the antibody can 'leap' the membrane, the germnali elements would be primarily involved in the immune process[6].

Riassunto. Gli Autori hanno dimostrato mediante immunofluorescenza la positività immunologica della parete tubulare del testicolo umano, in un caso di azoospermia. Questo reperto si presta ad interessanti considerazioni riguardo al ruolo svolto dalla membrana basale della parete tubulare nelle malattie autoimmunologiche del testicolo.

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[6] A. Isidori, F. Dondero, L. A. Garufi, Folia Endocr. *21*, 3 (1968).

The Effect of Electrical Stimulation of Olfactory Tract on the Nucleus Preopticus in the Catfish *Heteropneustes fossilis* (Bloch)

It is well established, at least in mammals, that the hypothalamus is the focal point at which the neural stimuli converge[1] to influence the adenohypophysial secretion by the neurosecretory material which is released by the hypothalamic nuclei[2]. Experimental studies on the significance of the hypothalamic nuclei in teleost fishes are very few[3–9], and the information available on the mechanisms which regulate the activity of these nuclei is still scanty[10]. The present communication reports the effect of electrical stimulation of the olfactory tract on the nucleus preopticus in the catfish *Heteropneustes fossilis*.

The dorsal part of the brain and the olfactory tracts of the fish were carefully exposed after immobilising the fish. They were then wrapped in a wet cloth and placed over a cotton pad which was kept in a small tray containing normal saline. Electrical stimuli (2 volts, 3 msec 10 cy/sec) were delivered to the olfactory tract for a total period varying between 30 sec and 10 min with a steel microelectrode and the grounded electrode in the normal saline bathing the brain. The olfactory tracts of the control fish were exposed and sham-stimulated by touching with electrodes without flow of current. The brains were fixed in Bouin's fluid immediately after stimulation. The sagittal sections, cut at 4–6 μ thickness, were stained with Halmi's modification of paraldehyde fuchsin (AF) technique.

The preoptic nuclei of *H. fossilis* are situated in the walls of the third ventricle on either side of the optic recess antero-dorsal to the optic chiasma. The constituent neurons are arranged as compact groups in the form of arcs extending along a postero-dorsal to an antero-ventral angle. The neurons in the dorsal portion of the nucleus preopticus are large (diameter, 1–2 μ) and form the pars magnocellularis; the neurons of the ventral part are small (diameter, 0.5–1.0 μ) and constitute the pars parvocellularis. The neurosecretory cells are mostly oval with round or oval nuclei; the nuclei are generally situated away from the axonal end. Most of the neurons are monopolar, but a few are bipolar. Some of the axons have beaded appearance because of the presence of tiny droplets of neurosecretory material. Initially most of the axons run antero-ventrally, while others extend in different direc-

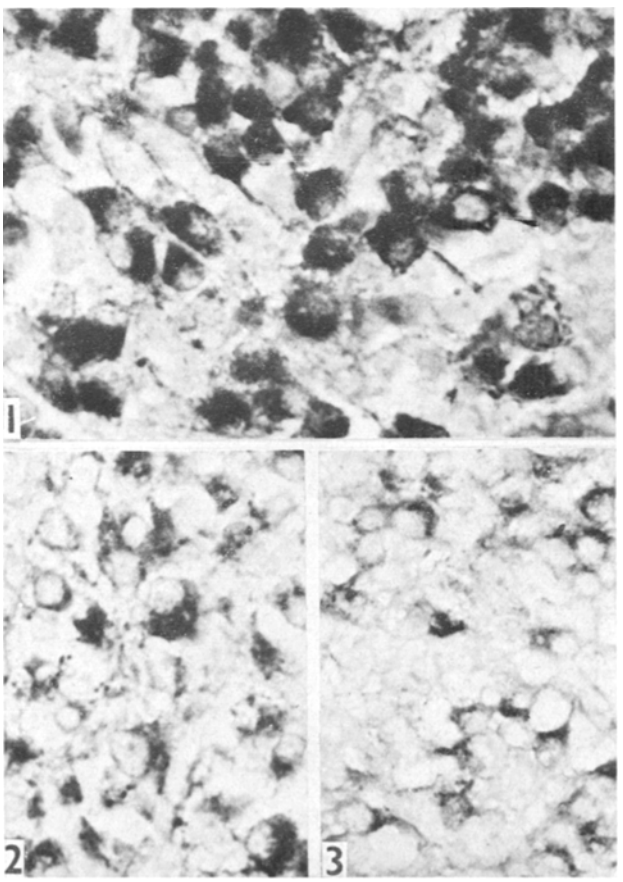

Fig. 1. Nucleus preopticus of untreated control showing perikarya stained deeply due to accumulation of neurosecretory material. × 795.

