PARALLEL BIOCHEMICAL AND HISTOCHEMICAL STUDIES OF AN ADRENOCORTICAL ADENOMA FROM A PATIENT WITH PRIMARY ALDOSTERONISM

By
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ABSTRACT
An adrenocortical adenoma surgically removed from a patient with primary aldosteronism was investigated by histological, histochemical and incubation-chromatographic techniques and compared to intact adrenal tissue excised from the contralateral gland. The tumour was composed almost entirely of fasciculata-like tissue and released \textit{in vitro} cortisol, corticosterone, aldosterone, cortisone and 17-hydroxy-11-deoxycorticosterone but no measurable amounts of 11\beta-hydroxy-androstenedione. In contrast to the contralateral gland with prominent zona glomerulosa and atrophic zona fasciculata, which responded poorly to corticotrophin (ACTH) stimulation, the tumour responded by a striking increase in the formation of cortisol, corticosterone and aldosterone. An attempt was made to correlate the morphological aspect with the biochemical findings \textit{in vitro}.

Primary aldosteronism is now recognized as a specific clinical entity and most of the features of the syndrome have been ascribed to the overproduction of aldosterone (Conn 1960). A single adenoma of the adrenal cortex has been found in about 70% of the reported cases (Delorme & Genest 1959; Conn 1960), and the analysis of the tumoural tissue for steroidal substances has re-

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revealed a high content of aldosterone (Conn & Louis 1955; Louis & Conn 1958) and wide variations in the amount of cortisol, cortisone or corticosterone (Louis & Conn 1958; Neher 1958). Different patterns of secretion »in vitro« have been reported for these tumours (Ayres et al. 1958; Bailey et al. 1960). In addition, pathological examination has revealed the majority of these adenomas to be composed of fasciculata-like cells although some were claimed to resemble the zona glomerulosa of normal glands (Delorme & Genest 1959).

Few correlations were made between the pathological aspect of the diseased adrenal tissue in primary aldosteronism and the pattern of secretion »in vitro«, and there is a lack of information concerning the response of these tumours to ACTH stimulation. The purpose of this paper is to report parallel biochemical and histochemical studies performed on an adrenocortical adenoma and on a biopsy specimen of the contralateral adrenal gland in a patient with primary aldosteronism. The data obtained indicate that such an adenoma may be highly responsive to ACTH stimulation »in vitro«.

CASE REPORT

A 35 year old, thin, white, housewife was admitted to the Hotel-Dieu Hospital in May 1958 because of arterial hypertension. Having a familial history of hypertension, she had been followed sporadically by her family doctor for the previous twelve years for high blood pressure ranging from 150/90 to 190/130 mm Hg. She had been complaining, during that period, of irritability, nervousness, tiredness, occipital headaches and a feeling of fullheadedness. She was known to drink »large« amounts of water since the age of 15; a mild polyuria had been present for years. Apart from a recumbent blood pressure of 220/140 mm Hg, generalized hyperactive deep tendon reflexes and a slight degree of arteriosclerosis in the fundi, the physical examination was essentially negative.

The diagnosis of primary aldosteronism was considered because of persistent hypokalaemia (2.3 to 3.6 meq./l), slightly elevated serum bicarbonate in venous blood, and a urine with low specific gravity and alkaline pH. The renal function as shown by creatinine clearance and phenolsulfophthalein excretion, was moderately impaired, without nitrogen retention or abnormalities of the urinary sediment. Laparotomy, however, was delayed because of incomplete clinical picture and failure to find a substantial elevation in daily aldosterone excretion. Four determinations of urinary aldosterone gave 6, 3, 18 and 8 µg/d (normal range 2–10 µg/d by the procedure of Nowaczynski et al. 1957). The patient was discharged and kept under observation at regular intervals for 18 months in the Hypertension Clinic of the Hotel-Dieu Hospital.

During this period she received potassium supplement and antihypertensive treatment. She kept complaining of asthenia, irritability and occasional headaches. The hypokalaemia persisted in spite of increased potassium intake. The hypertension proved difficult to control despite combined use of hydrochlorothiazide, ganglion-blocking drugs, hydralazine, reserpine and phenobarbital (Fig. 1). In the last days of November 1959, she was re-admitted in emergency because of severe epistaxis, and anaemia (haematocrit 29 %).
Fig. 1.

Blood pressure and serum potassium of the patient during nineteen months prior to surgery. Effects of the combined use of several hypotensive agents.

