

Gill ventilation and oxygen consumption in *Balistes capriscus*

	I. 700 g 17.9°C		II. 395 g 17.9°C		III. 320 g 17.5°C	
	Typical result	Range	Typical result	Range	Typical result	Range
Minute volume cm ³	210	200–330	111	90–165	90	65–175
Frequency/min	52	40–60	60	48–64	60	40–66
Stroke volume cm ³	4.04	3.8–8.25	1.85	1.5–2.6	1.5	1.25–4.3
pO ₂ inspired water mm Hg	151.0		154.5		150.7	
pO ₂ expired water mm Hg	32.8	29.5–62.5	37.7	12.3–70.2	41.9	17.4–73.0
% Utilization	78	58–81	76	55–92	72	51–89
Oxygen consumption cm ³ /kg/h	75.7	79.8 (Ave)	70.5	69.9 (Ave)	55.4	62.5 (Ave)
Gill area	484 sq mm/g		329 sq mm/g			

It seems probable that this preparation and technique will be useful in more detailed studies on the ventilation and gaseous exchange of bony fishes⁷.

men. Es wurden Sauerstoffausnützungen von 90% beobachtet.

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Zusammenfassung. Druck- und Bewegungsregistrierungen haben gezeigt, dass die Kiementilung beim plectognathen Fisch *Balistes capriscus* primär denselben doppelten Pumpmechanismus benützt, wie andere Knochenfische. Messungen des Ventilationsvolumens wurden durch Zufuhr von Wasser unter konstantem Druck zur Mundöffnung durch einen Gummischlauch, der an der steifen Haut des Fisches festgeklebt wurde, vorgenom-

⁷ This work was done during a visit to the Stazione Zoologica at Naples as part of the European Programme of the Royal Society, to whom I am grateful for their financial support. I wish to thank Dr. E. R. TRUEMAN for the loan of his recording equipment and Dr. D. BAUMGARTNER for her help with the gas analysis.

PRO EXPERIMENTIS

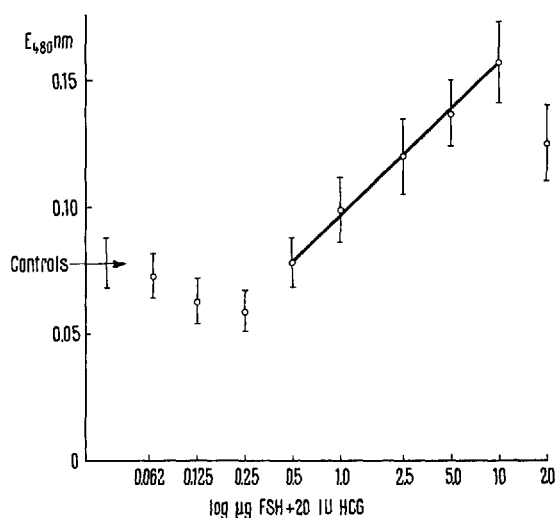
A Rapid, Sensitive Method for the Determination of Follicle-Stimulating Activity

We have developed a new method for the determination of follicle-stimulating (FSH) activity in the mouse. The method consists in the evaluation of the steroidogenesis, induced by FSH administration in impuberal female mice treated with a saturating dose of luteinizing hormone (human chorionic gonadotropin) (HCG).

The steroids produced by FSH administration were evaluated, as in the method for estrogens proposed by MARTIN¹, through the increased activity of some vaginal dehydrogenases which transform the colourless 2,3,5-triphenyltetrazolium chloride (TTC) into the reduced red form (formazan).

Groups of 19-day-old (± 12 h) Swiss strain impuberal female mice, weighing 10.5–11.5 g, received in 2 s.c. injections (0.25 ml \times 2, at 09.00 and 17.00) serial doses of FSH (lyophilized pig FSH, Mann Research Laboratories, New York) containing a saturating dose of LH (HCG Ormonoterapia Richter, Milan; 2830 IU/mg). A control group received only 20 IU of HCG, and a second control group was treated with physiological salt solution. 30 h after the first injection, 0.5 mg TTC dissolved in 0.01 ml distilled water were administered intravaginally, using a 'Hamilton' syringe graduated to 1 μ l, with No. 16 needle ending in a teflon tube.

Exactly 30 min later the animal was killed by decapitation and the vagina removed and washed with distilled



Evaluation of FSH activity by intravaginal TTC reduction. The log dose-response curve. Vertical lines indicate the standard error of the mean.

¹ L. MARTIN, J. Endocr. 20, 187 (1960).

water to remove the excess of unreduced TTC. The vagina was then dried with filter paper and weighed to ± 0.1 mg.

The organ was then extracted with 2 ml absolute ethanol-tetrachloroethylene mixture (3:1), in according to JARDETSKY and GLICK², and the reduced red TTC evaluated quantitatively in 5 mm cells at 480 nm by a Beckman DU spectrophotometer.

Previous experiments have shown that 20 IU HCG represent the optimal LH saturating dose. The best quantitative response, at equal dose, was obtained at ca. 30 h, the values for the s.c. route being slightly higher compared with i.p. administration.

