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Some effects of Antuitrin S on the ovaries of immature hamsters

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SOME EFFECTS OF ANTUITRIN S ON THE OVARIES OF IMMATURE HAMSTERS

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TABLE OF CONTENT

CHAPTER I

INTRODUCTION

A relationship between the pituitary gland or hypophysis, and sexual development has been recognized for sometime. Much literature has been compiled on work which has been done on such mammals as the albino mouse, rat and guinea pig but relatively little has been compiled on similar work on the Syrian golden hamster, a comparatively new laboratory mammal.

It is the purpose of this experiment to note some of the effects of the sex hormone from the pituitary gland on the ovaries of immature hamsters and attempt a verification of some of the findings of other workers.

CHAPTER II

REVIEW OF LITERATURE

The fact that there is a relationship between the anterior lobe of the pituitary gland and the sexual development has been established by experimentation and recognized for sometime. The observation leading to the establishment of this important influence of the anterior pituitary on females gonads originated with Comte (1909) who noticed the increased size of the pituitary during pregnancy in women. He attributed this increase to "pregnancy cells."

Lounois and Moulon (1903) ascribed the enlargement of the pituitary to the proliferation of the basophil cells.

Erdheim and Stumme (1909) described the "pregnancy cell" as chromphobe Cells.

In 1912 Aschner produced genital hypoplasia by excision of the pituitary in rats.

Early works included feeding and transplanting the whole pituitary gland or part of it, and injecting extracts from the gland.

Evans and Long (1921, 1922) reported increased size of the ovaries in rats which have been given daily injections of extracts from the pituitary.

Evans (1924) by injections of saline extracts of bovine anterior pituitary, inhibited the oestrus cycle in rats and produced striking alterations in the ovaries, namely, luteinization of the follicles.

In 1927 Smith and Engle used transplants from rats, mice, guinea pigs, rabbits, cats and pigeons, sexually mature and placed them on immature rats and mice. The implants rapidly induced precocious sexual maturity in the females.

Zondek and Aschheim (1927) injected mice with anterior pituitary extract and activated the ovaries to follication and the formation of ovarian hormones.

Johnson and Hill in 1930 gave injections of 0.1% bovine anterior pituitary extract to immature female albino mice and produced a marked increase in the size and maturation of the ovaries.

Wolfe and Cleveland (1931) obtained increased ovarian follication in rabbits by an implantation of anterior lobe of the pituitary on immature rats.

Guyénot et al. (1932) produced similar results with guinea pigs by prolonged treatments with extracts from the pituitary gland.

Zondek and Aschheim (1928) described two gonadotropic principles from the hypophysis. Prolan A. which was said to ripen ovarian follicle and stimulate them to the secretion of the female sex hormone known as oestrin, folliculin or theelin, which produces the phenomenon of oestrus. Prolan B. was considered to luteinize the graafin follicle and to lead to the secretion of a hormone-lutin, corpora or progestrin (Corner and Allen, 1929) which prepares the endometrium for the reception of the ovum.

Evans and Simpson (1928) prepared an alkaline aqueous extract of the anterior hypophysis which contained only the growth hormones, and an acid aqueous extract which contained only the gonads-stimulating hormone.

Teel and Cushing (1930) found that the hypophysis provides a growth hormone as well as a sex maturing hormone.

Leo Loeb (1932-1933) found that there are at least two types of anterior pituitary hormone that act on the ovaries, one of which affects also the thyroid.

The two hormones are (1) one which is responsible for the increased size of the ovarian follicle and for the maturation processes that take place in the granulosa of the follicles. (2) the hormone which acts in the opposite manner on the follicles.

Engle (1934) obtained a luteinizing effect as well as follicular activation by injections on immature monkeys.

Allen in 1928 also produced sexual precociousness in the monkey.

Reiss and Langendorf obtained precociousness in dogs and rabbits. Evidence for two distinct hormones influencing the ovarian cycle was also brought forward by Weisner and Crew (1930) who called them rho I and rho II, and in 1931 by Fevold, Hisaw and Leonard; Hill and Parkes; and a method for separating the follicle-stimulating and the luteinizing hormones has been described. (Fevold and Hisaw).