On the second day of hospitalization, bilateral carpal spasm appeared spontaneously; serum potassium was 2.0 meq./l, sodium 140.0 meq./l, chloride 98.5 meq./l, total calcium 5.0 meq./l, ionized calcium 2.2 meq./l and magnesium 1.3 meq./l. Trousseau's sign was present but there was no Chvostek sign. The carpal spasm disappeared rapidly upon intravenous infusions of calcium gluconate and potassium chloride. In the following days it could be easily reproduced by 1 or 2 minutes of hyperventilation.

The diagnosis of primary aldosteronism became more certain. On close questioning, the patient recalled one previous episode of carpal spasm and periodic nocturnal paresthesias in hands and forearms. Repeated determinations showed consistently low serum potassium (2.1 to 2.6 meq./l); low specific gravity (1.010) and alkaline pH of urine (8.0). The venous blood pH was 7.51 and serum bicarbonate 37 meq./l. Urine specific gravity reached a maximum of 1.027 after 23 hours without fluid intake. The free plasma 17-hydroxy-corticosteroids, total urinary 17-hydroxy-corticosteroids and 17-ketosteroids were normal, and the response to ACTH stimulation was also normal. A determination of aldosterone in urine showed a value of 58 μg/d.

Surgical exploration through an upper abdominal transverse incision demonstrated a left adrenal tumour. Excision of the tumour and a biopsy of the contralateral adrenal were performed. Fifteen months post-operatively, the patient is asymptomatic; serum potassium, venous blood pH, and serum bicarbonates are in the normal range. After an interval of normal readings of several months, the blood pressure is showing a tendency to rise again to slightly hypertensive levels (145/105 mm Hg recumbent).

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MATERIAL AND METHODS

The tumour of the left adrenal gland measured 1.6 cm × 1.4 cm × 1 cm and weighed with a very small adjoining fragment of adrenal tissue, 1.955 g. On section, it was well circumscribed and homogeneously yellow. The biopsy specimen of the right adrenal gland weighed 0.898 g. The tumour and the biopsy specimen of the right adrenal were both sectioned into three portions. One part was fixed in 10% formalin for histological examination; the second fragment was frozen with dry ice and placed in a cryostat; the third portion was used for incubation studies.

Sections 8 μ in thickness were cut from the frozen block and mounted on coverslips. Diphosphopyridine nucleotide (DPN) diaphorase, triphosphopyridine nucleotide (TPN) diaphorase, and glucose-6-phosphate dehydrogenase were determined by the techniques of Scarpelli et al. (1958) and Hess et al. (1958) with 2,2'-di-(p-nitrophenyl)-5,5'-di-phenyl-3,3'-[(3,3-dimethoxy-4,4'-biphenylene) ditetrazolium chloride (Nitro BT) as the final electron acceptor. Strong activity of the two latter enzymes in the adrenal cortex of the rat has been demonstrated by Cohen (1959). Recent histochemical investigations in our laboratory on sixteen surgical biopsies of adrenal gland have confirmed this finding in human cortex and have revealed in addition that DPN diaphorase is also very active. The activity of these enzymes was found to be intense throughout the cortex but particularly in the zona glomerulosa and in the zona reticularis. It was felt that the histochemical determination of these three enzymes would help to evaluate the functional state of the tumour.

A 1.096 g portion of the adenoma and a 0.462 g biopsy specimen from the contralateral adrenal were used for »in vitro« studies. Immediately after their excision they were placed in ice-cold saline and prepared for incubation in less than 45 minutes. They were dissected free from fat and other tissues, blotted, weighed and sliced with a Stadie-Riggs microtome. No effort was made to separate the cortex from the medulla in the intact adrenal tissue. The tumoural and contralateral non-tumoural tissue slices (0.5 mm thickness) were incubated separately in Krebs-Ringer bicarbonate medium, with added glucose (200 mg%) (Umbreit et al. 1957) using one milliliter of this solution per 100 mg of tissue.

The incubation was carried out in a Dubnoff metabolic shaker incubator, at 37 ± 1°C, in an atmosphere of oxygen-carbon dioxide 95:5 (v/v). The flow of gas and the shaking were regulated at 8 litres per minute and 100 oscillations per minute, respectively. After a preliminary »pre-incubation« of 30 minutes (Saffran & Bayliss 1953), the slices were transferred to fresh medium and the incubation »per se« allowed to proceed for 2 two-hour periods during which quantitative measurements were made, and a final four-hour period for qualitative studies of steroid formation. ACTH* (100 milliunits per 100 mg of tissue) was added to the medium during the second (two-hour) and the final four-hour periods of incubation.

