EFFECTS OF VITAMIN B-DEFICIENCY ON THE VITAMINS OF FOWLS

by

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B. A., Concordia College, 1936

A THESIS

sub-itted in partial fulfillment of the

requirements for the degree of

MASTER OF SCIENCE

Department of Zoology

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1940

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INTRODUCTION AND REVIEW OF LITERATURE

During recent years considerable effort has been made to determine the effect of vitamin E-deficiency on the development of animals. Mason (1926) fully described the testicular changes produced in male rats fed on an E-free diet. Peculiar chromolysis, fusion of the spermatozoa, and the bead-like appearance of the spermatid nuclei were characteristic of E-deficient animals, and usually appeared after 50 to 100 days. Mattill and Clayton (1926), Nelson (1933), and Rowland and Singer (1936) showed that some critical period, from 90 to 150 days, occurred, beyond which the administration of vitamin E seldom restored degenerated testes to normal function. Such a critical period, however, did not occur in female rats, even though they exhibited the same type of sterility. Adamstone and Card (1934) kept Rhode Island Red cocks on E-free diet for two years. After one year, mating experiments showed that all the cocks were fertile; and after two years, the degree of sterility varied.

A close interrelationship between the function of the gonads and the pituitary is definitely known to exist. Verzar (1929) first stated that vitamin E acted like anterior hypophysial hormone in inducing precocious sexual maturity in young female rats, and that vitamin E might be necessary

for the formation of hypophysial hormone. These views, however, have found experimental contradictions in the work of many investigators. Van Wagenen (1925), Nelson (1933), and Mattill (1938) disclosed that vitamin E-deficiency caused castration cells in the pituitary. Diakov and Krizenecky (1933) and Mattill (1938) maintained that vitamin E did not exhibit gonadotropic nor luteinizing effects; nor did anterior pituitary extracts, pregnancy urine nor corpus luteum extracts prevent reproduction failure in rats on vitamin E-free diets. But since Bouin and Ancel identified the cells of Leydig in 1903 as the cells in the testes as the endocrine cells, it has been agreed that development and activity of the gonads and sexual response in animals are controlled by hormones. Smith and Engel (1927) showed that daily transplantations of anterior pituitary tissues from mice, rats, cats, rabbits and guinea pigs rapidly induced precocious sexual maturity in the female rats; but in the male, aside from a possible increase in libido, no response of the genital system was manifested. Moore and Price (1932), after injecting hypophysectomized rats with testis hormone, found that the injections maintained the reproductive accessories in a normal state, but that the injections did not promote the normal testis development. The testes degenerated as if no testis hormone had been introduced. Schweizer, Charipper

and Kleinberg (1940) disclosed that anterior pituitary grafts in hypophysectomized male guinea pigs maintained the reproductive systems. The interstitial tissue was less abundant than that in the normal guinea pig, but the individual cells in this tissue were normal.

Thus, it seemed evident that gonads functioned only when they were stimulated by certain secretions that were normally provided by the anterior lobe of the pituitary. The pituitary, on the other hand, was to some extent controlled by gonadal secretions, for when these were present in effective amounts, pituitary activity was lowered. One or the other, or both, was seemingly affected by vitamin E-deficiency.

Drummond, Noble and Wright (1939) found that vitamin E had no effect on the reproductive organs of immature or hypophysectiomized rats, that hypophysectomy of vitamin E-deficient rats caused a further decline in weight of testis, and that the pituitaries of vitamin E-deficient rats contained an increased amount of gonadotropic hormone. The evidence described by him did not support the suggestion that the effects of vitamin E-deficiency are due to hormonal imbalance.

