

dante exprimée par la formation de 98 colonies qui, vers la 72<sup>e</sup> h ont commencé à se mêler formant une couche à la surface de la gélose, alors que chez les cultures normales un tel développement sur la gélose n'est apparu qu'au bout de 48 h, d'ailleurs assez réduit – environ 16 colonies dont le fusionnement n'a commencé qu'après le 6<sup>e</sup> jour. Les jours suivants la surface de la géloseensemencée de bactéries soumises à l'action de la capsicine s'est recouverte d'une couche caractéristique pour la croissance du *A. tumefaciens*, et dans les cultures normales, seulement après 10 jours.

Il convient de relever que cette nouvelle propriété ainsi obtenue s'est conservée aussi dans les générations suivantes.

Nous avons effectué des recherches aussi sur la virulence des cultures, tant des unes, que des autres. Nous avons utilisé la réaction de BERNAERTS et DE LEY<sup>6</sup> pour la détection du 3 céto-lactose. Cette réaction a montré que

la culture traitée par la capsicine possède une virulence plus grande que la normale.

**Summary.** It was established that the treatment of liquid cultures of *Agrobacterium tumefaciens* with capsicine (in doses of 0.0025–0.01) in the course of 6 h, causes a more abundant and quicker multiplication of the microorganism and an increase of its virulence, as measured by the method of BERNAERTS and DE LEY.

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<sup>6</sup> M. J. BERNAERTS and J. DE LEY, *Nature* 197, 406 (1963).

### Experiments on the Respiration of the Trigger Fish (*Balistes capriscus*)

Although several authors (WILLEM<sup>1,2</sup>, BERTIN<sup>3</sup>) have recognized that the Plectognathi have special problems in relation to their respiratory movements, relatively little experimental work has been carried out on this group of fishes. The possession of a markedly ossified skin confers rigidity and makes the body relatively indeformable and contrasts with the flexibility so characteristic of the locomotory and respiratory movements of other fishes. Largely on the basis of morphological studies, WILLEM<sup>2</sup> suggested that changes in volume of the buccal and opercular cavities mainly take place in a dorso-ventral direction as a result of movements of the branchiostegial apparatus in a lymphatic space beneath the skin. He considered that lateral movements of the palatal arch and operculum play a relatively minor role.

A preliminary study of gill ventilation in *Balistes capriscus* has been made by recording pressure changes in the buccal and opercular cavities of unanaesthetized fish by means of cannulae inserted dorsally through the nasal region and laterally through the operculum. Lateral expansion of the opercular region was found to be quite definite in this fish and its time course was recorded by means of an impedance pneumograph (HOGGARTH and TRUEMAN<sup>4</sup>) simultaneously with the pressure changes. These recordings have indicated that gill ventilation in this fish is achieved by the combined action of a buccal force pump and opercular suction pumps, as is typical of other groups of fishes (HUGHES<sup>5</sup>).

Specialized features of *Balistes* such as the stiffened skin with little mucous and the small mouth which scarcely moves during ventilation, suggested a new way of measuring the ventilation volume of a fish (Figure). A finger cut from a rubber glove was fixed round the mouth by means of Braun Histocoll T100B cement. Water was fed to the mouth from a plastic cone which floated at the surface of the water. The rate at which water was supplied was adjusted so that the level within the cone remained constant and the same as that in the aquarium. The flow rate was measured when a steady state had been reached. *Balistes* proved to be an ideal animal for this type of experiment as when confined in a small part of an

aquarium, it often remains stationary by means of the combined action of its fins. Samples of water drawn from the opercular cavity were used to measure the pO<sub>2</sub> of the expired water by means of an Eschweiler oxygen electrode.

The results of measurements made on three specimens are given in the Table and show % utilizations of oxygen as high as 92% and resting oxygen consumption of 55–75 cm<sup>3</sup>/kg/h. A number of different ventilation patterns, including unilateral ventilation as previously observed in another species (HUGHES and SHELTON<sup>6</sup>) were recorded and these will be discussed later.