Hamlett (1935) gave large doses of Antuitrin S to armadillos-non pregnant but mature and caused the follicle to rupture and form corpora.

Fremery (1939) found that gonadatropic substances of pituitary origin extracted from hog's pituitary, human pituitary, and human menopausal urine produced growth of ovarian follicles but no ovulation or luteinization in immature monkeys.

August (1941) in an attempt to cause sheep to come in heat in the spring so that fall lamb could be produced had 43.1% of 4281 ewes, which had been given intraveneous injections of follicular hormones either alone or with prolan, produce lamb.

CHAPTER III

MATERIALS AND METHODS

The Syrian golden hamster (Cricetus Auratus) was used in this experiment because it is closely related, phylogenetically, to the animals mentioned in the literature and because they mature at an earlier age than any of these animals (Selle 1945).

Antuitrin S- a name employed for a proprietary extract of the anterior lobe of the hypophysis (Parke, Davis) - is known to induce maturity in the gonads of animals. This hormone was obtained, indirectly, from the laboratories of Parke, Davis for use in this experiment.

A pair of six-weeks old hamsters was obtained from the General Biological Supply House, Chicago, in October, 1948. They were kept in separate cages and fed a diet of rabbit pellets, cracked grained which had been soaked in cod liver oil, brown bread, pecans and white potatoes. At night they were given such fresh vegetables as lettuce, collards, cabbage and carrots. Fresh water was supplied daily and the cages were cleaned and given a fresh supply of shredded paper twice a week.

The hamsters were bred in November at the age of ten weeks. Sixteen days after breeding, a litter was born.

At the weaning age of twenty-one days the youngs were separated from the mother and from each other. The young females to be used as experimentals were kept together in a cage and the controls were kept together in another cage. All of the young males were kept together in a single cage as they were not used in the experiment.

Three subsequent litters were obtained in February, May and July and separated like the first.

Beginning at the age of twenty-one days and continuing through the twenty-sixth day, the experimental females were given daily injections of 0.3cc of a 0.1% solution of Antuitrin S.

To make the injection the animal was picked up in the left hand by catching the skin just behind the head with the thumb and index finger and held so that the animal's back rested in the palm of the hand. A twenty-five gauge needle was used on a 1cc syringe to make the injection subcutaneously in the abdominal region.

The controls were given a daily injection of a saline solution for the same period of time.

On the twenty-seventh day the animals were killed. The ovaries were taken, fixed in Bouin's, washed in 50% alcohol and stored in 70% alcohol. The ovaries were prepared for histological study by Guyer's paraffin method. They were cut at 10 microns, stained in Erlich's haemotoxylin and counter-stained with eosin.

The ovaries from one control were taken at twenty-one days of age. An adult ovary was also sectioned.

CHAPTER IV

EXPERIMENTAL RESULTS

A count was made of the mature or near-mature follicles found in the ovaries of six twenty-seven day old hamsters which had received injections of Antuitrin S. A similar count was made of the follicles, mature and near-mature, found in the ovaries of three twenty-seven day old untreated hamsters. If a follicle had a two layered granulosa in tract it was considered primary. If the two layered granulosa was loosened or if there was an antrum present, the follicle was considered near-mature or mature.

The largest number of mature follicles- those with an antrum and the ovum eccentrically located - found in any one experimental ovary was eight. The average for all of the ovaries was approximately four. There was an average of approximately nine follicles per ovary with well loosened granulosa.

The twenty-seven day old untreated ovaries had an average of two mature follicles and approximately five with the granulosa well loosened. By actual count the number of primary follicles found in the normal twenty-seven day old was about the same as the number found in the experimental ovary. When the two ovaries are compared with a normal twenty-one day old ovary neither one seems to have a greater rate of follicular formation than the other. In other words, the Antiutrin S seems to have increased the rate of maturity of the follicle already present rather than increase the number of follicles.

There were more mature follicles found in the experimental ovaries which seemed ready to rupture than were found in the normal ovaries. There were three follicle s found in the experimental ovaries which seemed about

ready to rupture. The normal ovaries did not contain any which seemed ready.