Schooley and Riddle (1938) have described the pituitary of pigeons, and Rahn (1939) the pituitary of fowls. They

agreed that the anterior lobe of the pituitary developed from a single undifferentiated oral epithelium, and the stomodeal epithelium, which soon became a closed sac, Rathke's pouch. This united with a ventral outgrowth of the infundibulum of the diencephalon, the pouch of Soessel. The posterior lobe, usually showing a cavity in young birds, was separated from the anterior lobe by a fibrous capsule, which became compressed to a crescentic cap over a considerable area of the anterior lobe and which nearly or wholly disappeared in the adult. This probably represented the nearest approach to the intermediate lobe to be found in pigeons and fowls. The anterior pituitary of birds roughly separated into two lobes, the cephalic and caudal, on the sixth day of incubation. At about the eighth day a basophilic tendency, not true basophils, of this lobe was revealed; at the tenth day signs of acidophils arose at the most anterior end of the cephalic lobe; and at the eighteenth day the intensely granular acidophils had spread to the caudal lobe. At the same time distinguishable basophils, few in number, had appeared. After the eighteenth day the caudal lobe represented typical anterior pituitary tissue; the cephalic lobe was characterized by exceedingly faint staining acidophils of a very fine granulation. In the pigeon this latter lobe was definitely basophilic, but not so in the fowl where the

cells were relatively smaller and more crowded than the cells of the caudal lobe, the typical anterior pituitary.

Soon after hatching the granules of the majority of acidophils began to decrease in number and staining intensity. No true basophils, but cells exhibiting a metabolic center characteristic of basophils were present. Approaching the ages of one and one-half, two and one-half and three and one-half months in the fowl, pigeon and dove, respectively, the acidophils again increased in density of granulation. The birds became somatically mature during this time, but the gonads reached only a sub-functional state, a state which probably could have been reached without the aid of gonadal stimulation. At the end of this period, true basophils developed. Riddle and Schooley (1935) and Rahn (1939) inferred that since the birds showed no evidence of comb growth, there was no sex hormone elaboration before fully differentiated basophils appeared.

The anterior pituitary was composed of three cell types: two types of chromophilic cells, basophils and acidophils, and one type of chromophobe cells. Sample counts in the pigeon by Schooley and Riddle (1938) yielded the ratios 24-30-40 for acidophils, basophils, and chromophobes. In the human hypophysis Rasmussen (1929 and 1933) found the count 36.8 $\pm$ 0.52 acidophils, 10.9 $\pm$ 0.25

basophils, and 52.2 ± 0.54 chromophobes. Other investigators have found that the relative proportions of cells in most mammalian species followed the numbers presented by Rasmussen. These figures would have had little more than academic interest were it not for the discovery that proportions were changed with certain physiological alterations in the organism.

Basophils and acidophils were distinguishable not only by their staining reactions, but also by size, shape, position of nucleus and position of a structure which most investigators designate as Golgi apparatus, but which Schooley and Riddle (1938) designated as the "metabolic center", within which were found the Golgi apparatus and the mitochondria. The acidophils took a deep acid granular stain, their average size was from eight to nine microns with slight variation, they were of quite regular shape, the nucleus was generally large with two or more nucleoli, and the metabolic center capped the nucleus on the side nearest the cell membrane. The basophils had less distinct cytoplasmic granules and took a basic stain, they were more irregular in size and shape, ranging from six to fifteen microns and averaging nine to ten microns, the nucleus was large and eccentric, and the metabolic center was located somewhat away from the nucleus on the side where the bulk

of the cytoplasm was found. The cytoplasm of the chromophobes was devoid of specific granulation. These cells were the basic type from which the granular cells developed, and the type into which the chromophils developed upon losing their granules.

In addition to these three types of cells, others have been described. Severinghaus (1936) described the "pregnancy cell" which was found during and after pregnancy as degranulated basophils. Crooke (1935) described a hyaline change in the cytoplasm of basophils varying from small crescentic areas to greater areas almost completely obliterating the basophilic granules. This change, he suggested, may have been a result of altered physiological activity. The hyaline cell type has since been called the Crooke cell. Addison (1917), Van Wagenen (1926), Nelson (1933), Wolfe and Cleveland (1933), and Cate (1938) mentioned the castration or "signet-ring" cells which are modified basophils. After castration, the basophils increased in size and number and later the largest ones become vacuolated, presenting a ring-shaped colloid-containing vacuole in the central part of the cell with the cytoplasm and the nucleus at the periphery. The investigators thought this enlargement as indicating the storage of gonad-stimulating hormone, because they were able to show that the pituitaries of the

