## Effect of Corticosterone on the Incorporation of Tritiated Orotate into Adrenocortical Cells of Hypophysectomized ACTH-Treated Rats

Numerous lines of evidence indicate that the steroid-hormones inhibit the protein synthesis in adrenal quarters in vitro and also the ACTH stimulation of corticosterone production[1-4]. It seems advisable to verify, by quantitative autoradiographic methods, whether also in vivo the corticosteroids have a direct inhibitory effect on the protein synthesis of adrenocortical cells. In order to avoid a possible indirect action of corticosteroids at the hypothalamo-hypophysial axis, hypophysectomized ACTH-treated rats were used. The autoradiographic approach was chosen because it also allowed a morphological assessment of the action of the corticosteroids on the adrenocortical cells.

*Materials and methods.* 40 male rats, Sprague-Dawley derived (200 g), were hypophysectomized by the parapharyngeal approach and treated with ACTH (10 IU/kg/die) for 9 consecutive days. They were divided into 4 experimental groups. One group served as a control. The others were given i.p. injections of 5, 10 and 20 mg/kg of corticosterone, respectively, 3 h before being sacrificed. In addition, each group of animals was further divided into 2 sub-groups which were given i.p. injections of 90 μc of orotate 3H and leucine 3H (New England Nuclear Corp.), respectively, 2 h before the sacrifice. The adrenal glands were fixed in 5% glutaraldehyde, post-fixed in 1% OsO4 and embedded in an epoxy resin. The autoradiography was performed on 0.5 μ thick sections by methods similar to those we have previously described[5]. The degree of incorporation of the tracer was evaluated by the method of the mean grain count. Karyometric[6] and cytometric[7] assessments were also carried out on these same thick sections. Thin sections obtained from the same tissue blocks were checked under the electron microscope.

*Results and discussion.* The quantitative autoradiographic data, shown in Figure 1, a, demonstrate that the percentage of inhibition of the incorporation of orotate 3H into the rat adrenocortical cells (zona fasciculata) is statistically significant ($P < 0.01$ for all the doses of corticosterone) and correlated with the dose of corticosterone administered. The specificity of the effect of corticosterone on adrenocortical cells was verified by evaluating the incorporation of orotate 3H into the fibroblasts of the connective capsule of the adrenal glands in both normal and treated rats. No significant variations were found in the incorporation of the tracer in these cells; infact, even with the maximum doses of corticosterone, the inhibition was found to be less than 7%. This very low inhibition may be ascribed to the effect of corticosteroids on the protein synthesis in the connective cells in vitro[8].

Figure 1, b shows that the incorporation of leucine 3H into the rat adrenocortical cells is only slightly decreased ($P < 0.1$) by the treatment with corticosterone.

Karyometric analysis shows that the volume of nuclei in the adrenocortical cells of hypophysectomized ACTH-treated rats decreases significantly ($P < 0.01$ for all the doses of corticosterone) in relation to the dose of corticosterone (Figure 2). This indicates a reduced nuclear functional activity[9] and is in agreement with the results of the autoradiography. On the other hand, for such a short exposure, no significant quantitative variations were observed in the ultrastructural characteristics of the adrenocortical cells.

Our autoradiographic and morphometric data therefore indicate a direct and specific inhibition by the corticosterone of the nuclear activity (RNA synthesis) in the rat adrenocortical cells. The slight reduction in the incorporation of leucine 3H may be due to the fact that the RNA present in the cell at the moment of the corticosterone administration continued to exercize its function in the protein synthesis during the following 3 h before the sacrifice of animals. The fact that, even with the maximum doses of corticosterone, a total inhibition of the incorpora-


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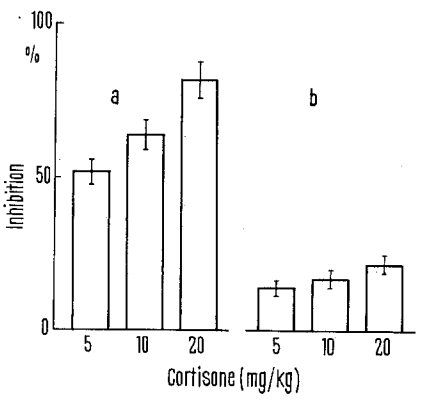


Fig. 1. Ordinate: % of inhibition; abscisse: mg/kg of corticosterone. Histogram showing the % inhibition of the incorporation of orotate 3H (a) and leucine 3H (b) into the adrenocortical cells of hypophysectomized ACTH-treated rats after administration of various doses of corticosterone. Standard errors are indicated.