At the end of each incubation period, the medium was decanted and extracted five times with an equal volume of chloroform. The emulsion formed during extraction, was reduced by centrifugation. After evaporation under partial vacuum in a flash evaporator at less than 45°C, the dry residue obtained was purified by partition chromatography on a 2.5 g silica gel column according to the method of Neher & Wettstein (1955). The first fraction eluted from the column (50 ml of chloroform: acetone 99:1, v/v) was discarded and the second fraction (100 ml of chloroform: acetone 1:1, v/v), containing the polar corticosteroids was evaporated to dryness under a stream of

* Adrenocorticotropic hormone. Parke & Davis, Lot No. 228781.
air and transferred to the B 5 paper partition system of Bush (1952), (methanol:water, 55:45/benzene).

Because of the small quantity of material available, the purified residue obtained from the incubated non-tumourous adrenal tissue was chromatographed from a dot-sized application. Scanning of the paper-gram under a 254 m\(\lambda\) emitting light, showed a fairly good separation of the aldosterone and cortisol spots, and no further chromatographic separation was attempted. This may account for slightly higher aldosterone values for the gland since we have shown (Davignon 1960) that this zone of Bush B 5 system is contaminated by small quantities of other UV-absorbing substances. For the tumour, the aldosterone-cortisone zone from Bush B 5 system was eluted and re-chromatographed in the system C of Bush (1952) (methanol:water, 1:1/toluene:ethyl acetate, 9:1) in order to obtain a better separation for these two steroids.

Cortisol (Compound F), aldosterone and cortisone (Compound E) were measured for their maximum absorption at 238–242 m\(\lambda\) in a Beckman DU spectrophotometer, and this value controlled by quantitative reaction with isonicotinic acid hydrazide (Weichertselbaum & Margraf 1957). The corticosterone - 17-hydroxy-11-deoxy-corticosterone zone »B + S zone« was measured from its absorption maxima at 240 m\(\lambda\), then quantitatively transferred to the system E2B (tertiary butanol:water, 250:450/isooctane 500) of Eberlein & Bongiovanni (1955) where corticosterone separates from compound S. Measurement of these two substances by ultraviolet light (UV) absorption was performed after a final chromatographic separation in a modified Bush B 3 system (methanol:water, 4:1/isooctane:benzene, 1:2, Davignon 1960).

The steroids were identified from their mobilities in various paper partition systems, from the mobilities of their acetylated products formed after reaction with acetic anhydride in pyridine at room temperature and from the absorption spectrum of their chromogens in concentrated sulfuric acid.

**RESULTS**

1) **Histological and histochemical findings**

Histologically the tumour was composed of large, polyhedral, finely vacuolated cells (Fig. 2, A) which stained intensely with the fat stains. The cells did not reproduce the arrangement in parallel rows of the normal zona fasciculata but were disposed in small groups and irregular cords. However, the tumour cells individually resembled the fascicular cells because of the extensive vacuolization of their cytoplasm. There were also scattered, smaller, more acidophilic cells isolated or in small islands. Many of the nuclei were round and small, but some were irregular, voluminous, and hyperchromatic, occasionally with a large nucleolus. The pathological diagnosis was cortical adenoma.

In the remainder of the left adrenal as well as in the resected fragment of the right adrenal (Fig. 2, B) the cortex showed a prominent irregularly thickened glomerulosa. In contrast, the fasciculata appeared thinner than normal, but was rich in lipids. The zona reticularis appeared normal.

The tumour gave a moderately strong reaction for DPN (Fig. 2, C) and TPN diaphorase, and for glucose-6-phosphate dehydrogenase. There were.
Fig. 2.

A – Microscopic appearance of the cortical adenoma of the left adrenal. The tumour is composed of vacuolated, clear cells resembling those of the fasciculata. (haemalum-phloxine-saffron × 80).

B – Cortex of the right adrenal. The glomerulosa (top layer) is thickened. The fasciculata is thin. The reticularis appears normal (H. P. S. × 80).

C – DPN diaphorase reaction in the cortical adenoma. The enzymatic activity is moderately intense and limited to the narrow threads of cytoplasm between the lipoid droplets. (× 80).

