18-Hydrocorticosterone Biosynthesis in an Aldosterone Secreting Tumor and in the Surrounding Nontumorous Adrenal Gland* (33976)

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A greater than normal concentration of aldosterone has been found in adrenal tumors from patients with primary aldosteronism (1). 18-Hydroxycorticosterone is probably an intermediary product in the conversion of corticosterone to aldosterone (2). This conversion takes place in the zona glomerulosa. The parallel changes in secretion rates of aldosterone and 18-hydroxycorticosterone which have been observed in man under a variety of conditions support the intermediary role of 18-hydroxycorticosterone (3). However, 18-hydroxycorticosterone is also synthesized in the fasciculata-reticularis zones of the cow adrenal gland (4), and to a lesser degree, in the inner zones of the rat adrenal gland (5).

The present report describes the conversion of corticosterone-4-^{14}C to 18-hydroxycorticosterone and aldosterone by an aldosterone producing tumor and the surrounding nontumorous adrenal tissue. Only the mitochondrial fraction of both tissues contained converting activity. The tumor converted 4 times more corticosterone-4-^{14}C to aldosterone than did the surrounding adrenal tissue but the conversion rate to 18-hydroxycorticosterone was the same for both tissues. Since it is likely that the zona glomerulosa of the nontumorous adrenal tissue of a patient with primary aldosteronism is inactive, these data suggest that the zona fasciculata-reticularis of the human adrenal gland can synthesize 18-hydroxycorticosterone.

Case report. The patient was a 60-year-old white male with a history of benign hypertension and hypokalemia. Aldosterone excretion and secretion rates remained elevated, 27 and 306 μg/24 hr respectively, after the patient was eating a 350 meq sodium diet for 4 days, and the plasma renin concentrations were depressed after 6 days of a 10 meq sodium diet. Plasma cortisol, 17-hydroxycorticoid and 17-ketosteroid excretion were normal. Laboratory tests ruled out the diagnosis of pheochromocytoma and renal artery stenosis.

At surgery, the left adrenal gland appeared grossly normal. The right adrenal gland contained a 1 $\times$ 1-cm adenoma; a right adrenalectomy was performed. Postoperatively the patient's hypertension improved and the hypokalemia was corrected. One month postoperatively, the response of aldosterone excretion and plasma renin levels to a low sodium diet was impaired.

Adrenal tissue. The tumor and the adjacent nontumorous adrenal tissue were placed on crushed ice immediately after surgical excision.

Incubation procedure. The tissue was freed of fat and homogenized in 0.25 *M* sucrose. Medullary tissue was not removed. Subcellular fractions were obtained by the method of Schneider (6). Fractions obtained from 100 mg of tissue were incubated as described by Marusic and Mulrow (7). The incubation started 3 hr after the removal of the glands. At the end of a 30-min incubation period a tracer amount of aldosterone-^{3}H was added to correct for losses during extraction and purification.

Extraction and identification of steroids. The incubation media were extracted with 10 vol of methylene chloride, the extracts were washed with 0.1 vol of 0.1 *N* NaOH, 0.1 *M* acetic acid and distilled water and evaporated in vacuum. Stable aldosterone and cortisol were added to each sample. The dry residues were applied to a paper chromatogram and run in the chloroform–formamide system for 3 hr. The aldosterone area was located

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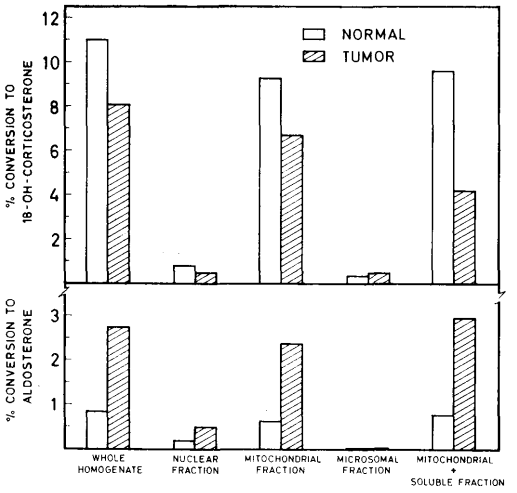

FIG. 1. Percentages of corticosterone-4-^{14}C converted to aldosterone and 18-hydroxycorticosterone.

by its absorption of ultraviolet light. The 18-hydroxycorticosterone area was located by radio-chromatogram scanning and its mobility with respect to the reference steroid cortisol (4). After elution of the radioactive zones from the chloroform–formamide system, aliquots of the samples were taken for counting. Further purification of 18-hydroxycorticosterone and aldosterone areas were carried out.