gonadectomized rats were more potent than glands from normal rats in their capacity to stimulate the immature ovaries. The same type of castration cells have been produced by ligation of the ductile efferentia. Mason (1926), Evans and Burr (1927), Nelson (1933), and Mattill (1938), while studying the pituitaries of male rats that had been kept on vitamin E-free diets for at least 120 days, found these castration cells. Such cells did not occur in vitamin E-deficient female rats. Severinghaus (1936) stated that castration cells do not develop in guinea pigs nor rabbits. Payne (1940) disclosed that "signet-ring" cells appeared in control instead of castrate chickens. Moreover, they appeared in both sexes and in the young, not the old, chickens. Castration suppressed the formation of "signet-ring" cells, but at the same time hastened the growth and development of the basophils to typical castration cells. In chickens then, contrary to mammals, the large basophil and "signet-ring" cells are probably formed in response to different stimuli and are of two distinct cell types. Though variations occurred in different animals, cell changes were found in the pituitary with changes in physiological activity. Wolfe and Cleveland (1933) presented evidence to show that there are cyclic histological changes in rat anterior pituitaries that are qualitative rather than quantitative.

Bryant (1916) was the first investigator to describe ciliated epithelial cysts in the pituitary. These cysts he found in the human pituitary in the region of the pars intermedia. The maculae were composed of tall columnar, ciliated, sensory cells interspersed with bipolar cells which had their nuclei toward the periphery whereas in the ciliated cells the nuclei were near the base. Vanderburgh (1917), Collins (1926), Rasmussen (1929a), Rahn (1939) and Opper (1940) described similar cysts in the pituitaries of guinea pigs, men, rats, and fowls. The cysts varied greatly in size, some being minute, others occupying more than one-fourth the diameter of the gland. The cells varied. They were either columnar, cuboidal, or squamous cells and were interspersed with mucus-secreting cells. Rasmussen (1929a) suggested that the origin of the cysts may be looked upon as unusual differentiation of hypophysial tissue or as migrations of nasopharyngeal elements during the early stages of development. No suggestion has been made as to a possible function of the cysts.

MATERIALS AND METHODS

In these experiments two different groups, totaling 21, of White Leghorn cockerels were fed a vitamin E-deficient diet for a period of six to seven weeks. An equal number of

control birds were fed the same diet but to which had been added two percent wheat germ oil.

The diet used was a modification of that prescribed by Mason (1926) and consisted of the following ingredients:

Casein, dry	18%
Osborne-Mendel salt mixture	4%
Corn Starch	38%
Rice, whole grain	20%
Dried Brewer's Yeast	10%
Lard	5%
Oat hulls, ground	5%
Cod liver oil	2%

The Osborne-Mendel salt mixture used was a modified form of the original salt mixture, and contained only inorganic constituents as developed by Wesson (1932). The true Osborne-Mendel salt mixture, as used by Osborne and Mendel (1919), contained, in addition to inorganic salts, citrates and citric acid. In salt mixtures used in compounding synthetic diets for experimental animals it has seemed desirable to use purely inorganic salts because of the possibility that the citrates may contain unsuspected vitamins. The modified Osborne-Mendel salt mixture contained inorganic radicals in the proportions prescribed for the original mixture. It was easily prepared from readily

available chemically pure chemicals, and it did not cake upon standing. The various chemicals and quantities of each used in the modified Osborne-Mendel salt mixture for 100 pounds of feed were as follows:

Chemicals	Grams
NaCl	187
KCl	214
KH_2PO_4	550
$Ca_3(PO_4)_2$	265
$CaCO_3$	374
$MgSO_4$ (anhydr.)	160
$FePO_4 + 4\ H_2O$	26.2
$MnSO_4$ (anhydr.)	0.36
$K_2Al_2(SO_4)_4 + 24\ H_2O$	0.16
$CuSO_4 + 5\ H_2O$	0.69
NaF	1.01
KI	.089

The first experimental and control groups, of six birds each, were started on the E-deficient diet when the birds were four and one-half weeks of age, and were fed the diet for a period of fifty-two days. The control groups were kept under the same conditions, save the addition of two percent wheat germ oil to the diet. The second two groups of birds, fifteen in each group, likewise were started on

the diet when four and one-half weeks of age and were continued for a period of fifty-nine days. At the end of the feeding periods the birds were killed and the pituitary glands and testes were removed. The left testis of each bird was measured and weighed. The tissues were fixed for sectioning.