Fig. 2. Nucleus preopticus after stimulation for one minute. Some of the perikarya have become degranulated, while others contain only very little amount of neurosecretory material. Note the presence of neurosecretory granules in the axons. × 795.

Fig. 3. Nucleus preopticus after stimulation for 2 min. Most perikarya have become almost completely degranulated, while only traces of neurosecretory material are present in others along the nuclear margins. Note the absence of neurosecretory granules in the axons. × 795.

[1] S. A. D'Angelo, J. Snyder and J. M. Grodin, Endocrinology *75*, 417 (1964).
[2] E. Scharrer and B. Scharrer, in *Handbuch der Mikroskopischen Anatomie des Menschen* (Eds. U. Mollendorf and W. Bargmann; Springer Verlag, Berlin 1954), vol. 6, p. 953.
[3] L. Arvy, M. Fontaine and M. Gabe, C. r. Soc. Biol., Paris *148*, 1759 (1954).
[4] P. Rasquin and L. M. Stoll, J. comp. Neurol. *107*, 273 (1957).
[5] G. Fridberg and R. Olsson, Z. Zellforsch. *49*, 531 (1959).
[6] H. Kobayashi, S. Ishii and A. Gorbman, Gunma J. med. Sci., Japan *8*, 301 (1959).
[7] H. Korn, Z. Zellforsch. *52*, 45 (1960).
[8] T. H. Schiebler and J. Hartmann, Z. Zellforsch. *60*, 89 (1963).
[9] A. G. Sathyanesan, J. Morph. *117*, 25 (1965).
[10] A. Jasinski, A. Gorbman and T. J. Hara, Science *154*, 776 (1966).

tions. In the normal and sham-stimulated fish, the perikarya of the neurons are deeply stained by AF because of the accumulated neurosecretory material (Figure 1).

Stimulation of the olfactory tract for 30 sec has caused incomplete degranulation in the perikarya of the neurons of the nucleus preopticus. 60 sec after stimulation a large number of vacuoles are formed in the perikarya due to acute degranulation. Some of the neurosecretory cells have become completely degranulated and only very little amount of neurosecretory material is present along the nuclear margin (Figure 2). Most of the neuronal cell bodies are either AF-negative or weakly-stained because of depletion of the neurosecretory material. Electrical stimulation appears to have caused a higher degree of depletion of the neurosecretory material from the neuronal cells of the pars magnocellularis than from those of the pars parvocellularis. The axons in the vicinity of the nucleus preopticus contain a large number of neurosecretory droplets that appear to have been discharged from the neuronal cells (Figure 2).

After stimulation for 2 min, almost the entire neurosecretory material seems to have been discharged from the neuronal cells. Most of the perikarya have attained AF-negative nature due to which it becomes difficult to locate the boundary of the neurons in stained sections. However, traces of neurosecretory material may exist along the nuclear margin of a few neurosecretory cells (Figure 3). A 2-min stimulation led to depletion of the neurosecretory material even from most of the neurons of the pars parvocellularis. The axons also lose their identity, since the neurosecretory material which was present in them after 60 sec stimulation appears to have been transported further away from the vicinity of the nucleus preopticus (Figure 3).

The response of the nucleus preopticus to electrical stimulation of olfactory tract in *H. fossilis* has resulted in depletion of the neurosecretory material as reported for the goldfish by JASINSKY et al.[10]. 60 sec stimulation evoked complete depletion in the goldfish[10] while in *H. fossilis* it required a 2-min treatment, although the characteristics of the stimuli are the same. This may be because of higher capacity of tolerance of the catfish to electrical stimulation. The difference in responsiveness of the neurons of the pars magnocellularis and pars parvocellularis to stimulation of the olfactory tract is suggestive of their functional difference[11].

