

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 JARDETZKY 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.

M. M. CASELLATO, G. LUGARO
and K. WEYDANZ

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

² C. D. JARDETZKY 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. PROVENGI 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

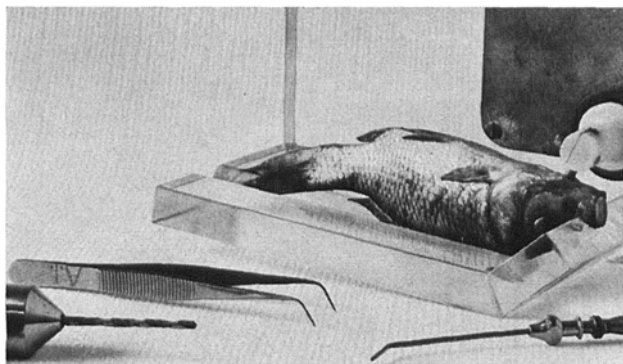


Fig. 1. The equipment.

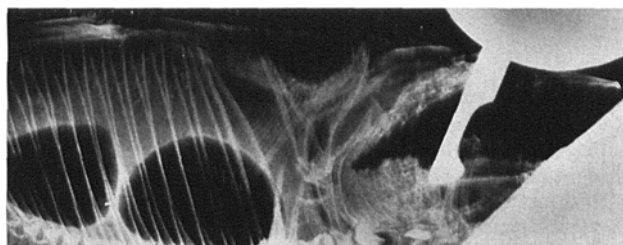


Fig. 2. A radiographic lateral projection of the fish in position. The guide is correctly centred on the parasphenoid prominence. Note that the guide prevents closure of the fish's mouth.

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).

plate is joined to an acrylic base plate so as to form the vertical branch of an L-shaped structure. The arrangement permits the operator to slide the drill guide in a plane parallel to the steel plate. This plane coincides with the median plane of the fish, which is accommodated in a V-shaped groove in the base plate.

A drill of 2.4 mm diameter is connected to a high-speed drilling machine. The drill shaft should be provided with an adjustable stop, preventing the drill from protruding excessively from its guide. A suction cannula, O.D. 1.0 mm, bent 60° 5 mm from its free end, is connected to a water suction pump. A finely pointed forceps serves as a self-retaining retractor.

Procedure. After anaesthesia in MS-222 (Sandoz, 1:3000), the fish is placed back downwards in the base plate's groove. By help of the closed forceps, the thin midline membrane of the lower jaw is perforated just in front of the lingual apex. The opening is bluntly enlarged to admit the drill guide. The guide is directed to the mid-point of an imaginary line connecting the caudal orbital rims, where the parasphenoid prominence is concealed by a mass of soft tissues. The prominence must be captured by the oblique opening of the guide, which is facilitated by carefully sliding the guide along the parasphenoid ridge. When the prominence is securely hooked within the tube's opening, the tube is depressed slightly to fix the skull rigidly. When correctly positioned, the tube forms an orally acute angle of 60° with the base of the skull.

The drill stop is adjusted to permit cutting of a 0.5–2.0 mm deep hole, depending on the size of the fish. The drill must traverse the soft tissues and the parasphenoid prominence but it should not damage the contents of the myodome. With experience, the drill stop may be discarded.

When the myodome has been opened, the drill is removed without changing the position of its guide. The

suction cannula is introduced via the fish's mouth and the guide's aperture. Tissue debris is removed under supervision from the guide's upper end, through which the parallel bellies of the rectus externus muscles then are discernible. The closed forceps is introduced between the muscles and allowed to separate and retract them. The white spheroidal hypophysis presents itself in the field once the muscles are retracted. It is removed with the suction cannula. The drill guide is then removed and the fish returned to its tank without further treatment.

Evaluation. The operation is performed in less than 2 min and craves no particular skill. If the guide is correctly positioned, there is small risk of damaging the large vessels posterior and lateral to the prominence. It is not necessary to close the entrance to the myodome as the soft parts heal rapidly. The extent of trauma is minimal. Asepsis is unnecessary, as is gill washing.

The mortality was less than 4%. Most deaths occurred on the day of surgery and were mainly due to hemorrhage from within the myodome. The fishes were observed up to 3 weeks postoperatively. Less than 2% proved incompletely hypophysectomized when checked under high magnification at autopsy. To date, more than 300 fishes have been operated on.

Zusammenfassung. Eine Schnellmethode (2 min) für Hypophysektomie an Kleinfischen wird beschrieben. Durch die Benutzung eines gesteuerten Bohrers wird das Freilegen der Hypophyse vereinfacht. Die kombinierte Mortalität und Fehlschlaghäufigkeit beträgt weniger als 5%.

L. FRISÉN

Department of Human Anatomy, University of Gothenburg SV, (Sweden), 13 June 1967.

CONGRESSUS

Germany

2nd International Symposium of Pharmaceutical Chemistry

in Münster (Westfalen), 22–26 July 1968

Principal themes: (1) Non-steroid drugs with anti-phlogistic effect. (2) Drugs with analgetic effect. (3) Drugs with effect on circulation and heart function. (4) Chemotherapy of parasitic infections. (5) Metabolism of drugs.

Programme and further information from: Sekretariat des 2. IUPAC-Symposiums "Pharmaceutical Chemistry", Hitdorfstrasse 58–62, 44 Münster, Westfalen (Germany).

Poland

10th International Congress of Internal Medicine

in Warsaw, 10–14 September 1968

Principal themes: (1) Enzymatic mechanisms in the pathogenesis of internal disorders. (2) Disturbances in protein metabolism.

Secondary themes: (1) Ethical, legal and social problems in modern therapy and clinical research. (2) Mathematical methods in internal medicine. (3) Rehabilitation in internal medicine. (4) Recent developments in internal medicine.

Programme and further information from: Department of Medicine, Institute for Postgraduate Medical Education, ul. Solec 93, Warszawa 30 (Poland).