Removal of the Pituitary

The comb and skin were first clipped from the head. With a hack-saw and scissors the upper part of the skull was removed. The brain, in turn, was removed with care, exposing the optic chiasma. The anterior pituitary, located in the sella tursica ventrally to the optic chiasma may have been removed with the brain in the young bird, had not the brain been removed with care. The pituitary, however, could usually be seen in the sella. The cancellous bone posterior to the sella was snipped away with small scissors to make a larger opening into the sella tursica. With a probe the meninges enveloping the pituitary were broken and the anterior lobe of the gland was removed.

Fixing and Slide Making

Immediately upon removing the pituitary from the bird the tissue was fixed in Heidenhain's "Susa", sublimate-

trichloracetic-formalin, as described by Gallgher (1934b). The testis, after being measured and weighed, was also fixed in "Susa". Heidenhain's "Susa" was prepared by the following method:

Mercuric chloride (saturated solution in physiological salt solution)	50 cc.
Trichloracetic acid (20 cc. of 10% trichloracetic acid	2 gm.
Formalin (40%)	20 cc.
Glacial acetic acid	4 cc.
Distilled water	10 cc.

The following method was used in fixing the tissues:

1.	"Susa"	6-10 hr.
2.	50% dioxan-50% water	4 hr.
3.	dioxan (From time to time a few drops of iodine were added until no more decolorization occurred.)	4 hr. or longer,
4.	dioxan (1 part)-warm paraffin (2 parts) (45°-50° C)	2-4 hr.
5.	paraffin (45°-50° C) 5 to 6 changes 20 min. each,	
6.	imbed,	
7.	section, (pituitary at 2, 3, and 4 microns; testis at 6 microns)	
8.	stretch on glass slide covered with a thin layer of egg albumin.	

When thoroughly dry the tissues on slides were stained with Heidenhain's "Azan" triple stain, a modification of

Mallory's triple stain in which Azocarmine G replaces acid fuchsin. This is a precise and powerful nuclear stain which keeps well. The following method of staining was used:

1. Remove paraffin with xylol.
2. Place in 50% xylol-50% absolute alcohol.
3. " " absolute alcohol.
4. " " 95% alcohol. each 1 min.
5. " " 85% alcohol.
6. " " 70% alcohol.
7. " " distilled water 5 min.
8. Stain at 55° C in the following solution: 1 hr.

   Azocarmine G or G_x (Hollborn) 0.2 gm.

   Distilled water 100.0 cc.

   Boil for 5 minutes, cool, filter and add Glacial acetic acid 1.0 cc.

   (At room temperature this solution contains fine crystals which dissolve at staining temperature.)
9. Rinse in distilled water.
10. Place in 0.1% aniline in 85% ethyl alcohol and destain until inspection shows that the cytoplasm and connective tissue are a pale pink color while nuclei remain bright red. Time varies.
11. Rinse in 96% alcohol containing 1% glacial acetic acid. 1 min.

12. Place in 5% aqueous solution of phosphotungstic acid. 2 hr. (May remain overnight).

13. Rinse in distilled water.

14. Counterstain with the following solution:

Orange G	2.0 gm.
Aniline blue, (Gruebler's water soluble)	0.5 gm.
Acetic acid, glacial	8.0 cc.
Distilled water	100 cc.

(For use dilute one part of above solution with two parts distilled water. Allow stain to act until the finest collagenous fibers are sharply stained.) 3-10 min. or longer.

