

THE EFFECT OF INTRAPERITONEAL INJECTIONS OF ANTUITRIN  
GROWTH ON AVITAMINOSIS A IN THE ADULT AND  
YOUNG FEMALE ALBINO RAT

by

JOHN CARR AYERS

A.B., Kalamazoo College, 1934

A THESIS

submitted in partial fulfillment of the

requirements for the degree of

MASTER OF SCIENCE

KANSAS STATE COLLEGE  
OF AGRICULTURE AND APPLIED SCIENCE

1935

Doc  
ment  
LD  
260  
74  
1936  
A95  
C.2

TABLE OF CONTENTS

	page
INTRODUCTION .....	1
REVIEW OF LITERATURE .....	1
Vitamin A .....	1
Anterior Pituitary Gland Secretion .....	8
MATERIALS AND METHODS .....	16
RESULTS .....	21
DISCUSSION .....	28
ACKNOWLEDGMENTS .....	31
REFERENCES .....	32

## INTRODUCTION

Certain hormones and vitamins have similar effects on the body, e.g. vitamin A by its absence can be responsible for lack of growth and similarly the secretion of the anterior lobe of the hypophysis is, by its absence, also responsible for lack of growth.

The work recorded in this paper was undertaken in an attempt to determine whether the pituitary growth hormone could, to any degree, counteract the effects of avitaminosis A, or act as a substitute totally or partially for the vitamin in animals suffering from a deficiency of vitamin A. Some previous unpublished work has been done by Dr. E.J. Wimmer on the effect of intraperitoneal injections of the pituitary growth hormone into rats depleted of vitamin A, but, this was only preliminary. This problem is meant to substantiate and expand the results of those experiments.

## REVIEW OF LITERATURE

### Vitamin A

In 1913 McCollum and Davis (42) and Osborne and Mendell (46), working independently, simultaneously reported that there were certain natural fats which were essential to the growth and development of young animals and without which

growth stasis occurred and led eventually to the sickening and death of those animals deprived by synthetic diets of those lipoids. The fats to which reference is made in these reports are; lard, which was deficient in these necessary lipoids; butter-fat, which contained these lipoids; egg-yolk, which was found to contain the natural fats. The following year Osborne and Mendell(47) reported codliver oil as being very rich in these fats and confirmed the findings of McCollum and Davis in respect to butter-fat and egg-yolk.

In 1915 Osborne and Mendell(48) reported further studies on these fats and in addition found that beef fat and oleomargarine gave curative reactions when fed to animals which had reached growth stasis. These writers confirmed McCollum and Davis's findings that lard lacked these essential fats. By careful rendering and subsequent treatment they produced a lard which had never been heated past the melting point and had been subjected to no treatment capable of destroying the active fats. With this lard they found no more response than with the commercial product and concluded that the required lipoids are not a part of lard and so could not have been destroyed by the methods of preparation. In this paper Osborne and Mendel reported finding that heat does not destroy the active principle of

butter-fat and that this principle is found in the "oil" left after fractional crystallization from alcohol. The fractions having high melting points do not contain the growth restoring principle.

In 1913 Osborne and Mendell (46) reported that animals which do not receive sufficient amounts of the lipoids become almost without exception the victims of an eye disease which has since been called variously xerophthalmia, ophthalmia, and conjunctivitis. McCollum and Davis (43) reported an increased susceptibility to lung disease in their vitamin A-free animals. Steenbock et al. (61) in 1918 confirmed this and suggested that lung disease and eye disease be regarded as symptoms of deficiency of the necessary lipoids.

A characteristic and invariable disturbance of reproductive functions in 100 per cent of animals fed on diets low in the necessary fats was reported in 1922 by Evans and Bishop (23). This disturbance takes the form of failure of ovulation and of persistent maintenance of the oestrous portion of the sexual cycle.

Steenbock and Gross (62) in 1919 published results showing that the fat-soluble vitamin was present in carrots, sweet potatoes, rutabages, red beets, parsnips, sugar beets, mangels, and dasheens. In 1921 Steenbock et al (65) suggested that the fat-soluble vitamin was "most likely to

occur with yellow pigmentation" and in the same year Steenbock, Sell and Boutwell (64) reported finding that green peas bearing yellow pigment contained more of the vitamin than did yellow peas bearing less of the pigment.

In a paper by Steenbock, Nelson and Black (63) published in 1924 is found the first reference to the fat-soluble anti-ophthalmic vitamin as "vitamin A" although Evans and Bishop in 1922 referred to it as "fat-soluble vitamin A". In this publication Steenbock announced the discovery of other fat-soluble principles in the necessary fats as the reason for this change.

Following the discovery by Evans and Bishop (23) that animals fed diets low in vitamin A suffered an inhibition of ovulation and consistently maintained a prolonged oestrous vaginal condition, several workers studied the vaginal smear as a method of determining avitaminosis A. Chief among these were Baumann and Steenbock (4), Aberle (1), Ellison (20), and Macy et al. (40).

It was generally agreed by these investigators that the persistent presence of cornified (or keratinized) epithelial cells in the vaginal smears of animals fed a vitamin A deficient diet over a period of time was a reliable index and a delicate indicator of the depletion of the animals' bodily stores of vitamin A. While avitaminosis A produces its most

definite and most easily recognized symptoms in the vaginal smear, it acts also upon the body epithelia in general. Harris, Innes, and Griffith (34) in 1932 reported definite keratinization and desquamation of the epithelium of the salivary glands and their ducts.

Avitaminosis A produces in addition to the constant cornified smear several other conditions which are typical, among these the occurrence of xerophthalmia is prominent. Osborne and Mendel (49) found that this disease appeared more promptly and frequently among rats fed highly purified foods than among those on less thoroughly purified diets. They reported also in the same paper that xerophthalmia was not found in animals other than those on rations deficient in vitamin A.

In relation to these findings Frederica and Holm (32) in the same year reported that the lack of vitamin A had no effect on the amounts of visual purple in the retina of the rat, but that in rats whose visual purple was completely bleached by strong light regeneration was slower in the vitamin A-free animals than in the controls. Night-blindness is reported to be worse after exposure to strong light.