The aldosterone area from the first chromatogram was run in the Bush B_5 system, eluted, and an aliquot was taken for counting, and the remainder was acetylated with pyridine–acetic anhydride 1:1(v/v) for 24 hr. The acetylated derivative was chromatographed sequentially in the CBM system (cyclohexane–benzene–methanol–water, 100:50:100:25) and DCM system (dioxane–cyclohexane–methanol–water, 75:100:75:25). A constant $^3H/^{14}C$ ratio for the aldosterone diacetate was obtained between these two systems. The 18-hydroxycorticosterone area was oxidized with periodate and identified as described by Marusic and Mulrow (4). Radioactive measurements were made in a Packard Tri-Carb liquid scintillation counter. For calculation of percentage converted, aliquots of 18-hydroxycorticosterone were counted after the first system. Aldosterone counts were obtained after the DCM system and corrected for losses with the tritium tracer.

Results and Discussion. In Fig. 1 are represented the percentages of corticosterone-4-^{14}C converted to aldosterone and 18-hydroxycorticosterone by different cell fractions of an aldosterone producing tumor and the adjacent nontumorous tissue. Whole homogenate, nuclear, mitochondrial, microsomal, and soluble fractions obtained from 100 mg of tissue were incubated in the presence of 20 μg of corticosterone-4-^{14}C. All the converting activity was present in the mitochondrial fraction of both the tumor and the normal gland. The addition of the soluble fraction to the mitochondrial had no further effect on the activity of the mitochondrial fraction. These results with the subcellular fractions are similar to our findings with the rat adrenal gland (7). As shown in Fig. 1, the conversion of corticosterone to aldosterone by the mitochondrial fraction of the tumor was 366% greater than the conversion by the mitochondrial fraction of the normal gland. No such difference was observed in the conversion of corticosterone (B) to 18-hydroxycorticosterone (18-OH B). The amount of 18-OH B produced by the normal gland was slightly higher than that produced by the tumor.

It is not clear why the conversion of B to 18-OH B by the tumor is similar to that by the normal gland while the conversion of B to aldosterone is enhanced. It is possible that either B or 18-OH B was converted to other metabolites by the tumor. These were not apparent in the radioactive scan of the initial chromatography system. The ratio 18-OH B/aldosterone produced by the tumor mitochondria was 2.7. This ratio is similar to the ratio of the secretion rates of 18-OH B and aldosterone in the normal human. Sandor and Lanthier (8) and Ulick *et al.* (2), have incubated slices of a normal adrenal gland with radioactive corticosterone and found a ratio of 18-OH B/aldosterone of 3 and 2.3, respectively. In the present study, the ratio of 14 was obtained from the mitochondria of the nontumorous portion of the adrenal gland.

Unfortunately, in the human adrenal gland, it is extremely difficult to separate the inner zones completely from the zona glo-

merulosa. However, there are several reasons to suspect that the inner zones, the zona fasciculata-reticularis of the nontumorous portion of the adrenal gland converted B to 18-OH B. Firstly, the fasciculata-reticularis zones of the cow and, to a lesser extent, the rat can produce 18-OH B. Secondly, the zona glomerulosa of the adrenal glands associated with aldosteronomas are usually inactive because of the suppressed renin activity (9–11). This appeared to be the case in our patient. There was little conversion of B to aldosterone by the nontumorous tissue, and, as long as 1 month postoperatively, he had a poorly responsive renin–aldosterone system.

These results may have relevance in explaining the normal 18-OH B and low aldosterone secretion rates in patients with 17-hydroxylase deficiency (12). In this syndrome, there is excessive production of corticosterone and deoxycorticosterone, probably by the inner zone fasciculata-reticularis. Through the mineralocorticoid action of these hormones, the renin system is suppressed and hence aldosterone secretion (13). Since these patients synthesize little aldosterone, the zona glomerulosa is probably atrophied. The normal secretion rate of 18-OH B may be explained by biosynthesis of 18-OH B in the zona fasciculata-reticularis.

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