tion of orotate 3H is not obtained – as was obtained in vitro by other authors [10] – may be explained by considering that in our experiment the ACTH continued to exercise its action on the adrenal gland. Infact it is well known that ACTH stimulates the protein synthesis in the target cells [11, 12].

In conclusion, our data in vivo support the hypothesis of a negative feed-back control mechanism at the adrenal level, mediated by an inhibition of the RNA synthesis in the adrenocortical cells by corticosteroid-hormones.

Riassunto. Con metodi autoradiografici e morfometrici è stato studiato l'effetto del corticosterone sulle cellule corticosurrenaliche di ratti ipofisectomizzati trattati con ACTH. I dati ottenuti in questa ricerca indicano che il corticosterone inibisce direttamente la funzionalità nucleare delle cellule corticosurrenaliche. Viene posta l'ipotesi dell'esistenza di un meccanismo di controllo diretto a feed-back negativo a livello corticosurrenalico, mediato dall'inibizione da parte del corticosterone della sintesi di RNA nelle cellule corticosurrenaliche stesse.

G. G. Nussdorfer and G. Mazzocchi

Istituto di Anatomia Umana Normale, Laboratorio di Microscopia Elettronica, Università di Padova (Italy), 10 June 1970.

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Antitesticular Immunity. Role of the Basement Membrane

Emphasis has recently been put on the role played by the basement membrane of the seminiferous tubule in the anti-testis immunization process. The question is whether the membrane is passively 'passed through' by the circulating antibodies on their way to the cells of the seminal line, or if it behaves as a 'barrier' against the same antibodies [1]. In the latter instance, being the precipitation site of the immune complexes, the basement membrane could act as the pathogenetic factor damaging the cellular elements it encircles, in a way similar to that found in other immune organ diseases (e.g. in the nephrotic syndrome).

Actually, immune complexes have been demonstrated experimentally by Edwards [2] in the basement membrane

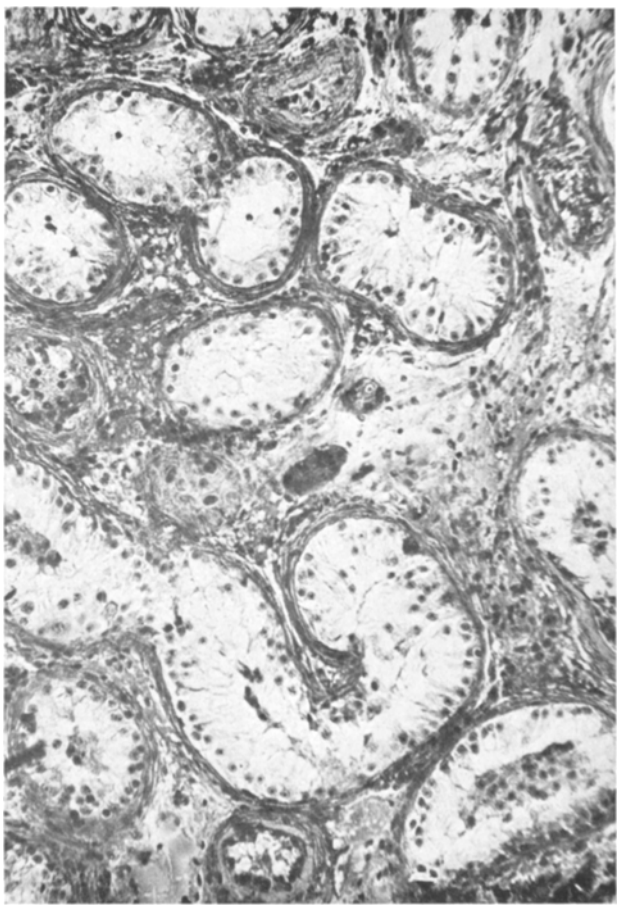

Fig. 1. Microphotograph of the histologic preparation. Note severe degenerative changes of the membrane of the tubular wall as well as of the germinal epithelium. Marked parvicellular infiltration of the interstitial spaces. HOPA staining; original magnification × 300.

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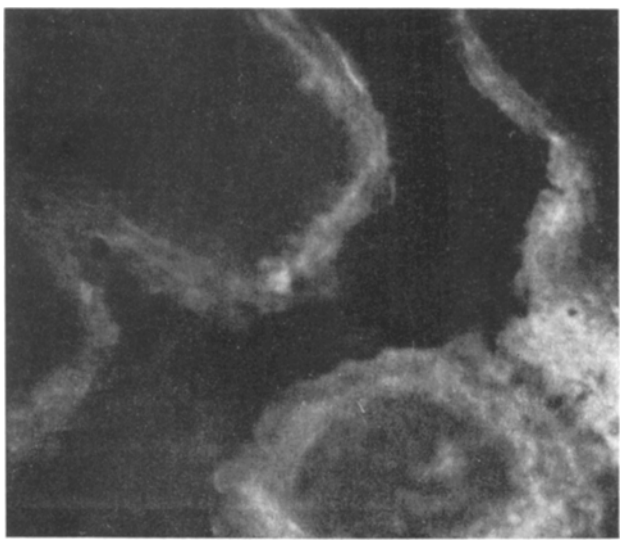

Fig. 2. Immunofluorescent staining. Anti-human γ-globulin rabbit serum. The positiveness is clearly confined to the tubular wall. Original magnification × 500.

