van der Maas[5] working upon *Xiphophorus helleri* castrated fourteen males by a single lateral incision upon the side of the fish. Of these only one large male survived. In this individual no effect was noted upon the secondary sex characters, and an autopsy indicated regeneration of the testis containing active sperm. They also tried implantation of testis into the abdominal cavity of a female. The ovaries were left intact. Of the eighteen cases only six survived, and upon these no effect was noted nor did autopsy indicate any testis tissue remaining. They were unable to demonstrate any hormonal relation between the gonads and the secondary sex characteristics in Xiphophorus.

Bock[6] castrated the stickleback *Gasterosteus oculeatus* and presents a successful record of post operative life. He removed the compact gonads through a small ventral slit on the abdomen. For anesthesia he used ether and water. The stickleback is a fish that shows secondary sex coloration in the male appearing in breeding season. Bock definitely found that castration prevented the appearance of that nuptial coloration. If one gonad was left intact the fish still developed the full male coloration, but the intensity was not as great as that in a fish containing both gonads. No generation of gonadal tissue was found.

Tozawa[7] shares with Bock the honor of a conclusive piece of work. He used the Japanese Bitterling, *Acheilognathus intermedium*, and performed gonadectomies both unilateral and total upon both sexes. This fish likewise develops a nuptial color during breeding season with a rather distinct reddening on certain parts of the body. He finds that the appearance of the nuptial coloration and the pearl organs is partially inhibited in the incompletely gonadectomied individuals, and more completely inhibited in the totally gonadectomied group. He agrees with Bock that the nuptial coloration is definitely influenced by a substance or substances produced by the sex glands.

Such work indicates to some degree the adaptivity of fish to operative procedure and the present status of experimental results of gonad removal and transplantation upon fish.

I. B. HANSEN

WESLEYAN UNIVERSITY

[5] G. J. van Oordt and C. J. J. van der Maas, "Castration and Implantation of Gonads in *Xiphophorus helleri* Heckel (Teleost)," Koninklije Akad. van Wetenschappen te Amsterdam. *Pro. of the Sect. of Sciences*, 29: 1172–1175, 1926.

[6] Friedrich Bock, "Kastration und sekundäre Geschlechtsmerkmale bei Teleostiern," *Zeit. für Wissen. Zool.*, 130: 455–468, 1928.

[7] Tomizyu Tozawa, "Experiments on the Development of the Nuptial Coloration and Pearl Organs of the Japanese Bitterling," *Folia Anatomica Japonica*, 7: 407–417, 1929.

THE RELATION BETWEEN THE ESTRUS-PRODUCING HORMONE AND A CORPUS LUTEUM EXTRACT ON THE GROWTH OF THE MAMMARY GLAND

IN connection with a study of the physiological cause of the growth of the mammary gland and the initiation of milk secretion, it has been demonstrated at this station that during pregnancy cattle excrete in the urine increasing amounts of the estrus-producing hormone.[1]

A study was therefore made of the effect of this hormone on the growth of the mammary gland in the rabbit.[2] In the normal rabbit after continued estrus the mammary glands show extreme extension of the duct systems resembling the naked branches of a tree. If pregnancy or even pseudo-pregnancy now ensues, the ducts develop lobules containing large numbers of alveoli, resembling the budding of leaves from the smaller branches. These two types of growth can be distinguished macroscopically in the fixed gland.

It was found that the daily injection of 20 rat units of the estrus-producing hormone recovered from pregnant cow's urine for 30 days in male castrate rabbits and in female rabbits castrated previous to puberty caused growth of the duct system of the glands equal to that produced during continued estrus in the normal female. A slight milk secretion resulted in these cases. The injection of greatly increased amounts of the hormone did not carry the development beyond this stage.

The purpose of the present communication is to report our recent success in developing the type of mammary growth characteristic of pregnancy and pseudo-pregnancy.

In continuing our effort to stimulate experimentally the growth of the mammary gland equal to that observed during pregnancy, it seemed logical next to determine the action of the hormones of the corpus luteum. In our experiments the method of extraction of the crude extract of the corpora lutea of the sow described by Allen[3] was followed. In an attempt to simulate the normal hormonal stimulation at the time of ovulation, an ovariectomized rabbit

[1] C. W. Turner, A. H. Frank, C. H. Lomas and C. W. Nibler, "A Study of the Estrus Producing Hormone in the Urine of Cattle during Pregnancy," *Mo. Agr. Exp. Sta. Res. Bul.* 150, 1930.

