TABLE III

Material	Units received	Mortality percentages
Control 1 { Covered with }	0	0
Control 2 { glass slide }	0	0
Slide 1	1/4	8.22
Slide 2	1/4	*
Slide 3	1/2	53.43
Slide 4	1/2	54.75
Slide 5	3/4	98.83
Slide 6	3/4	100.0
Slide 7	1	100.0
Slide 8	1	100.0

* Slide 2 was accidentally destroyed.

employed was to suspend the oocysts in the reagent for the desired time, wash thoroughly, resuspend in a 2½ per cent. solution of potassium dichromate and incubate at 20° C. At the end of 72 hours of incubation, the oocysts were examined and counted. Those failing to develop were considered dead. The results obtained are presented in Table IV:

TABLE IV

Reagent	Strength	Mortality percentages
$HgCl_2$	1 per cent.	100.0
$HgCl_2$	0.1 " "	18.4
Iodine suspensoid		
Merck	5 " "	100.0
NaOH	0.5N	0.5
NaOH	2N	1.1
HCl	0.5N	1.3
HCl	2N	4.5
Chlorazene	4 per cent.	0.0
Formol	2 " "	31.4
Formol	5 " "	40.0
Cresol	2 " "	100.0
Cresol	5 " "	100.0
Phenol	2 " "	99.4
Phenol	5 " "	100.0
Controls	2.5 " "	0.0
Potassium dichromate		

Time of exposure, 48 hours.

The comparative killing power of efficacious reagents are listed in Table V.

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TABLE V

Reagent	Strength	Time required for 100 per cent. mortality
Iodine suspensoid		
Merck	5 per cent.	1 hour or less
Cresol	2 " "	4 to 8 hours
Cresol	5 " "	4 to 8 hours
Phenol	2 " "	48 hours

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GONADECTOMY IN THE GOLDFISH CARASSIUS AURATUS

GONADECTOMY upon fish has not been practiced to any great extent until within the last few years, when the first truly successful operations have been performed. Other work has largely been done to determine the relation between the development of certain secondary sex characteristics, especially nuptial coloration, and the gonads. That relationship has been quite definitely shown to exist. Removal of gonads in the goldfish was undertaken for a different reason, namely, to determine the effect, if any, upon the color change of the young common goldfish from its youthful brown to the orange of the adult. Although there is no difference in the color expression and behavior of the sexes of the goldfish, it was hoped that an upset of a hormonic balance might prove to be of value in a better understanding of the phenomenon, perhaps by changing the rate of time of depigmentation, or even in the total inhibition of the degenerating influence. This work was done as a part of a program of the study of pigment development and pattern formation now in progress at Wesleyan University.[1]

During the autumn of 1928 and 1929 some thirty-nine gonadectomies and twelve operative controls were performed upon young goldfish about five months old. Such fish, hatched in May, were over four centimeters long in October and early November, and no fish under four centimeters was used. Of the complete gonadectomies, twenty-one were upon males and eighteen upon females.

The gonads are paired organs, relatively large and decidedly soft in the goldfish, so that complete removal demands large incisions and careful manipulation. Unfortunately, they do not permit of tearing,

[1] H. B. Goodrich and I. B. Hansen, "The Post-embryonic Development of Mendelian Characters in the Goldfish *Carassius auratus*," in press.

but instead must be carefully severed from their connectives and removed with considerable delicacy.

The method of procedure was somewhat as follows. The fish was placed in a finger-bowl and sufficient saturated aqueous solution of chloretone added to anesthetize the individual in five to ten minutes. When the fish became unconscious it was placed in a paraffin dissecting plate previously hollowed to fit in general the shape of the fish. Two strips of absorbent cotton, moistened well with the solution from the anesthetizing bowl, placed over the head and the caudal peduncle served well to hold the fish in place. The presence of an abundance of the solution permitted the fish to remain in position without attention until the operation was complete upon that side.