15. Rinse in distilled water

16. Rinse in 95% alcohol 30 sec. or less.

17. Rinse in absolute alcohol 1 min. or less.
(If blue stain is too heavy it may be destained by leaving in absolute alcohol for a longer period.)

18. Place in carbol-xylol. 5 min.

19. Place in pure xylol. 3-5 min.

20. Mount in balsam or clarite.

The action of this stain produced the typical basic blue cytoplasmic stain and the acidic red nuclear stain in the basophils. The cytoplasm and nuclei of the acidophils

were stained typically red. The nuclei of many of the acidophils took also a partial basic stain. The nuclei in cells of the testis tubules stained distinctly red, the lumina a pale blue, and the connective tissue a darker distinct blue. Thus, with no difficulty, the size of the tubules could be readily determined.

The tissues were studied and compared with the aid of a bi-ocular compound microscope. The general morphology of the pituitary sections were first studied under low power, 125x magnification, and then a detailed study of the various cells was made under high power, 535x magnification, and oil immersion, 1225x magnification. The ocular micrometer was used with high power magnification to measure the thickness of the epithelial wall, the diameter of the lumen and the entire diameter of each of three representative tubules of each testis. The total measurements of the testis tubules of the experimental and control birds were averaged. These averages were used to determine the effect of vitamin E-deficiency on the gonads.

RESULTS

In the pituitaries of immature birds the acidophils were by far the dominating cell type. The basophils were in various stages of development as could be seen readily by the variations in the density of staining. A few appeared

as typical basophils with the blue cytoplasm and red eccentric nucleus, a number appeared to have practically no cytoplasm, but a large pyknotic nucleus; while still others took practically no cytoplasmic stain but could be designated as basophils by the position of the nucleus.

Acidophilic Cell Differences

The first morphological difference noted in the pituitaries of the experimental and control groups was the presence of large cavities partially surrounded by deep stained acidophils in the E-deficient pituitaries. These acidophils were very compact, the nuclei were large, taking both an acid and a basic stain; the cytoplasm, reduced in quantity, contained coarse, deep-staining granules. The cavities appeared clear, containing no substance which took either a basic or an acid stain. A few cavities were found in the pituitaries of the control birds, but they were not so large, nor were they bordered by the characteristic acidophils found in the pituitaries of the vitamin E-deficient fowl. Just what effect these cells had on other tissues was not noted.

Basophilic Cell and Testis Variations

In a further study of the pituitaries a marked difference was noted in the number of basophils in a later stage of development (Figs. 1 and 2). These basophils resembled the ones described by Severinghaus (1936) as being in the later stages of secretion. The nuclei were highly pyknotic, staining an intense red. The cytoplasm was greatly reduced in quantity and was stained a light blue. Thus, a number of such cells appeared as a cluster of pyknotic nuclei and which at first glance appeared identical with the acidophils bordering the cavities in the E-deficient pituitaries. However, marked clumping of nuclear material was evident and all the nuclei were surrounded by a small quantity of blue staining cytoplasm which distinguished them from the acidophils. These basophils were found in both the experimental and the control groups, but they were found in greater numbers in the control groups. Since they appeared to be in the later stages of secretion and the cytoplasmic content was small, the cells had likely given off the gonadotropic hormone before these stages were reached. Riddle and Schooley (1935), Severinghaus (1936), and Rahn (1939) attributed the gonadotropic hormone secretion to the basophils.

The correlation between the condition in the pituitaries with the development of the testes was striking. Comparing the testis weight and size of the two groups, the testes from the control groups were larger and heavier than those from E-deficient groups. The following were average sizes and weights of the two major groups:

	Experimental group	Control group
Size (mm.)	15.08 x 7.01	18 x 8.87
Weight (gm.)	656.6	971.4

Comparing the thickness of the epithelial wall and the diameter of the lumen and the total diameter of the tubules, the following averages were found:

	Experimental group (microns)	Control group (microns)
Thickness of epithelial wall	27.10	44.29
Diameter of lumen	28.88	17.19
Diameter of tubule	83.07	106.92