Sutton, Setterfield, and Krauss (66) and Aberle (3) found a definite nerve degeneration evidenced by loss of



coordination and due to degenerative changes of the myelin sheaths as another condition typical of avitaminosis A. The nerves most effected were the femoral and sciatic, producing a pronounced loss of control of the hind legs. They reported that this condition, which occurs at the same time as xerophthalmia and cessation of growth, is extensive before the external symptoms appear.

Another condition found in animals depleted of vitamin A was described by Smith and Lantz (59) in 1933. They observed that the incisors of rats deficient in vitamin A would, at the cessation of growth and appearance of the early signs of xerophthalmia, cease to grow at the normal rate, lose their luster and normal orange pigment, and become short and blunt as well as white and opaque. Chidester, Eaton, and Thompson (10) Chidester, Eaton and Speicher (11) in 1928 and 1930 published results showing that minute dosages of iron salts and iodine given rats on a vitamin A-free diet had curative effects on xerophthalmia and brought the weight of the experimental animals up to nearly that of the controls. The results reported by Chidester, et al. were directly contradicted in 1931 and 1932 by Mason (41) and Cameron (9). Mason found no curative or preventative effect on the xerophthalmia of vitamin A deficient rats. Cameron reported that ferrous



iodide was without effect on xerophthalmia, and further (her results) indicated that ferrous iodide could not substitute for vitamin A in the cure or prevention of terminal infections characteristic of vitamin A deficiency in rats.

Recently, Aberle (2) in 1933, found that injections of gonadotropic hormone (placental extract) are incapable of producing any effect on the vaginal cells of vitamin A-deficient rats, the cornified smears characteristic of avitaminosis-A remaining constant in the experimental animals. The vaginal epithelial cells were viable in both controls and experimentals.

Baumann, Rising, and Steenbock (5) in 1934 reported a series of investigations on the storage of vitamin A in the rat. They found that 95 per cent of the bodily stores of the vitamin are in the liver; the remainder is found in the lungs and kidneys. They also found that rats under three weeks of age had only traces of vitamin A in the liver but beyond that stage storage was rapid and regular.

In a publication on the transmission of vitamin A from parent to young in mammals, Dann (17) stated that the amount of vitamin A in the liver at birth is small but rather constant and was not increased by giving large amounts of carotene to the mother during gestation. The

amount present in the liver at weaning was larger and more variable depending upon the amount of carotene given the mother during lactation. Another paper by the same author in 1934 (18) reported, in reference to this same transmission of vitamin A, that the amount of vitamin transmitted by a female to her young during gestation, when she receives large amounts of carotene in her diet, depends upon the fat value of the diet and is increased as the fat content increases. The relation of the growth response to the dosage of vitamin A was reported by Coward (16) in 1934. Her results showed an agreement and relation between the dosages of the vitamin-containing food and the mean growth response.

Chidester (12) in 1932 reported that the iodine-fat imbalance produced by deficiencies of fat-soluble vitamins is a most fundamental part of the disturbances caused by this type of deficiency. He believed that the other effects shown by animals deficient in these vitamins were merely symptoms of the deficiency.

#### Anterior Pituitary Gland Secretion

The existence of the pituitary or hypophysis was known from the time of Galen (200 A.D.). The name "pituitary"

probably was given it by Vesalius who considered it a mucous secreting gland and so named it (Latin: pituita, mucous). The idea of mucous secretion was overthrown by Conrad Schneider as early as 1660 (36) and this invaluable gland was relegated to the position of a vestigial organ.

The first knowledge of the pituitary in its true light came when Verga in 1864 (36) and Klebs in about 1894 (36) described hypertrophy of the pituitary accompanying the condition of facial and digital overgrowth known as acromegaly. The French neurologist, Pierre Marie, was first to determine the relation of the gland to the disease. This discovery was reported in 1886.

The first attempts at the determination of the exact function of the pituitary were made by Evans and Long (21) in a series of investigations; the findings of which were published in 1921. They found no effect from the feeding of pituitary tissue (anterior lobe). Later during that year Evans and Long (22) found that when anterior lobe tissue was pulped and administered intraperitoneally into the rat, a greater rate of growth, a delay in sexual maturity and a lengthening of oestrous cycles would result.

In regard to feeding the tissue of the anterior pituitary to hypophysectomized rats, P.R. Smith in 1927 (58)

showed that daily feeding of fresh pituitaries had no effect on the body weight, body or tail length, and produced no change in the atrophied genital system of the animals; nor was the thyroid or cortex of the adrenal gland effected. The growth curves of these animals closely paralleled those of the controls which were fed muscle tissue.

In 1928 Putnam, Teel and Benedict (51) announced the preparation of a sterile active extract of the anterior lobe of the hypophysis which was used on dogs and rats with apparently specific changes. They found that this extract served to repair some and perhaps all of the disabilities resulting from hypophysectomy. This extract was used with apparent temporary benefits on one case of human hypopituitarism.

Also in 1928 Reichert (52) reported that replacement therapy in an hypophysectomized puppy by transplants of whole rabbit pituitaries over a period of four months resulted in the overcoming, in a large measure, of the disabilities resulting from hypophysectomy. There was, however, a failure to obtain increased size and weight which was attributed to the attainment of full growth before the therapy was begun. The experimental puppy was observed to be in oestrous throughout the four months while the control

animal, also hypophysectomized, never came into heat. Studies in replacement therapy yielding results much the same as those of Reichert were reported by several others (38) (37) (58).

In 1929 Johnson and Sayles (37) found that daily injections of a crude extract of the anterior lobe of the bovine pituitary into the young albino rat produced an acceleration of growth which did not cease as soon in the experimental animals as in the controls. The treated animals of each sex were heavier than the untreated controls. The gain in weight was found to be due to general bodily growth. The extract produced no change in the uterus or in the time of opening of the vagina, but reduced fertility and probably increased the size of the ovaries due to the large numbers of corpora lutea contained in them.