D – DPN diaphorase reaction in the adrenal cortex adjacent to the adenoma. The enzymatic activity is strong throughout the cortex but particularly in the thickened glomerulosa and in the reticularis. The degree of activity in the fasciculata is comparable to that observed in the adenoma (cf Fig. 2, C) (× 80).
in addition, occasional more deeply stained cells corresponding probably to the acidophilic cells identified in the sections stained with haematoxylin and cosin. The cortex in the attached fragment of adrenal gland (Fig. 2, D) and in the biopsy specimen of the right gland showed an intense enzymatic activity, especially in the thickened zona glomerulosa and in the reticularis. The reaction in the fasciculata was moderately intense and was comparable to that observed in the adenoma (Fig. 2, C & D).

2) *Incubation studies*

The steroids in the dry residue extracted from the incubation medium of the tumour, after preliminary purification on the silica gel column, separated in the Bush B 5 system in a pattern similar to that obtained with normal adrenals. There was the exception, however, that no UV-absorbing spot corresponding to the position of 11β-hydroxy-androstenedione was visible on the chromatograms, even after four hours of incubation with added ACTH. Subsequently the zone corresponding to 11β-hydroxy-androstenedione was eluted from the three chromatograms of tumour incubates, pooled and rechromatographed from a 3 mm spot in modified Bush B 3 system in order to increase the sensitivity of the method of detection. No UV-absorbing substance was seen on the papergram. This would indicate that after 2 hours of incubation followed by an additional 6 hours of incubation with added ACTH, less than 5 µg of 11β-hydroxy-androstenedione was produced by 1.096 g of tumour slices since 5 µg of αβ-unsaturated ketosteroid per square cm of paper surface can be visualized by ultraviolet scanning (*Nowaczyński*, unpubl.). Conversely, on the Bush B 5 chromatogram of the steroid extract obtained from the contralateral adrenal, following the same incubation and purification procedures, a UV-absorbing spot was present at the level of 11β-hydroxy-androstenedione, after ACTH stimulation; estimation from peak absorption at 239 mµ gave a value of 11.9 µg/g·h.

Among the more polar steroids released by the adenoma, cortisol was identified by a mobility identical to that of standard cortisol in Bush B 5, Bush C, E, B and toluene/ethylene glycol (*Nowaczyński & Koiv 1957*) systems; by a spectrum of chromogens in concentrated sulfuric acid coinciding with that of standard cortisol and by a chromatographic behaviour of acetylated products similar to that of standard F acetate in modified Bush B 3 system.

The aldosterone produced by the tumour was identified by its typical mobility changes in Bush B 5, Bush C and E, B systems. It was found to be blue tetrazolium-reducing, it reacted with isonicotinic acid hydrazide and gave a peak absorption at 239 mµ in ultraviolet light. The gamma-lactone prepared by chromic acid oxidation was found to migrate in Bush B 1 at the level of gamma-lactone formed from standard aldosterone.

Cortisone released by the tumour migrated at the level of standard com-
pound E in Bush B 5, Bush C and E2B systems. The product obtained after acetylation with acetic anhydride and pyridine at room temperature had the same mobility as standard cortisone acetate. However, identification was not pursued further because of the small amount of material available.

In E2B system the »B + S zone« separated into two UV-absorbing spots migrating respectively at the level of standard corticosterone and 17-hydroxy-11-desoxy-corticosterone (Compound S) used as pilots. Measurement of these steroids was performed after transfer in modified Bush B 3.

The steroid formation by slices of tumoural tissue obtained from the left gland are compared in Table 1 and Fig. 3, before and after ACTH stimulation, to the release of corticosteroids by slices of »intact« contralateral adrenal gland.

The basal production of cortisol, cortisone and corticosterone »in vitro« was greater for the gland than for the tumour. In contrast, aldosterone and compound S were released in larger amounts by the adenomatous tissue. This increased aldosterone secretion is even more striking if one considers that only one paper chromatographic separation was performed for the gland, while the usual rechromatography of »E + Aldosterone zone« was carried out for the adenoma before quantitative determination. The reason for performing only one paper chromatography for the gland, was that a small quantity of tissue has been incubated, and that a well separated spot appeared on the first

*Table 1.*

Release of corticosteroids* during incubation of surviving adrenal slices from a patient with primary aldosteronism.