The maximum intravaginal TTC reduction response is obtained with 10 μ g FSH (+ 20 IU HCG), as shown in the Figure. 1–10 μ g FSH can be easily assayed.

An interesting phenomenon is observed below 1 μ g FSH, i.e. a significant lowering of TTC reduction response as compared with controls treated with 20 IU HCG only. It should be pointed out that in the STEELMAN and POHLEY³ test this was revealed as a decrease in the ovarian weight in comparison with controls treated with HCG below 22.5 μ g FSH (LUGARO et al.⁴).

In summary, the main features of our methods are (1) the possibility of assaying FSH activity at levels 50 times

less than hitherto possible by the classical STEELMAN and POHLEY test, (2) the speed of performance (30 h) and (3) the significantly lower cost, due to the use of mice.

Riassunto. Gli Autori riportano un nuovo metodo di saggio sul topo di piccole quantità di ormone follicolo stimolante (FSH), basato sulla riduzione intravaginale del cloruro di 2,3,5-trifeniltetrazolio cloruro (TTC). È così possibile determinare quantitativamente in sole 30 h dosi di FSH almeno 50 volte inferiori a quelle valutabili con i metodi fino ad oggi proposti.

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and K. WEYDANZ

Institute of Organic Chemistry, University of Milano (Italy), 17 April 1967.

² C. D. JARDETSKY and D. GLICK, *J. biol. Chem.* 218, 283 (1956).

³ S. L. STEELMAN and F. M. POHLEY, *Endocrinology*, 53, 604 (1953).

⁴ G. LUGARO, R. PROVENCHI and A. CORBELLINI, *Folia endocr.* 18, 293 (1965).

Rapid Transmandibular Hypophysectomy of small Fish

Hypophysectomy of small fish is a delicate and time-consuming procedure. It is commonly performed by an opercular approach¹, i.e. the instruments are introduced through the opercular opening, which often has to be enlarged^{2,3}. The mortality approaches 10%. The well-trained surgeon needs 4–5 min, during which time the gills should be flooded with water.

The most difficult and time-consuming steps are the dissection of the roof of the oral cavity, the identification and protection of large vessels, and the trepanation. By the use of a drill and a guiding device centred on a suitable point of the skull, it should be possible to expose the pituitary simply by drilling through both soft tissues and bone. The guide might also serve to protect the large vessels and keep the field clear of soft tissues.

The procedure was worked out on crucian carps, *Carassius carassius* L., weighing 5–30 g. As in the common goldfish, *C. auratus* L.⁴, the pituitary occupies a bony chamber, the myodome, superior to the parasphenoid bone. It is enveloped by the origins of the rectus externus muscles of the eyes. The parasphenoid presents a median prominence well suited for supporting the drill guide. If the guide is introduced through the lower jaw close to the tip of the tongue and centred on the prominence, it will be concentric with the pituitary but separated from the gland by soft tissues and bone.

Equipment (Figures 1 and 2). The guide is made from a 35 mm length of cannula tubing, diameter 3.0/2.5 mm. It is bevelled 30° to make a close fit with the surrounding bones of the base of the skull when centred on the parasphenoid prominence. An aperture facing the fish's mouth is cut in the guide's wall close to its oblique end to admit instruments and light.

The tube's square end is fastened side-to-end to a permanent magnet, adhering to a soft steel plate. This

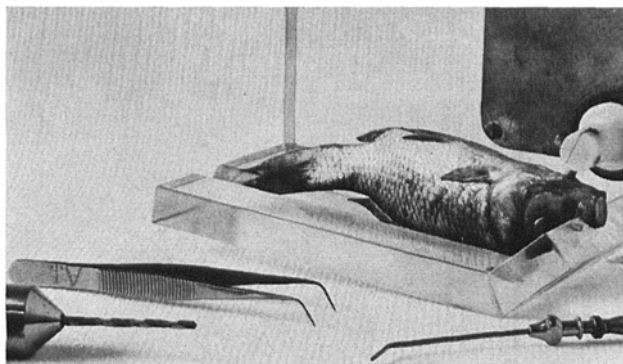


Fig. 1. The equipment.

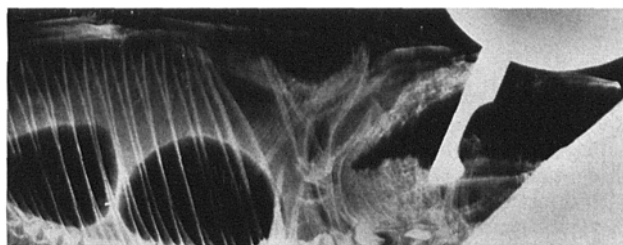


Fig. 2. A radiographic lateral projection of the fish in position. The guide is correctly centred on the parasphenoid prominence. Note that the guide prevents closure of the fish's mouth.

¹ A. A. ABRAMOWITZ, *Science* 85, 609 (1937).

² W. CHAVIN, *J. exp. Zool.* 133, 1 (1956).

³ S. N. AHSAN, *Can. J. Zool.* 44, 703 (1966).

⁴ W. R. BELL, *Zoologica* 23, 219 (1938).