In the same year Teel (58) observed diuresis in dogs given injections of neutralized alkaline extracts of the anterior hypophysis. This was not noticed in all cases and Teel mentioned the possibility of contamination by posterior pituitary as the inhibiting factor.

Evans, Cornish, and Simpson (27) in 1929 published a method of extraction of the anterior lobe for the growth hormone. With this extract they obtained results as fol-

lows: Evans and Simpson (28) found that injections of this extract markedly aids placentoma reactions in the uterine mucosa of adult virgin rats. These tumors were produced about silk threads in the mucosa when subcutaneous dosages of the extract were given. These investigators (26) found an impairment of the birth mechanism when administrations of this extract were given. The gestation period was prolonged from two to three days with an increased possibility of still-births, abortion, or resorption, or (rarely) live births. This disturbance was thought to be due to the formation of fresh lutein tissue in the ovary. These workers (25), continuing their investigations, found an invariable hyperplasia of the mammary tree of adult virgin females after a month of injections or other daily treatments with alkaline extracts of the anterior pituitary.

Benedict, Putnam, and Teel (6) in 1930 reported changes produced by injections of a new extract of the anterior lobe of the pituitary. With injections of this extract they found hypertrophy of the thyroid, of the uterus, of the ovaries, and of the vagina. Skeletal overgrowth and splanchnomegaly were observed in the animals which received treatment with this extract.

Evans (29) in his presidential address to the American Association of Anatomists summarized the knowledge of the growth hormone as it was in 1932 as having the following effects: (a) production of growth, (b) the production of mature-sized animals from hypophysectomized ones, these having only infantile genitalia, however, (c) production of diabetes when given to normal animals in overdoses. He also reported the growth hormone (d) as a necessary stimulus to some of the other glands, notable the adrenals, and (e) as being strangely converted into the sex-stimulating hormone by means of the chemical substances found in the urine of pregnancy.

In 1932 Bryan and Gaiser (8) reported on the influence of diet and the anterior pituitary growth hormone on the growth rate of rats. They found that the rate of growth was controlled by three factors: (a) the individual growth potential, (b) the quality of the diet, and (c) the injection of the growth hormone. In 1932 Hertz, Hellbaum, and Hisaw (35) found Phylene (the anterior pituitary extract of Van Dyke and Wallen-Lawrence) produced ovulation in adult rabbits and follicular development and extensive luteinization in young female rabbits.

Bischoff, Maxwell, and Ullman (7) in 1932 confirmed



the finding of Evans and Simpson (28) that neoplasms grow faster under the influence of the anterior lobe growth hormone.

The cure of the cachexia appearing after hypophysectomy by injections of the growth hormone and the relation of the hormone to the resulting repair of the adrenal cortex was reported in 1932 by Evans, Meyer, Pencharz, and Simpson (30). These investigators found that the injection of the hormone brought prompt relief from the cachexia, growth began, normal turgor returned to the tissues, normal strength and muscle tone returned, and autopsy showed repair (although not complete) of the thyroids and adrenals.

Riddle and Bates in 1933 (53) published the results of their researches on the contents of the various commercial extracts of the growth hormone. These studies related to the contamination of the extracts by other hormones.

The water and solid contents of the brains of rats given the growth hormone were reported in 1934 by Rubinstein and Fox (55). They found that the growth hormone failed to increase the size of the brain in proportion to the body and that the water and solid contents of the brains of animals artificially enlarged retained their normal proportions.

In 1934 Lee and Schaffer (39) published the results of their studies on the composition of the growth produced by the pituitary growth hormone. The growth was found to consist of almost normal proportions of all the constituents and the heat value was found unchanged. The treated animals were found to differ most from the controls in the contents of nitrogen and fat-ash-free tissues. The treated animals were found to oxidize more fat and less protein than the controls.

Rubinstein and Eolodner in 1934 (56) reported that administration of the growth hormone increased the body weight, tail lengths, and total lengths of the treated animals. They observed that the treated males responded somewhat less than the treated females and also showed smaller gains in body weight.

Gregory and Goss in 1934 (33) published their findings on the relation of the pituitary growth and glutathione concentration. They found that concurrently with the increase of weight induced by the hormone there was an increase in the concentration of glutathione in the muscles.

Rubinstein in 1934 (54) found a difference in the responses to the growth hormone by the pituitaries of male and female animals. The hormone increased significantly

the weight of the gland as well as the general body weight in the male animals. The pituitaries of the females, normally heavier than those of the males, did not increase in weight and were retarded in their growth although the general body weight increased.

Sontag and Manson (60) in the same year reported that the administration of the growth hormone (Antuitrin G) to pregnant rats resulted in the number of still-births being approximately trebled, the average weight of the young from the treated females being 7.3 per cent greater than that of the young of the controls. The period of gestation was increased in the majority of the cases. The course of treatments with Antuitrin G did not incapacitate the mother for subsequent reproduction.

Evans (31) in 1935 published the most recent summary of the knowledge of the growth hormone.

#### MATERIALS AND METHODS

Albino rats (*Mus Norvegicus albinus*), descended from rats originally supplied by the Laboratory of Mammalogy, University of Michigan, were used in this study. The young rats used in these experiments were the second generation of

brother-and-sister matings from selected breeding stock. The matings took place in small breeding cages and the males were removed as soon as the females became heavy. After parturition the young were left with the females until the twenty-first day of age at which time they were weaned and placed in especially constructed cages having false bottoms of half inch wire mesh. These covered the feces pans and formed the bottoms of the cages. In this manner all consumption of excreta was prevented and the maximum rate of depletion of the animals' bodily stores of vitamin A obtained.