These figures show, on the average, that the testes of the controls were more highly developed than those of the experimentals. Individual differences, however, were found within each group, but these differences, in turn, were in more or less direct correlation with the number of basophils in the later secretory stages in the pituitaries of the individuals. All birds did not reach the same degree of

maturity at the same age. In some cases, the testes of experimental birds, especially the larger testes, had abnormal cells: peculiar chromolysis was taking place, and the nuclei of the few developed spermatids and spermatozoa had a bead-like appearance. Possibly the tissues of these individuals were not affected by the stimulus of vitamin E-deficiency to any extent at the time sex cell differentiation was begun, but before differentiation had been completed the stimulus limited the sex cells from maturing normally. No castration cells were present in the pituitaries; this may suggest that the E-deficiency first affected the pituitary.

Thus, the evidence in these experiments showed that vitamin E-deficiency hindered the normal development of the basophils to the secretion stage. The number of basophils in the later secretory stages in the E-deficient pituitaries was considerably smaller than that in the control pituitaries. The number of these basophils showed a direct relationship to the stage of maturity of the testes. This evidence suggested that vitamin E-deficiency resulted first in retardation of basophilic secretions in the pituitaries, which, in turn, prevented full differentiation of the testes. Thus, the pituitary was directly affected and the testis indirectly affected by vitamin E-deficiency.

This was not in accordance with the results found by Van Wagenen (1925), Nelson (1933), and Mattill (1938) that vitamin E-deficiency produced typical castration cells in the pituitary. Possibly their results were due to the effect of total sterility on the pituitary which would be castration cells; but the evidence of these experiments was obtained before the birds were sexually matured, and before the testes were totally degenerated to produce castration effects. This evidence confirms the conclusion set forth by Verzar (1929) that vitamin E is necessary for proper hormonal production. Lack of gonadotropic hormone from the basophils retarded sex cell differentiation in the testes. In time, a castration condition would undoubtedly be developed in the testes which would result in the formation of castration cells in the pituitary.

Epithelial Cysts

The epithelial cysts as described by Bryant (1916), Vanderburgh (1917), Collins (1926), Rasmussen (1929a), Rahn (1939) and Opper (1940) were observed in pituitaries of different birds. Studies were made of adrenalectomized birds, of normally sexually matured birds, and of birds that had received male hormone (penandren) injections in addition to the vitamin E-deficient and control birds of this experiment.

No marked differences as to size or frequency of occurrence of the cysts among the various groups could be noticed. Variations within a group were as great as those among the various groups. Nearly all the 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. The nuclei of these cells were located in the central portion or towards the basal region of the cells. The cytoplasm took a red acid stain characteristic of the acidophils. These ciliated cells were interspersed with a number of non-ciliated cells which were relatively larger in size. These cells without cilia seemed to correspond to secreting cells of epithelial cysts described by other workers. The nuclei were located near the center or toward the outer border of the cells. Most of them took the acid stain, but a few took the blue basic stain. It is thought that these cells secrete the mucus of colloidal material contained in the lumina. The lumen contents took a medium blue basic stain even though it is possibly the product of both the acidophilic and basophilic secretory cells. A layer, usually of one cell thickness, bordered the epithelium. The cells of this layer appeared flat, forming a thin substratum under the epithelial lining.

Approximately three-fourths of the glands harbored one or more cysts. Their size, number, and location varied greatly. More frequently, they were found near the periphery of the glands and in the cephalic lobes. In some cases no outlet for the cysts could be traced; they began and ended blindly within the gland. In other cases, the cysts definitely opened to the exterior of the gland. The sizes of the various cysts were determined by measuring the shortest diameter of each one with the ocular micrometer while the tissues were focused under high power magnification. The majority of the cysts ranged from 30 to 70 microns in diameter, but some measured only a few microns, while others occupied almost one-fourth of the cross section of the entire anterior lobe. Typical cysts, as found in the fowl pituitaries, are illustrated in Figs. 3, 4, and 5.

The great variations in the cysts found in all the groups of birds studied suggests that vitamin E-deficiency has no effect on the production of epithelial cysts in the pituitary.