[2] C. W. Turner and A. H. Frank, "The Effect of the Estrus Producing Hormone on the Growth of the Mammary Gland," *Mo. Agr. Exp. Sta. Res. Bul.* 145, 1930.

[3] W. M. Allen, "Physiology of the Corpus Luteum. V. The Preparation and Some Chemical Properties of Progestin, a Hormone of the Corpus Luteum which Produces Progestational Proliferation," *Amer. Jour. of Phys.*, 92: 174, 1930.

whose mammary glands showed only the estrus type of development was injected daily with 20 r. u. of the estrus-producing hormone during a period of three days. Following this 1 cc of the crude corpus luteum extract was injected daily for 11 days. No change could be noted in type or extent of growth in glands before and after injection.

Similarly a castrate male rabbit was injected daily with 20 r. u. of the estrus-producing hormone during a period of 30 days. A check gland removed at this time showed the development of the estrus type of growth. The injection of 1 cc daily of the crude corpus luteum extract was begun 10 days later and continued for 30 days. Glands removed at 10-day intervals showed neither additional growth of the ducts nor the pregnant type of development.

A second castrate male rabbit which had received the same previous treatment was injected with 1 cc daily of the crude corpus luteum extract plus 12 r. u. of the estrus-producing hormone. In glands removed at 10-day intervals during a period of 30 days increasing development of both ducts and lobules was observed strikingly similar to that produced during pregnancy.

In a third male castrate rabbit which had received the same previous treatment somewhat greater growth of the ducts and lobules was observed following injection of the same amount of the corpus luteum extract but an increased amount (20 r. u.) of the estrus-producing hormone. Additional experiments are now in progress having as their object the further determination of the effect of increasing amounts of the estrus-producing hormone with constant amounts of the corpus luteum extract.

It will be noted in the previous experiments that the estrus type of development of the ducts of the mammary gland had been produced previous to the initial injections of the corpus luteum extract. In a fourth male castrate rabbit daily injection of 12 r. u. of the estrus-producing hormone and 0.5 cc of the crude corpus luteum extract was made over a period of 30 days. The size and development of the mammary gland characteristic of advanced pregnancy were observed at that time.

These observations lead us to believe that the growth of the mammary glands during pregnancy comes as a result of the combined action of the increasing amounts of the estrus-producing hormone and one or more hormones from the corpus luteum. It should be noted, however, that lactation was not produced in these animals. This may be due to the fact that the injections were continued up to the time of examination of the glands. The initiation of milk secretion may be stimulated by any one of several factors. It is possible that it follows the complete withdrawal of the growth stimulus or it may result from changes in the effective concentration of the two hormones. It is also possible that an as yet unidentified hormone is required. This phase of the problem is at present being studied.

In our study of the effect of the estrus-producing hormone on the growth of the mammary gland it was suggested that the growth observed during pseudo-pregnancy may be due to either one or both of these hormones (estrus-producing and corpus luteum) acting on the uterus, which may in turn produce a hormone or hormones which may be the active agent.

Two separate lines of evidence seem to indicate that the action of these hormones is directly upon the mammary gland rather than through the mediation of the uterus. The production of the growth of the gland in male castrates eliminates the possibility of the uterus acting as a gland of internal secretion. On the other hand, the development of the mammary gland characteristic of pseudo-pregnancy was observed in a hysterectomized rabbit after coitus.

Having obtained the type of growth characteristic of pregnancy by the combined action of the estrus-producing and the corpus luteum extract, it became possible to test for the presence of the active principle in the urine of pregnant cows. In this we have been successful. It has been found possible to produce the growth characteristic of pregnancy with a water and alcohol soluble extract in both castrate male rabbits and rats in combination with the estrus-producing hormone. By using this method it is proposed to trace the changes in the concentration of this active principle (corpus luteum?) in the urine of cows during the course of gestation.

C. W. TURNER
A. H. FRANK

MISSOURI AGRICULTURAL EXPERIMENT STATION

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