Part of the young were given a vitamin A-free diet\* ad libitum until the vaginas opened (at about the thirty-fifth day of age). Vaginal smears of these were taken daily until the vaginal smears had been definitely and persistently cornified for seven days at which time the

\* The vitamin A-free diet used was that described by Sherman (57) and was composed of:

alcohol-extracted casein.....	800 grams
dried yeast.....	400 grams
sodium chloride.....	40 grams
Osborne-Mendel salt.....	140 grams
viosterol.....	3.6 grams
cornstarch.....	2600 grams

All the animals used in these experiments were given only distilled water to drink to avoid the possibility of any iron salts (10) (11) being available to the animals. The water was supplied from bottles equipped with rubber stoppers bearing bent glass tubes which extended into the cages. The water fed down slowly as the animal lapped at the end of the tube and forced air up into the bottle.

animals were considered deficient in vitamin A. (Cornified smear--smear majority of whose cells are cornified.) This occurred about the forty-second to the forty-fifth day of age. Injections of 0.4 cc. of Antuitrin Growth (formerly Antuitrin G) (Parke-Davis and Company) were begun after the seventh day of the constant cornified smears and were continued until the animal died or was killed for autopsy on the fifty-sixth day from the beginning of the experiment or the seventy-seventh day of age.

Sisters, or at most cousins, of the rats receiving treatment as above were placed at weaning on the same vitamin A-free diet and at once were given daily injections of 0.4 cc. of Antuitrin Growth. Approximately equal numbers of animals were run in these two groups and were injected at the same time each day from the same vial of extract with the same hypodermic syringe. All injections were given intraperitoneally through the body wall posterior to the last ribs and in no case on the same side two days in succession.

The adult female rats used in these experiments were from the breeding stock of the colony. During alterations of the laboratory the males and females had been separated and placed in wire mesh cages in a rather crowded condition.

At the same time a change in the amount of cod liver oil added as a supplement to the diet was tried and proved insufficient by the appearance of xerophthalmia at about the fourth month. The animals showing this symptom and those which gave cornified vaginal smears were segregated and placed on a diet free of vitamin A. Daily vaginal smears were taken of all suspected of being deficient in vitamin A. When an animal had shown for seven days the constant cornified vaginal smear it was considered depleted and injections of Antuitrin Growth begun. The dosage was the same as given the young females (0.4 cc. daily intraperitoneally). Variations in rate at which the animals took on the constant cornified smears enabled them to be run in groups of five with the fifth animal serving as a negative control, i.e. receiving a diet free of vitamin A but no injections.

Controls were maintained on both the extract and the diet. The hormonal controls were fed a diet well supplied with vitamin A in the form of cod liver oil and given daily injections of 0.4 cc. of Antuitrin Growth intraperitoneally. The diet controls received the same diet as the hormonal controls but were given no injections. Additional control of the vitamin A-free diet was maintained by use of the de-

pleted animals themselves. Approximately half of the negative controls when they had become very emaciated and were showing clearly the loss of coordination and xerophthalmia typical of vitamin A deficiency were put on the vitamin A-free diet with cod liver oil added. An improvement in the general condition of the animal was regarded as proof that the synthetic diet used was indeed free of vitamin A.

The constant cornified vaginal smear as described by Aberle (1), Evans and Bishop (23) and others (14, 40, 20, 24, 50) was used as the indication of depletion, an animal being considered depleted after it had shown these smears consistently for a seven day period. In all the animals the smears were taken at approximately the same time of day.

The treatment given the depleted adult females was of two types: of the four experimental animals in each group all were given injections for two or three weeks after which two were kept on the daily injections while the other two were refused injections over periods of from three weeks to five weeks until they again showed emaciation and had approached the weight level of the controls. This loss of weight when the injected animals were refused the hormone served as an added control on the potency of the extract.



Upon the death of the control for the group or when the control had become extremely emaciated, autopsies were made upon it and the experimental animals and tissues preserved for histological study. The tissues saved were the ovaries, and the junction of the uteri with the vagina. Bouin's fixative was used and, after fixing, the picric acid was removed from the tissues by repeated changes of 70 per cent alcohol saturated with lithium carbonate. These tissues were sectioned and stained with Ehrlich's haematoxylin and counterstained with eosin. Tissues from both types of adult experimental and both types of young experimental animals as well as from diet controls, hormonal controls, negative controls, and normal stock animals were treated in this manner.

At the time of autopsy especial notice was taken of the following points: the condition of the eyes, the condition of the skin, the degree of roughness of the coat, the position of the hind limbs (before killing), the degree of general emaciation, and a study of the condition of the internal organs not preserved was made.

#### RESULTS

The adult control animals used in this experiment de-

veloped very definitely the xerophthalmia, emaciation, incontinence of urine, and loss of nervous coordination characteristic of vitamin A-free animals. The adult experimentals in a few cases developed these same symptoms before injections were begun.

In no case did total blindness develop among the controls although in five cases there was exudation of matter and blood from the eyes. Sensitiveness to light seemed to be evidenced by the apparent preference of the animals for the darker corners of the cages. The emaciated condition was very well developed in the controls, extending even to the thickness and smoothness of the coats, which were without exception rough, thin, and loosely held.

Incontinence of urine in the control animals was advanced to such a stage that the coat around the opening of the urethra was, after the third week of the experiment, always caked with salts from the constantly dribbling urine. No extreme loss of nervous coordination was observed but all the vitamin A-free animals were unsteady and awkward in the use of their feet after the third or fourth week of the experiment.

In the adult experimental animals the conditions of xerophthalmia, incontinence of urine, and emaciation char-

acteristic of avitaminosis A were decidedly different from those in the controls. In the experimentals a definite growth occurred after injections of Antuitrin Growth were begun. This was the first visible reaction to Antuitrin Growth injections. Some of the injected animals gained 65 grams or more while on the experiment. The average increase in weight for the experimentals was 40 grams for the six weeks of treatment. The control animals in a corresponding length of time lost ten grams. The greatest growth in the experimental group was 73 grams in six weeks and the least was -7 grams. The latter occurred in an animal which throughout this time suffered severe respiratory difficulty and autopsy revealed an advanced case of tuberculosis. This was the only instance of this disease found in the experiment.

A rather consistent change of the epithelial cells composing the vaginal smear was observed in the adult experimental animals injected with Antuitrin Growth. The vaginal smears of animals deficient in vitamin A are composed of non-nucleated keratinized epithelial cells (called cornified cells) which are the characteristic cells of the smears of the animals whose stores of the vitamin have been depleted. In some animals, only the metoestrous smears

(leucocytes and cornified cells) could be obtained, which is nevertheless an indication of avitaminosis.