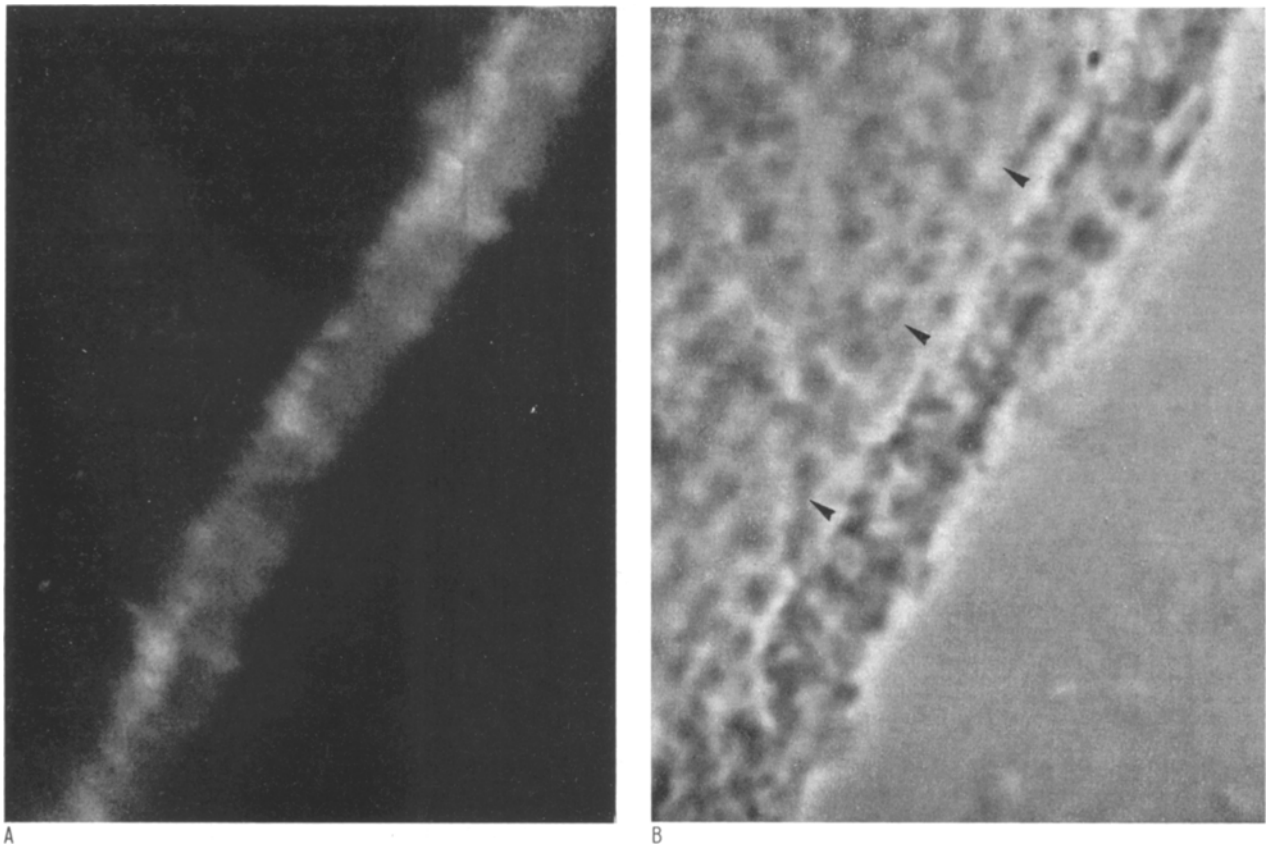

Fig. 3. These microphotographs show at high magnification the same portion of the tubular wall, observed after immunofluorescent staining (A) and in phase microscopy (B). Note how the inner part of the tubule (B, arrows) is absolutely free of fluorescein-positive material.

of the tubular wall of the guinea-pig testis; while in the experimental anti-testicular immunization in humans, MANCINI et al.[3] could detect no sign of immune localization in the basement membrane similar to what they found in the cellular elements.

In previous reports[4] we described some cases of anti-testicular autoimmunization in humans, and, more recently, we detected the exclusive localization of immunoglobulins in the tubular lining membrane.