Comparison between an adenoma of the left adrenal gland and a biopsy specimen of the right adrenal.

<table>
<thead>
<tr>
<th>Tissue from the right adrenal gland</th>
<th>Tissue from the right adrenal gland</th>
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<tr>
<td>Adenoma from the left adrenal gland</td>
<td>Adenoma from the left adrenal gland</td>
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<tr>
<td>+ ACTH**</td>
<td>+ ACTH**</td>
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<tr>
<td>Cortisol</td>
<td>8.2</td>
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<tr>
<td>Cortisone</td>
<td>4.4</td>
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<tr>
<td>Aldosterone</td>
<td>2.9</td>
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<tr>
<td>»B + S zone«</td>
<td>6.5</td>
</tr>
<tr>
<td>Corticosterone</td>
<td>3.0</td>
</tr>
<tr>
<td>Compound S</td>
<td>2.4</td>
</tr>
<tr>
<td>F/B + S ratio</td>
<td>1.2</td>
</tr>
<tr>
<td>Total steroids</td>
<td>22.0</td>
</tr>
<tr>
<td>% Aldosterone</td>
<td>13.1</td>
</tr>
</tbody>
</table>

* µg/g · h.

** 100 µU/100 mg of tissue. The ACTH was added during the second two-hour period of incubation.
papergram at the expected position of aldosterone. The values obtained with compound S should be considered with caution because of the additional manipulations and number of chromatographic runs performed with a small quantity of material.

The gland responded poorly to ACTH stimulation. A significant rise was observed only for cortisol (47 %) and »B + S zone« (58 %). The tumour, in contrast, responded by a striking rise in the formation of all steroids analyzed except compound S and cortisone. Cortisol showed a ten-fold increase. Aldosterone rose to three times its basal formation, and »B + S zone« to eighteen times. For compound S and cortisone, respectively, increases of 1.4 and 1.7 times the control production were observed. The total corticosteroid release went from 16.9 to 136.9 µg/g·h for the tumour, as compared with 22.0 to 28.8 µg/g·h for the gland. The mean percentage of aldosterone in respect to total steroid production was very high for the gland (13.1 %) and much higher for the contralateral tumour (21.3 %) as compared with values obtained in normotensive patients (4.0 %) (Davignon 1960). After ACTH stimulation the percentage was much lower for the tumour (7.2 %) than for the gland (10.7 %).

**DISCUSSION**

The cortical adenoma excised from a patient with primary aldosteronism was found to release »in vitro« aldosterone, cortisol, cortisone, corticosterone, and compound S. These hormones are also found in incubates of normal human
adrenal tissue (Cooper et al. 1958; Davignon 1960) and have been isolated by extraction procedures from adenomas in cases of primary aldosteronism (Neher 1958).

The tumour failed to produce 11\(\beta\)-hydroxy-androstenedione even under ACTH stimulation and prolonged incubation, while the intact contralateral adrenal tissue yielded a fair amount of this 17-ketosteroid. To our knowledge, the formation of this compound by »aldosteronomas« has not yet been reported; its secretion by hyperplastic adrenal tissue excised from a patient with Conn’s syndrome (Lucis 1959) was estimated to be 3.5 \(\mu g/g\cdot h\).

On an equal weight basis, the yield of steroids by the tumour was comparable to that of the intact adrenal tissue. From the data reported in the literature on the release of steroids by adenomas in primary aldosteronism it appears that the pattern of secretion »in vitro« may differ widely from one tumour to the other. Ayres et al. (1958) quoted two cases where incubation studies were performed separately on the tumour and on an intact portion of the adjacent adrenal tissue. In one case the slices of the tumour were shown to produce large amounts of aldosterone, some cortisol, and very little corticosterone whereas equivalent slices of the intact adrenal tissue yielded very much less aldosterone and appreciable amounts of cortisol and corticosterone. In the other case the tumour yielded some cortisol and much aldosterone but also large amounts of corticosterone. Recently Bailey et al. (1960) reported two adenomas that released »in vitro« small quantities of aldosterone and much larger amounts of cortisol and corticosterone. In a review of 5 cases, Neher (1958) reported that the aldosterone content of these tumours ranged from 1.05 to 8.7 \(\mu g\) per gram of tissue.

