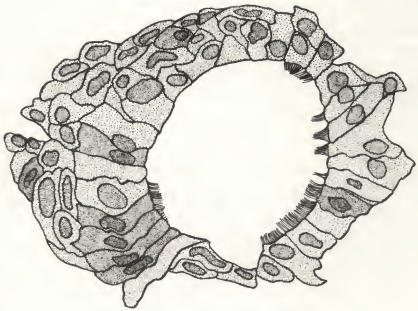

Fig. 2