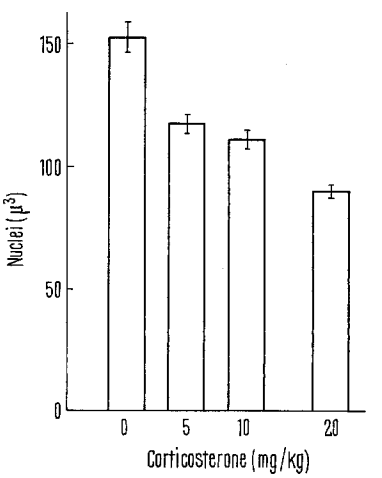


Fig. 2. Ordinate: volume of nuclei ($\mu^3$); abscisse: mg/kg of corticosterone. Changes in volume of nuclei of the adrenocortical cells of hypophysectomized ACTH-treated rats after administration of various doses of corticosterone. Standard errors are indicated.

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.

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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

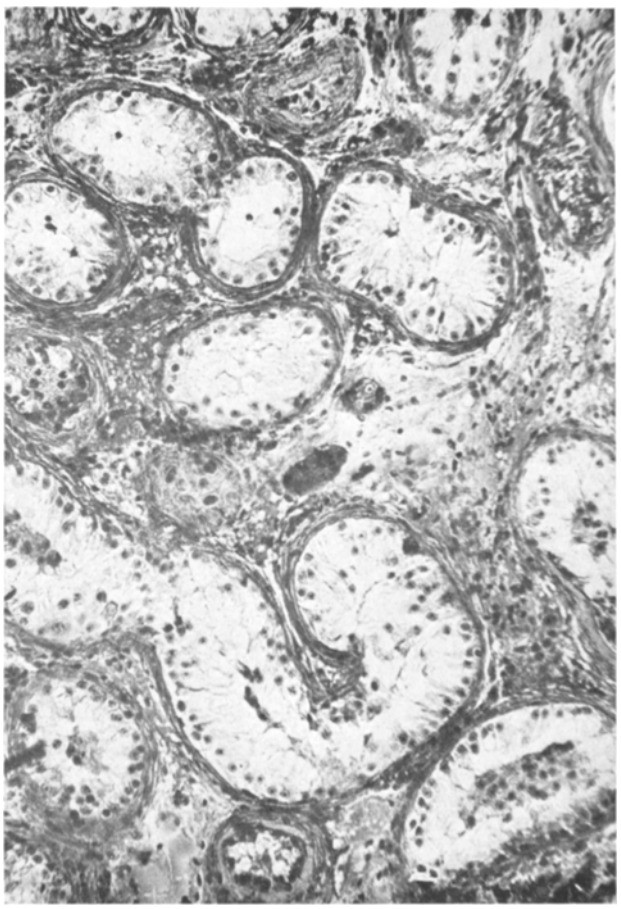

Fig. 1. Microphotograph of the histologic preparation. Note severe degenerative changes of the membrane of the tubular wall as well as of the germinal epithelium. Marked parvicellular infiltration of the interstitial spaces. HOPA staining; original magnification × 300.

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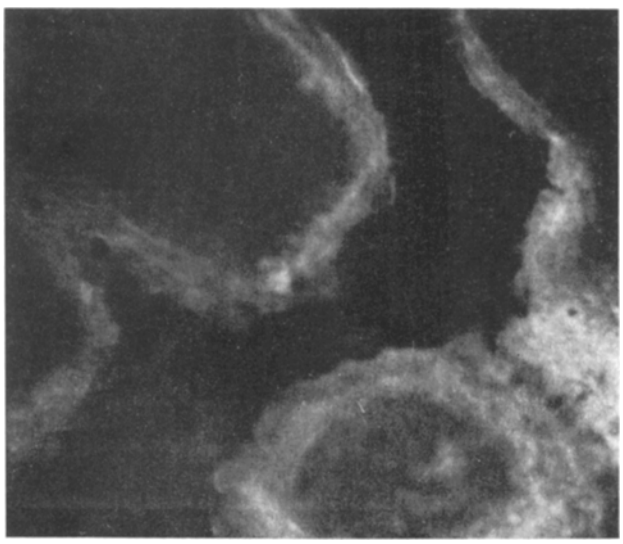

Fig. 2. Immunofluorescent staining. Anti-human $\gamma$-globulin rabbit serum. The positiveness is clearly confined to the tubular wall. Original magnification × 500.