It was found that a single median incision was inadequate to remove successfully both gonads. Consequently a lateral incision was adopted and performed upon both sides of the fish. The advantages of the double incision were more absolute certainty of removing all of both gonads, less injury and disturbance to the visceral organs, greater ease in operation and an intact ventral abdominal wall to remain as a firm support to the viscera. The objections are likewise several, the most serious of which is the double incision giving the fish a wound in aggregate twice as large as the median abdominal cut. It further has the disadvantages that practically all the abdominal ribs are cut on both sides and that a relatively large number of scales are removed. Operating time is increased likewise, averaging some twenty minutes.

With the fish securely fastened with the cotton strips, a line of scales was removed from the region just above the anus and extending upward and forward in a curve corresponding to the position of the gonad in the abdominal cavity. The primary break was made by piercing the abdominal wall with a needle and the incision was completed by fine scissors. With the wound open, further work was done with suitable instruments under a binocular dissecting microscope. After the gonad was removed, the wound was closed with one or two stitches of silk thread according to the size of the incision. The process was then repeated upon the other side of the fish. No aseptic methods were used as the probability of infection is slight. Ordinary care was used to keep the wound clean and free from scales.

The fish were kept isolated in finger-bowls and healing took place in about three weeks, by which time the stitches either had pushed out or were removed. Fungus infections were the chief cause of concern, and it was found that the quickest and surest remedy was surgical removal.

As a control, a group of twelve fish were treated similarly, but had no gonadal tissue touched.

Of the thirty-nine complete gonadectomies, thirteen died within a week or so of the operation. The remaining twenty-six healed perfectly, some being still alive and in good condition. The others were killed some six months later to note whether there had been any regeneration of gonadal tissue. Of the controls, only one died.

The results of the experiments were negative as far as the effect upon the pigment change was concerned. All the surviving fish subsequently passed through the color transformation in spite of the gonadectomy operation. The only noticeable effect was the lengthening of time before demelanization took place. This was due to operative shock, for the controls showed the same delay although to slightly lesser degree. Of those fish examined for regeneration tissue, it was observed that without exception some gonadal tissue was to be found, which indicates that regeneration to some degree does take place. In no case was the amount of regenerative gonadal tissue large.

The experiments are not entirely conclusive, but they indicate, first, that the gonads probably have no major rôle in the process of demelanization, and secondly, that the goldfish is excellent material for operative procedure.

Gonadectomy in fishes has been practiced by other workers and with some notable results. Kopeč[2] succeeded in castrating the minnow *Phoxinus laevis*, a fish that shows a nuptial coloration during the mating season. This nuptial change consists of a reddening of many parts of the body, especially the abdomen, and is more distinct in the male. He castrated these fish in a satisfactory fashion by a single abdominal incision just to the right of the mid line. Kopeč was not very fortunate in his post operative success, for all his fish had died at the end of three weeks. He reports evidence that gonad removal suppresses and removes almost entirely the nuptial hue, and believes that the development of the nuptial color in *Phoxinus laevis* depends upon the presence of the gonads.

Evidence of sex reversal in fish such as reported by Blacher[3] for *Lebistes reticulatus*, and Essenberg[4] for *Xiphophorus helleri*, in addition to Kopeč's work, led to three other important papers. Van Oordt and

[2] Stefan Kopeč, "Contribution to the Study of the Development of the Nuptial Color of Fishes," *Spraw. Z. Pos. Tow. Nauk. Warszawskiego*, 3, vol. 11–18, English summary, pp. 108–114, 1918.

[3] L. J. Blacher, "The Dependence of Secondary Sex Characteristics upon Testicular Hormones in *Lebistes reticulatus*," *Biol. Bulletin*, 50: 374–381, 1926.

[4] J. M. Essenberg, "Complete Sex-reversal in the Viviparous Teleost *Xiphophorus helleri*," *Biol. Bulletin*, 51: 98–111, 1926.

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.

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[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.