Histochemistry of Steroid-3β-ol Dehydrogenase in the Human Adrenal Cortex

Histochemical methods for demonstration of cell enzymes have been recently applied to the study of the human adrenal cortex so as to obtain a better knowledge of its function under normal and pathological conditions. These methods are mostly concerned with the demonstration of enzyme activities (such as non specific lipase and esterase, dehydrogenases of the Krebs' cycle, DPNH and TPNH diaphorases, acid and alkaline phosphatases), which are supposed to be indirectly related to biosynthesis of steroid hormones[1-3]; moreover, a suitable technique has been introduced for demonstration of an enzyme, the steroid-3β-ol dehydrogenase, which converts C_{19} and C_{21} steroids with the Δ^5-3β-hydroxyl structure to those with the Δ^4-3-ketone grouping characteristic of most of the active steroid hormones[4-9].

Biochemical researches have shown that this stereospecific enzyme takes part in an intermediate step of steroid-hormone synthesis which leads to the conversion of pregnenolone into progesterone and androstenolone into androstenedione[10-12]; thus, it appears to be indispensable for the production of all the cortical hormones.

In the present study, 24 human adrenal glands, surgically removed from female patients, have been examined: 10 having been obtained from subjects with inoperable breast cancer, partly following previous stimulation with exogenous ACTH (500 U.I. fractioned during the 5 preoperative days), and 14 from patients with Cushing's syndrome, (one case due to atypical adenoma and 13 to cortical hyperplasia), generally treated preoperatively with small doses of cortisol intramuscularly (not more than 100 mg), during 3 days before the operation. Surgical manipulation was reduced to a minimum, and the glands were immediately frozen after removal.

On cryostat-frozen 5 μ sections the following histochemical techniques have been employed: for steroid-3β-ol dehydrogenase the method of WATTENBERG[4] using 0.001 M-dehydroepiandrosterone as substrate, for non specific lipase Gomori's method (mod. by CURRI[13]) and for alkaline phosphatase the cobalt calcium method according to GOMORI[14].

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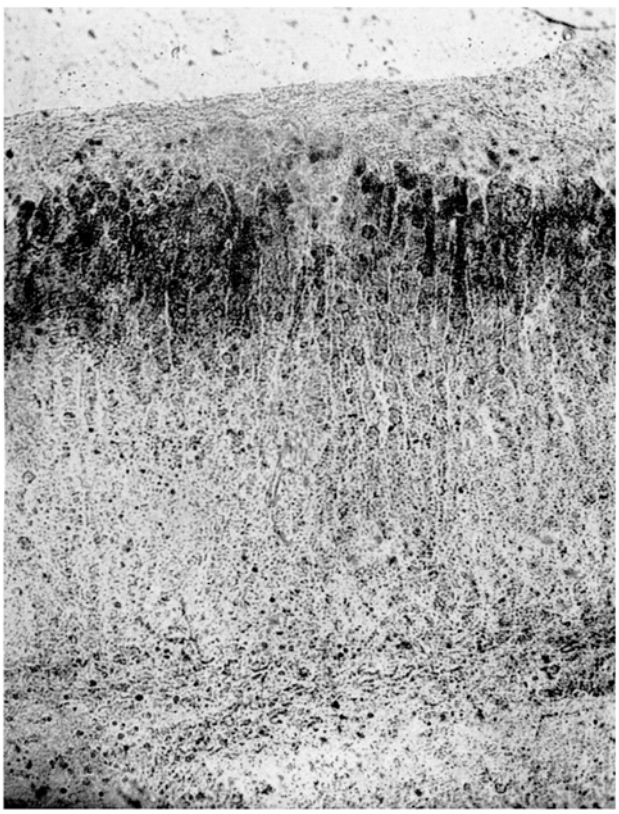

Fig. 1. Steroid-3β-ol dehydrogenase activity of 'normal' human adrenal cortex. ×60.

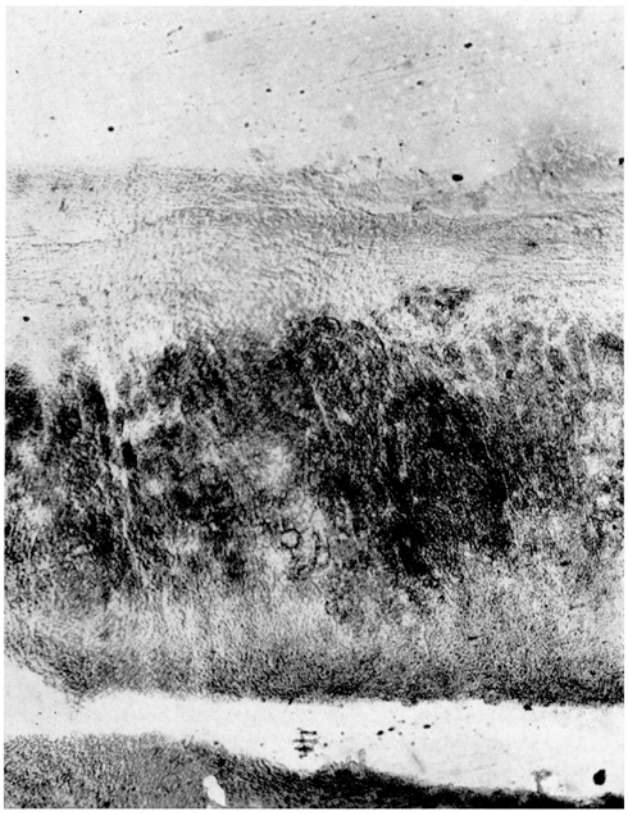

Fig. 2. Steroid-3β-ol dehydrogenase activity of human adrenal cortex after ACTH stimulation. ×60.