No corpus luteum was found in either the normal or experimental ovaries.

CHAPTER V

DISCUSSION

The increased size of follicles and the increased rate of maturity indicated in this experiment with the immature hamster is in agreement with the finding in the rat by Evans and Long (1921, 1922); in the mouse by Zondek (1927); in the rat and mouse by Smith and Engle (1927); in the guinea pig by Guyénot et al. (1932); in the rabbit by Wolfe and Cleveland (1931); in the monkey by Allen (1928) and also by Engle (1934); in dogs and rabbits by Reis and Langendorf (1929); in the armadillo by Hamlett (1935) and in sheep by August (1941).

Foster and Hisaw (1935) in trying to produce experimental ovulation in anoestrus cats used several different pituitary extracts but found that only the follicle-stimulating factor, Antuitrin S, gave constant follicular enlargment without atresia or hemorrhage. No atretic cells or indications of hemorrhage have been found in the ovaries examined in this experiment. This would seem to indicate that the follicle-stimulating hormone used in this experiment was free from luteinizing material and, therefore, produced enlargment of follicles without the formation of lutea.

Lane (1935) found that by administering various doses of the follicle-stimulating hormone to fifteen day old rats he could increase the number of follicles proportionately to the size of the doses used. The constant dosage used in this experiment did not seem to increase the number of follicles as we found no appreciable difference in the number of follicles found in the normal and in the experimental ovaries. The difference in the effect in the two cases may be accounted for by the fact that the rat at fifteen days of age is relatively less mature than is a twenty-one day

old hamster. Selye, Collip and Thompson (1935) found that age is a factor in the responsiveness of the ovary to gonadotropic hormones. They found that injections of gonadotropic substances prior to the formation of the two-layered granulosa in the ovary of the rat would cause prolification of the granulosa so as to produce an increased number of follicles, while injections made after the follicles had formed two-layered granulosa, had the effect of inducing precocity or development of these follicles.

The hamster's ovary seems to respond to the effects of Antuitrin S much in the same manner as that of the rat. The ovary of the twenty-one day old hamster has a predominance of follicles with the two-layered granulosa in-tact. So that the injections had the effect of inducing maturity in these follicles rather than increasing the number of follicles.

In experiments with the ovaries of immature rats, Fevold, Hisaw and Leonard found that by first stimulating the ovaries with the gonad-stimulating hormone and then treating them with the luteinizing hormone, the follicles would become luteinized. They concluded, therefore, that luteinization is the result of a one-two reaction of the gonadotropic substances of the pituitary and must take place in the order designated. That is, the follicle-stimulating hormone must act first and this action must be followed by the action of the luteinizing hormone if the follicle is to become luteinized.

In view of the preceding facts, it is possible, there, to elicit the development of small follicles in animals like the rat and hamster without the development of corpora lutea by injecting small doses of the gonad-stimulating hormone, Antuitrin S.

The absence of corpora lutea in the ovaries examined in this experiment may be accounted for by the fact that the injection of small doses

of Antuitrin S had the effect of interrupting the normal ratio of the follicle-stimulating hormone to that of the luteinizing hormone produced by the animal's own hypophysis so that luteinization did not keep pace with follicle maturity.

CHAPTER VI

SUMMARY

The results of this experiment with the ovary of the immature hamster would seem to justify the following conclusions:

(1) Injections of Antuitrin S in immature hamsters induce follicle maturity.

(2) Small, constant doses administered from the age of twenty-one days through the age of twenty-six days do not produce corpora lutea.

(3) There is a striking similarity in the response of the immature ovary of the hamster to Antuitrin S and that of the rat, mouse, guinea pig, armadillo and rabbit.

(4) At twenty-one days of age the hamster is more mature sexually than is either the rat, mouse, guinea pig, armadillo, or rabbit of similar age.

EXPLANATION OF FIGURES

Fig. 1. The ovary of a normal twenty-seven day old hamster.

Fig. 2. The ovary of a twenty-seven day old hamster after injections of Antuitrin S. Compare with figure 1.

Fig. 3. The ovary of a normal twenty-one day old hamster.

Fig. 4. The ovary of a normal adult hamster.

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