These smears after injections of Antuitrin Growth were begun changed from the oestrous or metoestrous type of smear (cornified or cornified-leucocytes) to the dioestrous type (composed of leucocytes and nucleated epithelial cells). This change varied in its degree. In the experimentals in which the true cornified smear could not be attained before injections were begun, the change away from the cornified type toward the dioestrous type was not so striking as the change from the oestrous type.

The change of the vaginal picture is the result of a decided change in the thickness of the vaginal mucosa. The control animals which were given the synthetic A-free diet and denied injections showed an average thickness of vaginal mucosa of 10.9 cells while the experimentals receiving both the A-free diet and the injections showed a thickness of the vaginal mucosa averaging 4.3 cells.

This change of thickness of the vaginal mucosa is concurrent with change in the ovaries indicative of hormonal conditions favorable to the type of mucosa found in the controls and experimentals. The ovaries of the controls (A-free but not injected) contain large, apparently normal

follicles, numerous young follicles, and some large corpora lutea which seem to be degenerating since they contain numerous blood lakes and vacuolation areas. This type of ovarian picture is identical with that of a normal ovary immediately after ovulation of part of the follicles and corresponds strikingly with the state of the normal ovary at the time of the thickened mucosa.

The ovaries of the experimentals show very large numbers of corpora lutea, a few small follicles, and a very few young follicles. This composition in itself differs from that of the control ovaries but is especially striking in that the corpora contain no blood spaces and exhibit no vacuolation. This ovarian picture is almost identical to that of the normal ovary when no fertilization has occurred and the thick mucosa has sloughed off leaving a low raw-edged mucosa.

The lack of vitamin A in young female rats leads to the development of the conditions of xerophthalmia, emaciation, incontinence of urine as described above in regard to the adult animals. The characteristic cornified vaginal cells appear in the first smears after the opening of the vagina, or at the latest after the completion, wholly or partially, of an abnormal first oestrous cycle.

In these young animals a very severe xerophthalmia which was observed resulted in the production of heavy rims of dried bloody exudate around the eyes. Pronounced loss of nervous coordination especially of the hind legs was also observed in these animals.

In the young animals which received the injections from the time of weaning, there was no change in the composition of the smear. In both controls and experimentals the cornified cells persisted throughout the experiment.

The ovaries of these animals which had received injections and the A-free diet from weaning showed no significant difference from those of the controls. The ovarian picture was one of many large apparently normal follicles, many young follicles, and a few corpora lutea. The few corpora which were present showed definite evidences of degeneration, large blood spaces, and definite vacuolation.

This would indicate a secretory condition in that organ favorable to the maintenance of the thickened vaginal mucosa which cross-sections of the vagina showed to be six to twelve cells in depth. The thickened mucosa was that typical of either avitaminosis A or the oestrous period of the sexual cycle. From such a mucosa only the cornified cells characteristic of avitaminosis A or of the oestrous

period could be obtained in the vaginal smear.

These experimental young females showed an improvement in the general condition of the body, the emaciation was relieved, and the urine was held. Definite growth was initiated by the injection of Antuitrin Growth and for awhile this was maintained in these animals while the controls consistently lost weight. The growth observed seemed to be largely visceral and due to storage of fat, although there was increase of skeletal length.

The animals of the group which received no injections until after the opening of the vagina showed conditions of xerophthalmia, incontinence of urine, loss of nervous coordination, emaciation, and shagginess of coat. The only external difference between this group and the previous group which received injections from weaning, and the controls was that these which were not injected until the vaginas had opened showed a xerophthalmia more severe than did the first group yet not so severe as did the controls. Some growth was observed in this group of young females and the animals were still increasing in weight when autopsies were made at the 56 day after weaning.

There was no change in the vaginal smears of the group, since the cornified smears persisted throughout the



experiment. There was also no difference between the ovarian composition of this group, the previous group, or the controls--the preponderance of follicles over corpora lutea was as definite as in any of the others. The same ovarian picture explains also the observed lack of difference in the thickness of the vaginal mucosae of these, the previous group, and the controls.

An apparent stimulation of tumors was observed during the course of these experiments, five tumors being found. Of these, two were connective tissue tumors found in and removed from the right axillae of two adult animals, two were vaginal connective tissue tumors; one, a small one, found in studying cross-sections of the vagina of an injected individual, and the other, a large one, was found protruding from the vaginal orifice of an animal being depleted. A large melanoid tumor was found on the left oviduct of an injected adult at autopsy. With two exceptions all tumors were found in animals receiving injections.

#### DISCUSSION

That animals kept on a diet free of vitamin A until they exhibit growth stasis and the cornified vaginal smears

characteristic of avitaminosis A resumed growth when given injections of the anterior pituitary growth hormone seemed to indicate a relation between the growth hormone and vitamin A. This was strengthened by the fact that there was observed, concurrently with this growth, a change in the type of epithelial cells composing the vaginal smears. These were cornified before injections were begun but became nucleated and infiltrated with leucocytes shortly after the first injections.

The reason for this change of smears was found to be a decided alteration in the thickness of the vaginal mucosa. The mucosa had a thickness of 10.9 cells (average thickness) in the controls and a thickness of only 4.3 cells in the injected experimental animals. There was a corresponding difference in the cells lining the lumen: cornified cells in the controls and small nucleated cells and leucocytes in the injected adult animals. The ovaries of the controls were composed of many apparently normal follicles and few degenerating corpora lutea while the ovaries of the experimental animals were made up of many large solid corpora lutea and a few small follicles. This altered composition was believed to be evidence of a change in secretory condition within the ovaries from a condition very

similar to that existing in the normal ovary at the time of the oestrous smear to one similar to that in the normal ovary at the time of sloughing off of the thickened mucosa of the oestrous period.

Further evidence for the belief that the pituitary growth hormone has an effect on avitaminosis A was found in the fact that there was a pronounced improvement in the coats of those animals receiving the injections.