Case report. Subject of 24 years, complaining of infertility. History of repeated infections of the genito-urinary tract. The examination of the seminal fluid showed complete azoospermia. A biopsy of the testis was performed, and histologically the basement membrane of the tubular wall appeared to be severely damaged, together with parvicellular infiltration of the interstitial spaces and severe degenerative changes involving the elements of the germinal epithelium (Figure 1).

Immunochemical study. The immunochemical study was carried out by means of the indirect COONS' method[4,5]. Ultrathin sections (4 μ) of testis have been obtained at the microtome cryostat (Ames Lab. Tech.), and fixed for 10 min with acetone. After rinsing twice with phosphate buffer (pH 7.2), they were incubated for 30 min with anti-human γ-globulin (γG) fluorescein-labelled serum, previously absorbed with acetonic liver powder in order to remove aspecific fractions. After incubation, the slices were again rinsed twice with phosphate buffer, air-dried and mounted with buffered glycerol. The fluorescence microscopy in dark field was carried out with a microscope Wild M20 KdG, equipped with quartz-mercury lamp HB0200 and with appropriate sets of exciter and barrier filters. The photographs were taken with an automatic photographic set (Wild MKa IV).

Results. At the immunofluorescent staining, the γ-globulin complexes appeared to be localized exclusively in the basement membrane of the tubular wall (Figures 2 and 3). The specificity of this finding was checked, employing fluorescein-labelled anti-goat γ-globulin serum and fluorescein-labelled anti-human complement sera (anti β1C-β1A); as well as with control tests on normal subjects.

This first finding of a localization of immunoglobulins in the testicular tubular wall in human pathology seems to be an important contribution to the understanding of the role played by the basement membrane of the seminiferous tubule in the anti-testicular immunization. Actually, while in our case the histological lesions were detectable in the basement membrane as well as inside the seminiferous tubules and in the interstitial spaces, only the basement membrane seemed to be involved in the immunological process, as a precipitation site of immune complexes. In this case the endotubular damage would have been 'trophic' in nature and secondary to the lesion of the basement membrane, while in other cases, in which

[3] R. E. MANCINI, I. A. ANDRADA, D. SARACENI, A. E. BACHMANN, J. C. LAVIERI, M. C. NEMIROWSKY, J. clin. Endocr. *25*, 7 (1965).

[4] A. KAWAMURA JR., *Fluorescent Antibody Techniques and Their Applications* (University of Tokyo Press; University Park Press 1969).

[5] A. H. COONS, F. M. LEDUC, J. M. CONNOLLY, J. exp. Med. *102*, 49 (1955).

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.

A. Isidori, F. Dondero and D. Lombardo

Istituto di Patologia Medica II, Università di Roma, I-00100 Roma (Italy), 1 June 1970.

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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-

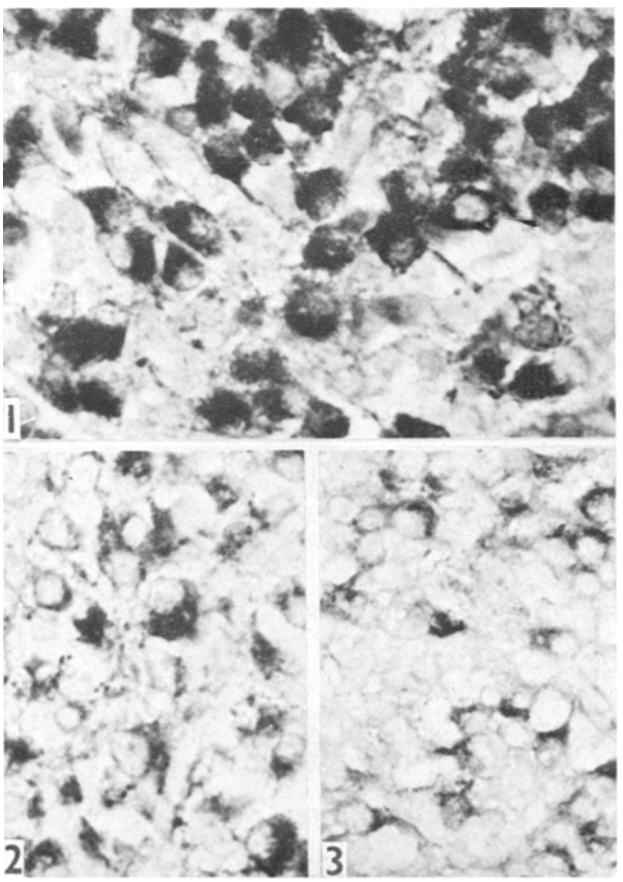

Fig. 1. Nucleus preopticus of untreated control showing perikarya stained deeply due to accumulation of neurosecretory material. × 795.

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.

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