Résumé. Chez le *Heteropneustes fossilis* normal le périkaryon des neurons du noyau préoptique montrent une grande accumulation de substance neurosécrétaire AF-positive. Stimulation électrique du nerf olfacteur pendant une minute menait à une dégranulation incomplète, tandis qu'un traitement de dux minutes produisait un épuisement presque complet de la substance neurosécrétoire. Les neurons de la portion magnocellulaire du noyau préoptique paraissent répondre plus rapidement que ceux de la portion parvocellulaire.

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Calcitonin Stimulation of Pyrophosphatase Activity in Rat Bone

Calcitonin exerts its hypocalcemic effect through an action on bone[1]. However, the exact mechanism of such an action is not fully understood.

In view of the fact that inorganic pyrophosphate plays a key role in the regulation of bone formation and resorption, as has been proposed by FLEISCH et al.[2,3], one possibility is that calcitonin exerts its effect through the alteration of inorganic pyrophosphatase, an enzyme which degrades pyrophosphate.

TENENHOUSE and RASMUSSEN[4] have recently shown that calcitonin stimulates inorganic pyrophosphatase activity of the Ehrlich ascites tumor cells incubated in vitro.

We have therefore determined the inorganic pyrophosphatase activity of the rat bone after the administration of porcine calcitonin. Female Wistar rats of 50 g body wt. fasted overnight were given s.c. injection of 200 mU of porcine calcitonin (Armour pharmaceutical. Co. 8MRC mU/mg) dissolved in 16% gelatin. Left tibiae were dissected out, freed from adherring soft tissues, and blood samples were obtained from the cervical vein before, 15, 30 and 60 min after injection. In another experiment in which graded doses of porcine calcitonin was given s.c. to thyroparathyroidectomized rats, tibiae and blood samples were obtained 3 h after the injection. Thyroparathyroidectomy was performed 1 day before the experiment. Serum calcium was determined by the colorimetric autoanalyzer method of GITELMAN[5], and inorganic pyrophosphatase activity of the tibia was estimated by a highly sensitive assay method using ^{32}P-labeled inorganic pyrophosphate[6]. After removing the bone marrow, 1% homogenate of the tibia was prepared by homogenizing frozen tibia in ice-cold 0.25 M sucrose with bone crusher and Waring blender. The homogenate was then mixed with 0.1% Triton X-100, centrifuged at 3000g 3 min and the supernatant was used for the assay of pyrophosphatase activity and for the determination of protein content by LOWRY's method[7].

Pyrophosphatase activity was estimated from ^{32}Pi released from ^{32}PPi in 0.033 M pH 7.5 veronal buffer or 0.05 M pH 2.5 glycine HCl buffer with 10^{-5} M $Na_4P_2O_7$ and 10^{-5} M $MgCl_2$ during 30 min incubation period at 37 °C. Reaction was stopped by the addition of 10% trichloroacetic acid and the aliquot was taken and mixed with 1 M acetate buffer (pH 5.0) and 0.1 M PPi. 0.1 M $MnCl_2$ was then added, shaken and centrifuged, thus precipitating the remaining ^{32}PPi. After the centrifugation, the released ^{32}Pi was counted by the liquid scintilla-

[1] P. F. HIRSCH and P. L. MUNSON, Physiol. Rev. *49*, 548 (1969).
[2] H. FLEISCH, R. G. G. RUSSELL and F. STRAUMANN, Nature, Lond. *212*, 901 (1966).
[3] H. FLEISCH, H. MAERKI and R. G. G. RUSSELL, Proc. Soc. exp. Biol. Med. *122*, 317 (1966).
[4] A. TENENHOUSE and H. RASMUSSEN, *Parathyroid Hormone and Thyrocalcitonin* (Ed. R. V. TALMAGE, Excerpta Medica Foundation, Amsterdam 1967), p. 392.
[5] H. J. GITELMAN, Analyt. Biochem. *18*, 521 (1967).
[6] M. OHATA, H. ORIMO and T. FUJITA, submitted to J. Biochem.
[7] O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, J. biol. Chem. *193*, 265 (1951).