That the growth hormone cannot completely substitute for vitamin A was shown by the persistence of some xerophthalmia after the completion of the course of injections.

This work confirms the unpublished findings of E.J. Wimmer who found that injections of an alkaline extract of anterior pituitary glands into rats fed a vitamin A-free diet promoted growth in young animals injected from the time of weaning, and brought about an increase in weight in adult animals whose weight had begun to fall as a result of vitamin A depletion.

## ACKNOWLEDGMENTS

The author wishes to thank Dr. E.J. Wimmer for suggesting this problem and directing the work. He is also indebted to Dr. R.E. Nabours of the Department of Zoology, and Dr. Martha M. Kramer and Miss Bernice Runerth of the Department of Home Economics for their advice and encouragement. The writer wishes to thank Dr. Oliver Kamm of the Parke, Davis and Company for the donation of part of the Antuitrin Growth used in this experiment, and Dr. E.H. Herrick of the Department of Zoology for the use of animals and laboratory facilities.

## REFERENCES

- (1) Aberle, S.B.D.  
Continual cornified vaginal cells as an index of avitaminosis A in rats. *Jour. of Nutrit.* 6: 1-10. 1933.
- (2) Aberle, S.B.D.  
The interrelation of a gonadatropic hormone and vitamin A. *Amer. Jour. Physiol.* 106: 267-272. 1933.
- (3) Aberle, S.B.D.  
Neurological disturbances in rats reared on diets deficient in vitamin A. *Jour. of Nutrit.* 7: 445-460. 1934.
- (4) Baumann, Carl A. and Steenbrock, H.  
The vaginal smear method of determining vitamin A. *Science*, 76: 417-420. 1932.
- (5) Baumann, C.A., Riising, B.M., and Steenbock, H.  
Fat soluble vitamins. XLII. The absorption and storage of vitamin A in the rat. *Jour. of Biol. Chem.* 107: 705-715. 1934.
- (6) Benedict, E.B., Putnam, T.J., and Teel, H.M.  
Early changes produced in dogs by injections of a sterile active extract from the anterior lobe of the hypophysis. *Amer. Jour. Med. Sciences*, 179: 489. 1930.
- (7) Bischoff, Fritz, Maxwell, L.C., and Ullman, H.J.  
Effect of anterior lobe growth-promoting principles on the growth behavior of neoplasms. *Jour. of Biol. Chem.* 97: sup. 7-8. 1932.
- (8) Bryan, A. Hughes and Gaiser, David W.  
The influence of diet and the anterior pituitary growth hormone on the growth rate of adolescent rats. *Amer. Jour. Physiol.* 99: 379-390. 1932.

- (9) Cameron, Hazel C.  
Ferrous iodide as a substitute for Vitamin A in rats.  
*Science*, 76: 18-19. 1932.
- (10) Chidester, F.E., Eaton, A.G., and Thompson, G.P.  
The influence of minute doses of iodine and iron on  
growth of rats furnished vitamin A-free diet.  
*Science*, 68: 432. 1928.
- (11) Chidester, F.E., Eaton, A.G., and Speicher, H.K.  
The influence of iodide of iron on rats receiving a  
vitamin A-free diet. *Proc.Soc.Exper.Biol. and Med.*  
28: 187-189. 1930.
- (12) Chidester, F.E.  
Vitamin A and the iodine-fat balance. *Science*,  
75: 286-287. 1932.
- (13) Collip, J.B., Selye, H. and Thomson, D.L.  
Preparation of a purified and highly potent extract  
of growth hormone of anterior pituitary lobe. *Proc.*  
*Soc. Exper. Biol. and Med.* 30: 544-546. 1933.
- (14) Coward, Kathrine H.  
The influence of vitamin A deficiency on the  
oestrous cycle of the rat. *Jour. of Physiol.*  
67: 26. 1929.
- (15) Coward, K.H., Cambden, H.R., and Lee, E.M.  
LXXXV. Astock diet for rats bred for vitamin tests.  
*Biochem. Jour.*, 26: 679-690. 1932.
- (16) Coward, K.H.  
The relation of the growth response to dosage of  
vitamin A. *Biochem. Jour.* 28: 865-869. 1934.
- (17) Dann, Wm. John  
The transmission of vitamin A from parents to young  
in mammals. *Biochem. Jour.* 26: 1072. 1932.
- (18) Dann, Wm. John  
The transmission of vitamin A from parents to young  
in mammals. III. Effect of the fat content of diet  
during pregnancy on the transmission of vitamin A  
to the foetal rat. *Biochem. Jour.* 28: 634-637. 1934.

- (19) Downs, Wm.G. Jr. and Gelling, E.M.K.  
Possible water balance; Effects of alkaline anterior pituitary extracts. Proc. Soc. Exper. Biol. and Med. 27: 63-64. 1929.
- (20) Ellison, E.T.  
The oestrous cycle in vitamin A deficiency. Anat. Rec. 51: 58. 1931.
- (21) Evans, H.M. and Long, J.A.  
Effects of feeding the anterior lobe of the hypophysis on the oestrous cycle of the rat. Anat. Rec. 21: 62. 1921.
- (22) Evans, H.M. and Long, J.A.  
The effect of the anterior lobe administered intraperitoneally upon growth, maturity, and oestrous cycles of the rat. Anat. Rec. 21: 62. 1921.
- (23) Evans, H.M. and Bishop, K.S.  
On an invariable and characteristic disturbance of reproductive function in animals reared on diet poor in fat-soluble vitamin A. Anat. Rec. 23: 17. 1922.
- (24) Evans, Herbert M.  
The effects of inadequate vitamin A on the sexual cycle of the female. Jour. of Biol. Chem. 77: 651-654. 1928.
- (25) Evans, H.M. and Simpson, M.E.  
Hyperplasia of mammary apparatus of adult virginal females induced by anterior hypophyseal hormones. Proc. Soc. Exper. Biol. and Med. 26: 595. 1929.
- (26) Evans, H.M. and Simpson, M.E.  
Impairment of the birth mechanism due to hormones from the anterior pituitary. Proc. Soc. Exper. Biol. and Med. 26: 595-598. 1929.
- (27) Evans, H.M., Cornish, R.E., Simpson, M.E.  
Potent, sterile, and low-protein extracts of the growth hormone from the anterior hypophysis. Proc. Soc. Exper. Biol. and Med. 27: 101. 1929.