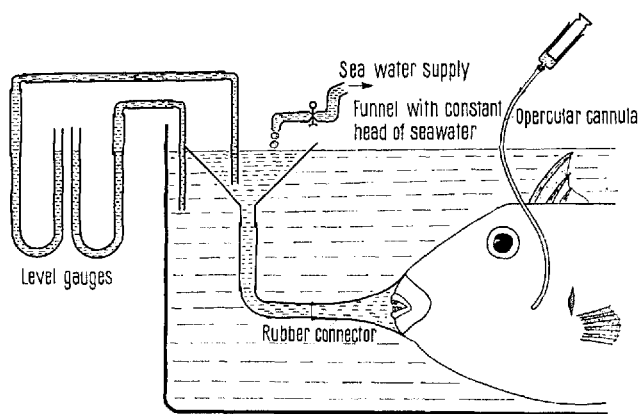


Diagram to show experimental arrangement used to measure ventilation volume and for sampling opercular water.

<sup>1</sup> V. WILLEM, *Bull. Mus. r. Hist. nat. Belg.* 78, 35, 1 (1942).

<sup>2</sup> V. WILLEM, *Bull. Mus. r. Hist. nat. Belg.* 23, 29, 1 (1947).

<sup>3</sup> L. BERTIN, *Traité de Zoologie* (Ed. P. P. Grassé; Masson et Cie., Paris 1958) vol. 13, p. 1303.

<sup>4</sup> K. R. HOGGARTH and E. R. TRUEMAN, *Nature*, Lond. 213, 1050 (1967).

<sup>5</sup> G. M. HUGHES, *J. exp. Biol.* 37, 28 (1960).

<sup>6</sup> G. M. HUGHES and G. SHELTON, in *Advances in Comparative Physiology and Biochemistry* (Ed. O. LOWENSTEIN; Academic Press, New York and London 1962), vol. 1, p. 275.

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 <sup>3</sup>	210	200–330	111	90–165	90	65–175
Frequency/min	52	40–60	60	48–64	60	40–66
Stroke volume cm <sup>3</sup>	4.04	3.8–8.25	1.85	1.5–2.6	1.5	1.25–4.3
pO <sub>2</sub> inspired water mm Hg	151.0		154.5		150.7	
pO <sub>2</sub> 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 <sup>3</sup> /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 fishes<sup>7</sup>.

men. Es wurden Sauerstoffausnützungen von 90% beobachtet.

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**Zusammenfassung.** Druck- und Bewegungsregistrierungen haben gezeigt, dass die Kiementilation 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-

<sup>7</sup> 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

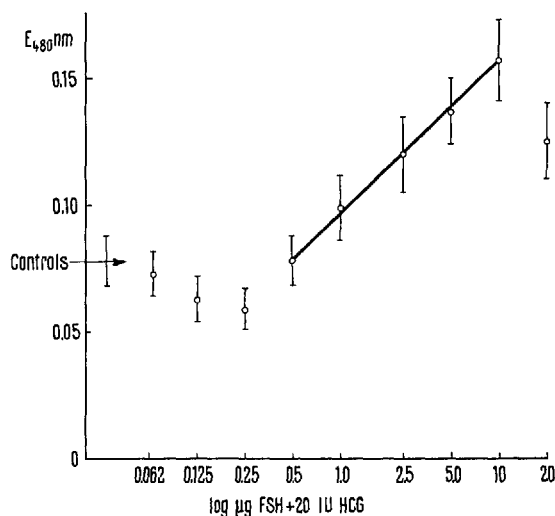
## A Rapid, Sensitive Method for the Determination of Follicle-Stimulating Activity

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 MARTIN<sup>1</sup>, 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 ( $\pm 12$  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  $\mu$ 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.

<sup>1</sup> L. MARTIN, J. Endocr. 20, 187 (1960).