The glands removed from patients with breast cancer without previous ACTH stimulation have been considered as functionally normal on hand of clinical and laboratory data; histologically, they showed the three classical zones of the cortex, with a clear cut separation of the zona fasciculata into an outer clear-celled and an inner dark-celled layer. Histochemically, a different pattern of distribution has been observed according to the type of enzyme studied: a strong steroid-3β-ol dehydrogenase activity was evident in the clear cells of the fascicular zone and a weak one in some cell groups of the glomerulosa (Figure 1), whereas non-specific lipase and alkaline phosphatase were prominent in the dark cells of the zona reticularis.

A short-term intensive treatment with exogenous ACTH has increased in these glands the width of the enzyme activities, together with a lipid depletion of the clear cells. The steroid-3β-ol dehydrogenase activity was found to spread to the whole of the zona fasciculata (Figure 2), whereas the non-specific lipase and alkaline phosphatase activities extended outwards up to the outer zona fasciculata.

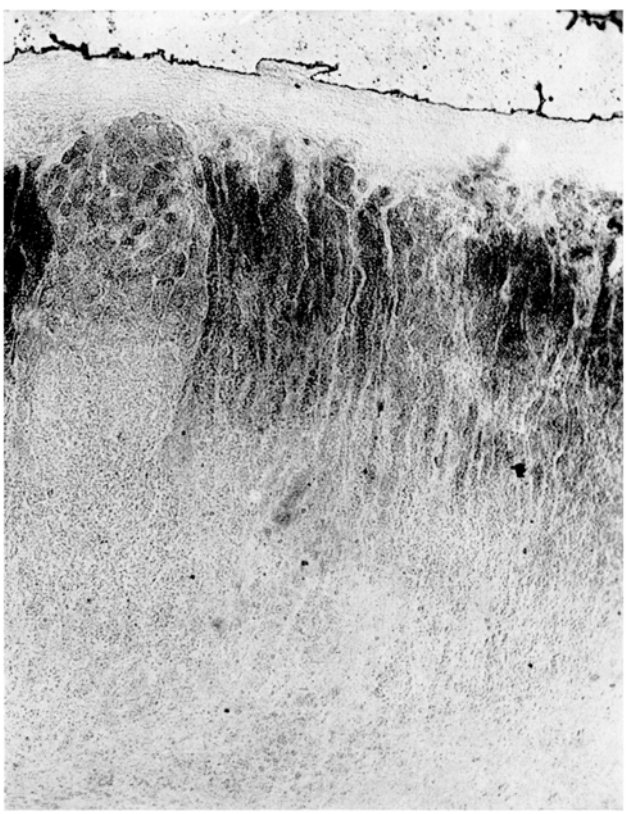

Fig. 3. Steroid-3β-ol dehydrogenase activity in adrenal cortex of a patient with Cushing's syndrome. ×60.

The findings in cases of Cushing's syndrome have been different according to their morphological back-ground. In the single case resulting from cortical tumour (adenoma), both clear and dark tumour cells showed a large quantity of all the enzymes studied; conversely, the tumour-free cortex was very poor or practically free of enzymes.

On the other hand, in cases resulting from hyperplasia of the adrenal cortex, no evident change in enzyme distribution has been observed; generally, the steroid-3β-ol dehydrogenase, as well as non-specific lipase and alkaline phosphatase, were within normal limits (Figure 3).

From the results outlined, the following conclusions may be drawn:

(1) In functionally normal glands, the presence of steroid-3β-ol dehydrogenase in the clear cells of the fascicular zone, as well as in cell groups of the glomerularis, supports the view that these elements are directly related to steroid-hormone synthesis or, at least, to an intermediate step of this process. Moreover, the presence in the reticular zone of some enzyme activities, such as non-specific lipase and alkaline phosphatase, poses the problem of the function of this zone, as it may be excluded that it is only a *zona consumptiva*.

(2) Exogenous ACTH leads to an increase of all the enzyme activities, which should be interpreted as a sign of functional stimulation.

(3) In Cushing's syndrome due to cortical adenoma, as expected, the tumour cells appear to be very active, whereas the tumour-free cortex is functionally at rest.

(4) Conversely, in Cushing's syndrome due to adrenal hyperplasia, the cortical enzymes show a pattern which is very near to normal or quite normal. This suggests that in this variety of the syndrome, there exists an interaction of some other still unknown factor or mechanism, apart from ACTH stimulation.

Zusammenfassung. Bericht über histochemische Untersuchungen an menschlichen Nebennieren, die chirurgisch bei Fällen von inoperablem Mammacarcinom (mit oder ohne vorhergehende Stimulation mit exogenen ACTH) sowie bei Fällen von Cushingscher Krankheit entfernt worden waren.

Anhand der Verteilung der verschiedenen Enzyme und insbesondere des Steroid-3β-ol Dehydrogenase wird auf Fragen der Beziehungen der Morphologie und Funktion der normalen Drüse eingegangen und die Cushingsche Krankheit interpretiert.

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Modifications of Myosin Caused by Adenosinetriphosphate, Adenosinediphosphate, and Pyrophosphate

In recent years, adenosinetriphosphate and pyrophosphate have often been used for the extraction of muscle proteins[1-3]. It was thought that they would help selectively extracting myosin from the myofibrils, but it was soon realised that, in addition to myosin, other protein material – extra-protein[4], X-protein[5], fraction T[6] – passes into solution and does not precipitate when the ionic strength is reduced to 0.04.

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