10869

Tests of Mammalian Gonad-Stimulating Hormones on Gonads of Fishes.

MYCHYLE W. JOHNSON AND OSCAR RIDDLE.

From the Carnegie Institution, Station for Experimental Evolution, Cold Spring Harbor, N. Y.

Houssay[1] implanted pituitary glands of fish and induced a prompt ovulation of the nearly ripe eggs of another species of fish, and Cardoso[2] and von Ihering[3] confirmed this result and further showed that extracts and suspensions of fish pituitary stimulate growth in testes and ovaries of various species of fish. Azevedo and Canale[4] reported that piscine pituitaries are effective while extracts from mammalian and avian pituitaries are not effective for gonad stimulation in fishes. Koch and Scheuring[5] injected prolan into 3- to 4-year-old rainbow trout with negative results. Matthews[6] obtained practically no stimulation of the testes of Fundulus with an unfractionated extract of sheep pituitaries, but demonstrated that removal of the pituitary in adult *Fundulus* is followed by inactivity or atrophy of the gonads. On the other hand, Boucher[7] and associates report slight evidence for the hastening of sexual maturity in eels by small amounts of pregnancy urine, while Calvet[8] and Damas[9] report success in stimulating the ovaries of lampreys with both prolan and whole extract of mammalian pituitary. It is evident that further studies are needed to resolve the question whether vertebrates higher than fishes have evolved a new and distinct gonad-stimulating hormone (FSH) or whether an apparent refractoriness of piscine gonads to mammalian FSH perhaps rests upon a basis hitherto unexplored.

We have tested the action of 3 types of previously assayed mammalian FSH preparations on 90 rainbow trout *(Salmo shasta?)* aged 11 and 16 months without any transfer of the fish from their normal habitat. For these trout and facilities for maintaining them

1 Houssay, B. A., *Rev. Soc. Argent. Biol.*, 1930, **6**, 686.

2 Cardoso, D. M., *Compt. Rend. Soc. Biol.*, 1934, **115**, 1347.

3 von Ihering, R., *Zool. Anz.*, 1935, **111**, 273.

4 Azevedo, P., and Canale, L., *Arq. Inst. Biol.* (Sao Paulo), 1938, **9**, 165.

5 Koch, W., and Scheuring, L., *Zool. Anz.*, 1936, **116**, 62.

6 Matthews, S. A., *Biol. Bull.*, 1939, **76**, 241.

7 Boucher, S., Boucher, M., and Fontaine, M., *Compt. Rend. Soc. Biol.*, 1938, **116**, 1284.

8 Calvet, J., *Compt. Rend. Soc. Biol.*, 1932, **109**, 595.

9 Damas, H., *Bull. Soc. Roy. des Sci. de Liège*, 1933, No. 4, 94.

under wholly normal conditions during our tests we are indebted to Mr. Stanley C. Walters, Foreman, New York State Fish Hatchery, Cold Spring Harbor, N. Y. A brief abstract of these results was published.[10]

In November, 60 11-month trout (weight about 30 g), divided into 4 equal groups, were injected with NaCl (control), pituitary FSH No. 429, mare serum powder, and prolan (Elberfeld). No. 429 was free from prolactin, was relatively rich in thyrotropin and contained little adrenotropin; a dose of 1 mg daily for 4 days gave a 400% increase in testes of young doves (weight 150 g), and in 20 mg daily doses for 3 days into 21-day rats gave ovaries of 48 mg, uteri of 55 mg, testes of 376 mg and seminal vesicles of 14.8 mg. The prolan contained 15 R.U. per mg. The mare serum powder, when administered in 10 mg doses daily for 4 days to young doves, increased testis weight by 300%. During the first 11 days the daily dosage per fish was as follows: No. 429, 0.5 mg; prolan, 2 mg; mare serum powder, 8 mg. During a final 10-day period all of these daily dosages were doubled. These quantities were injected intramuscularly in 0.2 cc of fluid.

Immediately upon first handling and injection of these small trout one or more males in all groups released sperm (milt) before injection, and a similar release of sperm was observed on many other days during the 21-day period of injection. Two such males were among the control and 8 among the 3 injected groups. At the end of the period of injection (or at death) 3 of 11 male control had testes filled with sperm, 3 such males were among the 7 males injected with No. 429, 3 among the 8 injected with prolan, and 3 among the 7 injected with mare serum. In addition, 3 treated males showed only a few sperm. Altogether 10 males injected with gonad-stimulating preparations had minute thread-like testes with no sperm and no developmental stage beyond spermatocytes undergoing their early period of growth.

Ovaries of injected females showed no detectable stimulation though ovaries of control and all treated groups contained oöcytes entering the growth period. There were some instances of oöcytes beginning to form yolk but no heavily yolked eggs were found. In view of the stage of ovarian development thus observed—a stage which might be considered especially sensitive to gonad-stimulating hormone—the definitely negative result of all these tests is especially notable.

In the following April, 30 rainbow trout of the same brood (then

[10] Johnson, M. W., and Riddle, O., *Year Book, Carn. Inst. of Wash.*, 1936, **35**, 53.

aged 16 mo, and slightly larger than the 11-mo group) were divided into 3 equal groups and injected daily for 15 days with NaCl (control), 4 mg prolan (15 R.U. per mg) or 1 mg FSH No. 474. This latter preparation, in doses of 1 mg daily for 4 days into 450 g pigeons, increased testis weight by 125%. Three fish of each group were injected intraperitoneally, the others intramuscularly. At autopsy the testes of the 7 treated males (only one with sperm) did not differ significantly from the 4 male controls. The ovaries of 13 treated fish were also insignificantly different from those of 6 control in both histology and in weight (control, 40.5 g; prolan, 39.8 g; No. 474, 44g).

It is notable that at the N. Y. State Hatchery where our fish were treated (and maintained at 51°F) these rainbow trout breed (at and after 2-years old) in November, not in the following March-April as they frequently do in some other localities.

Summary. (1) FSH from beef pituitaries, powdered serum of the pregnant mare, and a commercial preparation of human pregnancy urine (Prolan, Elberfeld) were otherwise proved potent and then injected daily for 21 or 15 days into 65 rainbow trout *(Salmo shasta?)* aged 11 or 16 months. An additional group of 25 trout of the same brood served as control (NaCl injected), and all fish were maintained in their normal habitat at 51°F while under test. (2) A fraction of the males had sperm-producing testes before injection but an equal or greater number had unstimulated minute thread-like testes at the end of treatment. The largest oöcytes in the ovaries of all groups were beginning to form yolk but no heavily yolked eggs were present. (3) Neither of the potent mammalian gonad-stimulating preparations used measurably stimulated either testes or ovaries in these trout. These results support the view that the FSH elaborated by pituitaries of fish differs qualitatively from that of mammals but much further study of the problem is needed.