- (28) Evans, H.M. and Simpson, M.E.  
Stimulation of placenta reaction in virginal endometrium by treatment with anterior pituitary hormone. Proc. Soc. Exper. Biol. and Med. 26: 595-598. 1929.
- (29) Evans, H.M.  
The growth hormone. Science, 75: x. 1932.
- (30) Evans, H.M., Meyer, K., Pencharz, R., and Simpson, M.E.  
Cure of cachexia following hypophysectomy by administration of the growth hormone and its relation to the resulting adreno-cortical repair. Science, 75: 442-443. 1932.
- (31) Evans, H.M.  
The growth hormone of the anterior pituitary. Jour. Amer. Med. Assoc. 104: 1232. 1935.
- (32) Frederica, L.S., and Holm, Eiler.  
Experimental contribution to the study of the relation between night-blindness and malnutrition. Influence of deficiency of fat-soluble vitamin A in the diet on the visual purple in the eyes of rats. Amer. Jour. Physiol. 73: 63. 1924.
- (33) Gregory, P.W., and Goss, H.  
The hypophyseal growth hormone and glutathione concentration; does the hormone influence the concentration concurrently with the increase in weight? Jour. Exper. Zool. 69: 13. 1934.
- (34) Harris, L.J., Innes, J.R.M., and Griffith, A.S.  
On the pathogenesis of avitaminosis A. Vitamin A as a keratinizing factor. Lancet. 2: 614-617. 1932.
- (35) Hertz, R., Hellbaum, A., and Hisaw, F.L.  
Gonadotropic action of Phyone on juvenile female rabbits. Proc. Soc. Exper. Biol. and Med. 30: 41-42. 1932.
- (36) Hoskins, R.G.  
Tides of life. New York. W.W. Norton & Company, p. 118-120. 1933.

- (37) Johnson, G.E., and Sayles, E.D.  
The effects of daily injections of bovine anterior pituitary extract upon the developing albino rat. *Physiol. Zool.* 2: 285-301. 1929.
- (38) Johnson, G.E., Gann, E.L., Poster, M.A., and Coco, R.M.  
The effect of daily hetero-pituitary implants into adult but sexually inactive male ground squirrels. *Endoc.* 18: 86-96. 1934.
- (39) Lee, H.O., and Schaffer, W.K.  
Anterior pituitary growth hormone and the composition of growth. *Jour. of Nutrit.* 7: 337. 1934.
- (40) Macy, Isie G., Outhouse, Julia, Long, M. Louisa, and Graham, Alice.  
Human milk studies. I. Technique employed in vitamin studies. *Jour. Biol. Chem.* 73: 153-174. 1927.
- (41) Mason, Karl E.  
Can ferrous iodide replace vitamin A in the diet? *Anat. Rec.* 51: 57. 1931.
- (42) McCollum, E.V., and Davis, Marguerite.  
The necessity of certain lipoids in the diet during growth. *Jour. Biol. Chem.* 15: 167-175. 1913.
- (43) McCollum, E.V., and Davis, Marguerite.  
Observations on the isolation of the substance in butter-fat which exerts a stimulating influence on growth. *Jour. Biol. Chem.* 19: 245. 1914.
- (44) Nelson, E.M.  
Regulating the storage of vitamin A in animals that are to be used for the determination of this vitamin. *Science*, 68: 212. 1928.
- (45) Orr, J.B., and Richards, M.B.  
Growth and vitamin A deficiency. *Biochem. Jour.* 28: 1259-1273. 1934.
- (46) Osborne, Thomas E., and Mendell, Lafayette E.  
The influence of butter-fat on growth. *Jour. Biol. Chem.* 16: 423-437. 1913.

- (47) Osborne, Thomas B., and Mendell, Lafayette B.  
The influence of cod liver oil and some other fats on growth. *Jour. Biol. Chem.* 17: 401. 1914.
- (48) Osborne, Thomas B., and Mendell, Lafayette B.  
Further observations of the influence of natural fats upon growth. *Jour. Biol. Chem.* 20: 379. 1915.
- (49) Osborne, Thomas B., and Mendell, Lafayette B.  
Ophthalmia as a symptom of dietary deficiency. *Amer. Jour. Physiol.* 69: 543-547. 1924.
- (50) Parkes, A.S., and Drummond, J.C.  
The effects of fat-soluble vitamin A deficiency on reproduction in the rat. *Brit. Jour. Exper. Biol.* 3: 251-275. 1926.
- (51) Putnam, T.J., Teel, H.H., and Benedict, E.B.  
The preparation of a sterile active extract from the anterior lobe of the hypophysis. *Amer. Jour. Physiol.* 84: 157-164. 1928.
- (52) Reichert, F.L.  
The results of replacement therapy in an hypophysectomized puppy: four months of treatment with daily pituitary heterotransplants. *Endoc.* 12: 451. 1928.
- (53) Riddle, Oscar, and Bates, Robert W.  
Concerning anterior pituitary hormones. *Endoc.* 17: 689-698. 1933.
- (54) Rubinstein, H.S.  
The difference of response of the pituitary glands of male and female albino rats treated with the growth hormone. *Anat. Rec.* 61: 131-139. 1934.
- (55) Rubinstein, H.S. and Fox, L.M.  
The water and solid content of the brains of albino rats treated with the growth hormone. *Jour. Compar. Neurol.* 60: 349. 1934.
- (56) Rubinstein, H.S., and Kolodner, L.J.  
The effect of the growth hormone on body and tail lengths. *Anat. Rec.* 58: 107-110. 1934.