When ACTH was added to the incubation medium, a striking difference was observed between the tumourous tissue and the intact gland. The latter showed a poor response to stimulation while the tumour responded by a marked increase in cortisol, corticosterone, and aldosterone release. An increase of much less magnitude was found for the other steroids measured. This finding suggests a possible dependance of adenomas to ACTH influences in primary aldosteronism. The dependance of these tumours to ACTH stimulation has been indicated previously from urinary determination of aldosterone following intravenous infusions of corticotrophin in some patients with primary aldosteronism due to an adrenocortical adenoma (Baulieu et al. 1959). Urinary aldosterone was investigated in our patient before and after ACTH stimulation prior to surgery. The results unfortunately were lost due to the ingestion of meprobamate that interfered with chromatographic separation of aldosterone in urine.

An attempt was made to correlate the biochemical findings with the morphological aspect of both normal and tumoural tissues. Histologically the tumour was composed almost exclusively of lipid-laden cells of the fasciculata type.
(«clear cells»). In contrast, the contralateral gland showed atrophy of zona fasciculata. Symington (1960) has proposed that the «clear cells» found in zona fasciculata represent storage cells for steroid precursors. Upon ACTH stimulation these cells would be depleted of their lipid content, the precursors being used for steroid synthesis, and would show an intense enzymatic activity with increased formation of corticosteroids, giving them the appearance of «compact cells» of the reticularis type. In our study the tumour responded markedly to ACTH stimulation while the gland showed a poor response. Since the tumour contained a higher population of «clear-cells» per mass unit of tissue than the gland, it was most likely to secrete more corticoids upon corticotrophin stimulation.

It is now well established that the glomerulosa zone is the site of aldosterone production in ox (Ayres et al. 1956) and rat (Giroud et al. 1956) adrenals. Some evidence has been brought up recently that this zone is also the site of aldosterone formation in man (Ayres et al. 1958; Siebenmann 1959). The fact that the gland contralateral to the tumour in our patient had a prominent zona glomerulosa and produced rather high amounts of aldosterone would tend to support this current concept. This correlation, however, could not be made for the tumour. The aldosterone formation of the latter was high and was increased three fold following corticotrophin stimulation. Microscopic examination of the tumour revealed a few isolated islets of acidophilic, non-pigmented cells with intense enzymatic activity (TPN and DPN diaphorase and glucose-6-phosphate dehydrogenase) in their cytoplasm. The nature of these cells and whether they are of glomerulosa or reticularis type, cannot be ascertained from the histological and the histochemical investigations. These nests of cells, however, were small and rare in the adenoma, which was chiefly composed of fasciculata-like tissue and gave enzymatic histochemical reactions similar to those of normal fasciculata. The fact that in the tumour the basal level of aldosterone was relatively high and that a striking elevation of this steroid occurred upon ACTH stimulation would suggest participation of «clear cells» in the formation of aldosterone. In their first patient with primary aldosteronism, Conn & Louis (1955) found a tumour composed of lipid-laden cells and containing large amounts of aldosterone. In addition, they observed atrophy of zona fasciculata in the contralateral adrenal gland, as in our case. These observations suggest that aldosterone may also be formed by cells of the zona fasciculata.

Aldosterone production by the contralateral adrenal gland with prominent zona glomerulosa remained unchanged upon ACTH stimulation. Stacheko & Giroud (1959) reported recently that when ACTH was added to the incubation medium of beef adrenal slices composed chiefly of glomerulosa tissue, it did not significantly effect the total production of corticosteroids nor that of aldosterone. In contrast, slices of fasciculata-reticularis tissue responded to
ACTH stimulation by a marked increase in total corticosteroid and cortisol formation. This, added to the fact that aldosterone and total corticosteroid production by a fasciculata-like tumour was increased after ACTH stimulation and to the observation that a gland with hypertrophy of glomerulosa and atrophy of fasciculata failed to respond to ACTH stimulation, gives indirect support to the hypothesis that aldosterone may be secreted by both zones and that the glomerular zone may be independent of ACTH control.

However, as emphasized by Ayres et al. (1958) and Ayres (1960) it seems precarious to relate disorganized cells of tumoural tissue to any normal zone of the adrenal cortex. One must await a specific histochemical means of detecting some »18-oxidase« activity before ascribing the role of aldosterone production to any kind of normal or abnormal adrenal cells. This is particularly true in man where the separation of the different zones is rendered difficult by the many convolutions occurring in the adrenal cortex and the irregular distribution of glomerulosa cells beneath the capsule (Symington 1960).

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