DISCUSSION

The pituitaries of immature fowls were directly affected by a lack of vitamin E in the diet. This deficiency hindered the secretory development of the basophils, likely reducing the production of gonadotropic hormone.

The testes reached a higher degree of development in the birds fed the diet with vitamin E than in the ones fed the E-free diet. The lack of development of the testes in the latter seemed to be caused by the lack of hormonal stimulation from the pituitaries rather than being caused directly from the diet. Had the testes been affected directly by vitamin E-deficiency, castration cells should have been produced in the pituitaries, but no castration cells could be found in these glands. This evidence suggests that the testes were affected by vitamin E indirectly through the pituitaries.

Large, irregular, and clear cavities with highly pyknotic acidophils on their borders were produced in the vitamin E-deficient pituitaries. Acidophils of this type were not found near the border of smaller, clear cavities found in the control pituitaries. Just what relationship these acidophils had to other tissues was not determined.

Ciliated epithelial cysts were found just as frequently in the pituitaries of the vitamin E-deficient fowls as in those of the control fowls. The cysts were more often found in the cephalic lobe and near the periphery than in the caudal lobe or near the central portion of the gland. A few cysts opened to the exterior of the glands, whereas others had no apparent outlet.

SUMMARY

Vitamin E-deficiency in immature experimental fowls gave the following results:

1. Fewer basophils in the later stages of secretion were present in the pituitaries of E-deficient than in control birds.

2. The number of such basophils present in the pituitary had a direct relationship with the degree of development of the testes. An increase in the number of basophils produced a higher degree of testis development.

3. The pituitaries deficient in vitamin E developed clear cavities partially bordered by highly pyknotic acidophils.

4. Vitamin E-deficiency did not affect epithelial cyst formations; great variations were found in all groups.

ACKNOWLEDGMENT

Indebtedness is acknowledged to Dr. E. H. Herrick for his generous and effective aid during the entire course of this study.

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EXPLANATION OF PLATE I

Fig. 1. Basophils in later stages of secretion under low power magnification distribution of cells in the pituitary.

Fig. 2. Basophils of Fig. 1 under oil immersion magnification showing the highly pyknotic nuclei and the small cytoplasmic content.