- (57) Sherman, Henry C.  
Chemistry of food and nutrition. Third edition.  
New York. The Macmillan Company, p. 444-471. 1928.
- (58) Smith, P.E.  
The experimental feeding of fresh anterior  
pituitary substance to the hypophysectemized rat.  
Amer. Jour. Physiol. 81: 20-26. 1927.
- (59) Smith, E.C., and Lantz, E.M.  
Changes in the incisors of albino rats accompanying  
a deficiency of vitamin A. Jour. of Home Econ.  
25: 411-415. 1933.
- (60) Sentag, L.W., and Munson, P.L.  
The effect on the weight of the offspring of  
administration of Antuitrin G to the pregnant rat.  
Amer. Jour. Physiol. 108: 593-598. 1934.
- (61) Steenbock, H., Boutwell, P.W., and Kent, Hazel.  
Fat soluble vitamin. I. Jour. Biol. Chem.  
35: 517-527. 1918.
- (62) Steenbock, H., and Cross, E.G.  
Fat soluble vitamin. II. The fat-soluble vitamin  
content of roots together with some observations  
on their water-soluble vitamin content. Jour.  
Biol. Chem. 40: 501-531. 1919.
- (63) Steenbock, H., Nelson, Mariana T., and Black, Archie.  
Fat soluble vitamins. XX. A modified technique for  
the determination of vitamin A. Jour. Biol. Chem.  
62: 275. 1924.
- (64) Steenbock, H., Sell, Mariana T., and Boutwell, P.W.  
Fat soluble vitamins. VII. The fat-soluble  
vitamine content of peas in relation to their  
pigmentation. Jour. Biol. Chem. 47: 303. 1921.
- (65) Steenbock, H., Sell, M.T., and Buell, M.V.  
Fat soluble vitamins. VII. The fat-soluble vita-  
mine and yellow pigmentation in animal fats with  
some observations on its stability to saponifica-  
tion. Jour. Biol. Chem. 47: 89. 1921.

- (66) Sutton, T.S., Setterfield, H.E., Krauss, W.E.  
Nerve degeneration in the albino rat associated with vitamin A avitaminosis. The use of the polarizing microscope in diagnosing degenerative changes in the myelin sheath. Jour. Biol. Chem. 105: lxxxix-xc. 1934.
- (67) A.A.A.S.  
Isolation of vitamin A. Science 75: sup. p. 9 Jan. 29, 1952.
- (68) Teel, Harold M.  
Diuresis in dogs from neutralized alkaline extracts of the anterior hypophysis. Jour. Amer. Med. Assoc. 93: 760. 1929.

## PLATE I

## Explanation of figures.

Fig. 1. Injected adult animal #36. Showing the thick smooth coat and the relatively good condition of the eyes.

Fig. 2. Photomicrograph of section of the ovary of #36, magnification of approximately 20 diameters. Showing the large solid corpora lutea and the comparatively few follicles.

Fig. 3. Photomicrograph of cross-section of the vagina of #36, magnification approximately 400 diameters. Showing the low vaginal mucosa.

Fig. 4. Photomicrograph of the vaginal smear of #36 on the day of first injection, magnification approximately 400 diameters. All cells are cornified.

Fig. 5. Photomicrograph of the vaginal smear of #36 on the day of autopsy showing leucocytes and nucleated epithelial cells. Magnification of approximately 400 diameters.



Fig. 1



Fig. 2



Fig. 3



Fig. 5

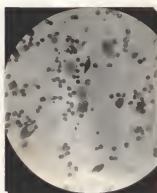


Fig. 6



## PLATE II

## Explanation of figures.

Fig. 1. Adult control animal #39C depleted of vitamin A but receiving no injections. Showing the rough thin coat and bloody exudate on the eyelids.

Fig. 2. Photomicrograph of section of the ovary of #39C, magnification of approximately 20 diameters. Showing the corpora lutea containing blood spaces and the greater number of follicles.

Fig. 3. Photomicrograph of the cross-section of the vagina of #39C, magnification of approximately 400 diameters. Showing the high vaginal mucosa.

Fig. 4. Photomicrograph of the vaginal smear of #39C on the first day of the experiment, magnification of approximately 400 diameters. All cells are cornified.

Fig. 5. Photomicrograph of the vaginal smear of #39C on the day of autopsy, all the cells are cornified. Magnification of approximately 400 diameters.

## PLATE II.



Fig. 1

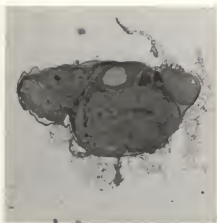


Fig. 2

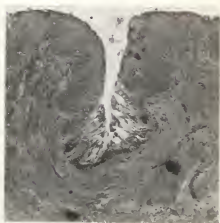


Fig. 3



Fig. 4

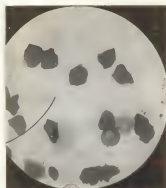


Fig. 5

## PLATE III

## Explanation of figures.

Fig. 1. Injected young animal #62. Showing the smooth thick coat and the fairly good condition of the eyes.

Fig. 2. Photomicrograph of section of the ovary of #62, magnification of approximately 20 diameters. Showing blood spaces in the corpora lutea and the large number of follicles.

Fig. 3. Photomicrograph of the cross-section of the vagina of #62, magnification of approximately 400 diameters. Showing the high vaginal mucosa.

Fig. 4. Photomicrograph of the vaginal smear of #62 on the day of the first injection, magnification of approximately 400 diameters. All cells are cornified.

Fig. 5. Photomicrograph of the vaginal smear of #62 on the day of autopsy, all the cells are cornified. Magnification of approximately 400 diameters.

## PLATE III.



Fig. 1

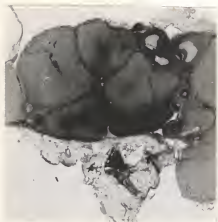


Fig. 2



Fig. 3

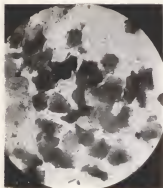


Fig. 4

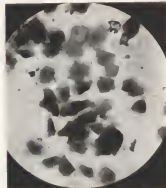


Fig. 5