PLATE I

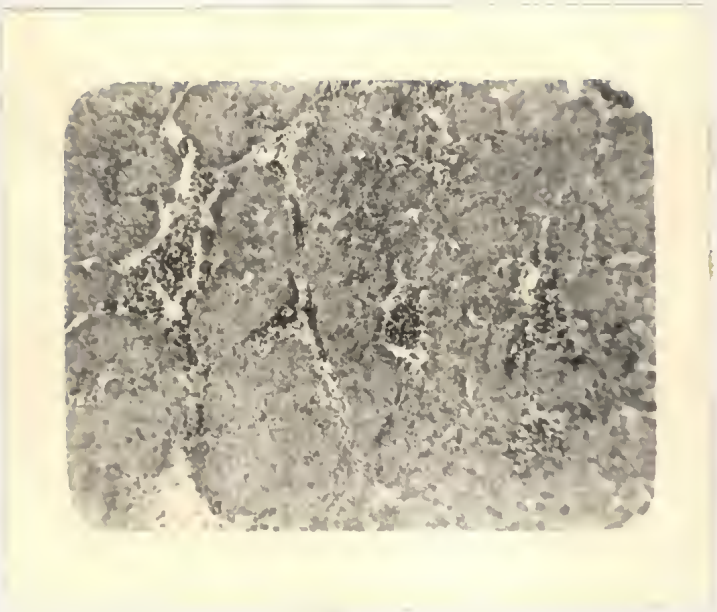

Fig. 1

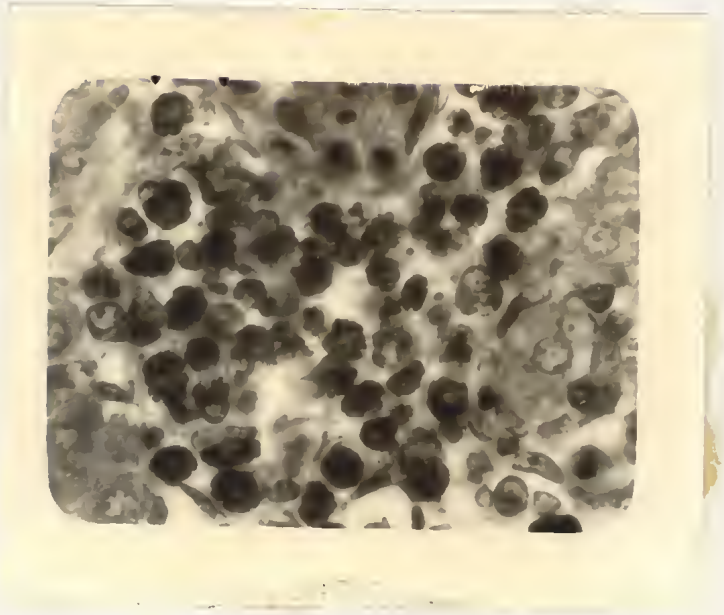

Fig. 2

EXPLANATION OF PLATE II

Fig. 3. Ciliated epithelial cysts under low power magnification.

Fig. 4. A portion of the cyst wall under oil immersion magnification showing the cell types, cilia, basophilic substratum and the colloidal contents of the lumen.

Fig. 5. A drawing, made with the aid of a camera lucida, of a typical epithelial cyst as found in fowls.

Fig. 3

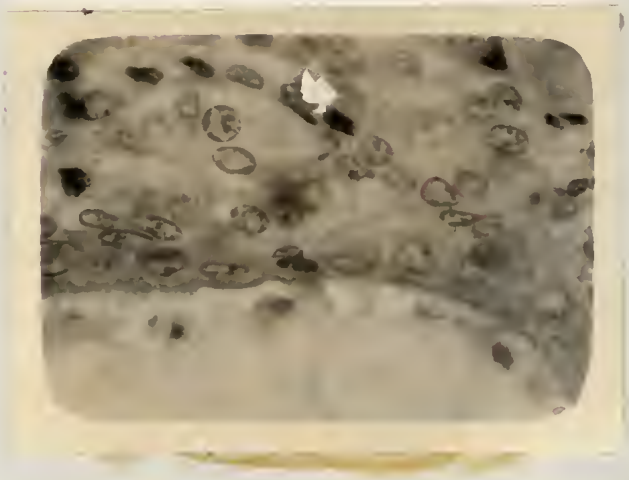

Fig. 4

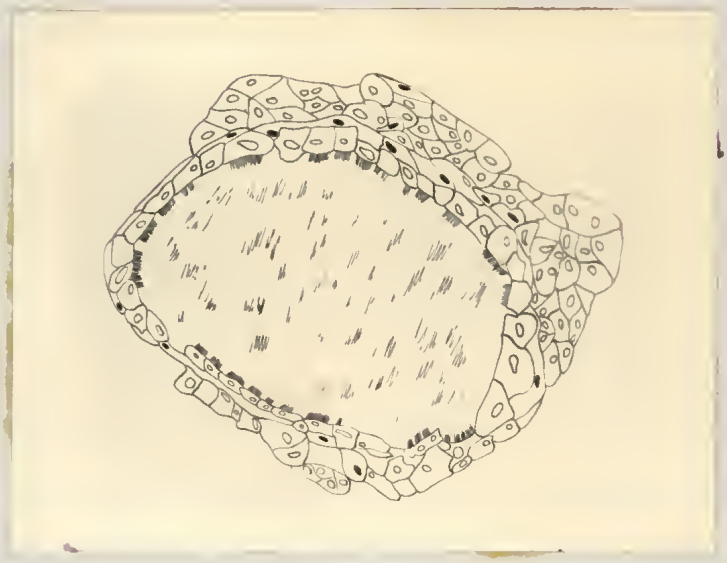

Fig. 5

Date Due
