EFFECT OF POTASSIUM INTAKE ON THE FINAL STEPS OF ALDOSTERONE BIOSYNTHESIS IN THE RAT

I. 18-Hydroxylation and 18-Hydroxydehydrogenation

By

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ABSTRACT

Incubated capsular adrenal glands (zona glomerulosa) of potassium-deficient rats converted approximately 30 times less tritiated corticosterone to aldosterone and 18-hydroxycorticosterone than capsular adrenals of potassium-replete rats. This difference was demonstrable over a large range of substrate concentrations. Capsular adrenal mitochondria of potassium-deficient rats also incorporated considerably less tritiated corticosterone into aldosterone and 18-hydroxycorticosterone than mitochondria of potassium-replete rats. Conversion to both corticosteroid fractions increased within 4 hours and became normal within 24 hours of resumed potassium intake. On the other hand, potassium intake only marginally affected the ratio of 18-hydroxycorticosterone formation to aldosterone formation by capsular adrenals and had no effect on the conversion of tritiated deoxycorticosterone to 18-hydroxydeoxycorticosterone and 18-hydroxydeoxycorticosterone by decapsulated adrenals (zona fasciculata-reticularis). These findings indicate that potassium intake enhances 18-hydroxylase activity selectively in the zona glomerulosa of the rat adrenal cortex, but yield no information concerning an effect on 18-hydroxydehydrogenase activity. Kinetic data suggest that the potassium-induced increase in 18-hydroxylase activity is most likely due to de novo enzyme synthesis.

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According to experimental evidence, physiological regulators of aldosterone production act at different stages of the biosynthetic pathway. During short-term incubation and perfusion experiments known stimulators such as angiotensin II, potassium ions, ACTH (adrenocorticotropic) and serotonin primarily act on an early step, i.e. a reaction preceding the formation of cholesterol (Lommer & Wolff 1966), pregnenolone (Müller 1966; Müller & Ziegler 1968), progesterone (Kaplan & Bartter 1962), or corticosterone (Blair-West et al. 1967; Baniukiewicz et al. 1968; Haning et al. 1970). On the other hand, alterations in sodium balance were found to lead to marked changes in the activity of one or both of the enzymes involved in the conversion of corticosterone to aldosterone (Vecsei et al. 1966; Marusic & Mulrow 1967b; Davis et al. 1968; Müller 1968, 1970; Müller & Huber 1969; Baniukiewicz et al. 1968; Blair-West et al. 1970a; Haning et al. 1970). The physiological mechanism by which this effect on late biosynthetic steps is mediated is as yet unknown.

Previous studies carried out in this laboratory have indicated that potassium deficiency can also markedly influence the activity of enzymes involved in the final stages of aldosterone biosynthesis. Thus, we found that in incubated quartered adrenals or capsular hemiadrenals of potassium-deficient rats serotonin, potassium ions, angiotensin II or ACTH did not stimulate aldosterone production as in the adrenal tissue of normal rats but elicited an increased deoxycorticosterone output (Müller & Huber 1969; Müller 1970). Since deoxycorticosterone is a known intermediate of aldosterone biosynthesis (Kahnt et al. 1955), we have suggested that the increased response in deoxycorticosterone output and the decreased response in aldosterone production by the zona glomerulosa of the adrenal cortex of potassium-deficient rats are most likely due to a decreased activity of one or more of the enzymes involved in the conversion of deoxycorticosterone to aldosterone, i.e. 11β-hydroxylase, 18-hydroxylase, and 18-hydroxydehydrogenase. The following experiments were carried out in order to study more closely the nature and extent of the alteration in the activity of each of these enzymes induced by potassium deficiency. The present report will describe the effects of alterations in potassium intake on 18-hydroxylation and 18-hydroxydehydrogenation. Effects on 11β-hydroxylase activity will be reported and discussed in a separate paper (Baumann & Müller 1972).

MATERIAL AND METHODS

Rats and diets

Pure-bred male Osborne-Mendel rats, weighing between 130 and 180 g, were kept on semisynthetic diets which were prepared with slight modifications according to the specifications of Hartroft & Eisenstein (1957) and had the following respective sodium and potassium contents:
- «complete»: 230 meq. Na+ and 230 meq. K+ per kg,
- potassium-deficient: 230 meq. Na\(^+\) and 0.7 meq. K\(^+\) per kg.
- normal: rats kept on the complete diet and demineralized water for two weeks.
  Serum K\(^+\) 4.4 ± 0.2, Na\(^+\) 135 ± 2 meq/l.
- potassium-deficient: rats receiving the potassium-deficient diet and demineralized water as drinking fluid for two weeks. Serum K\(^+\) 3.2 ± 0.2, Na\(^+\) 144 ± 4 meq/l.
- potassium-replete: rats kept on the potassium-deficient diet for two weeks and receiving an aqueous solution of KCl (0.154 mol/l) and sucrose (50 g/l) for 48 h before sacrifice. Serum K\(^+\) 4.6 ± 0.8, Na\(^+\) 138 ± 1 meq/l.

In one series of experiments the KCl-sucrose solution was given as drinking fluid for 4, 8, 12, 24, 48 and 96 h, respectively, to rats which had been kept on the potassium-deficient diet for two weeks.

**Incubation of adrenal tissue**

Groups of twelve rats were decapitated. The excised adrenals were bisected and decapsulated by the method of Giroud et al. (1956). The capsular and decapsulated portions, respectively, were evenly distributed into 4 homogeneous tissue pools (Müller 1970). Each pool of capsular or decapsulated hemiadrenals was incubated – without preincubation – in 6 ml of a modified Krebs-Ringer bicarbonate buffer containing 3.6 meq. of potassium and 2 g of glucose per litre (Müller 1965). Labelled steroid substrates were added to each incubation flask in 0.06 ml of ethanol. After flushing with 95% O\(_2\), 5% CO\(_2\) for 10 min, incubation was continued for 110 min at 37°C in a metabolic shaker. At the end of the incubation the medium was filtered through a small funnel with a glass bead. The tissue of each flask was blotted and weighed.

**Incubation of capsular adrenal mitochondria**

Capsular hemiadrenals of groups of 24 rats were gently ground in ice-cold sucrose solution (0.25 M) in a Potter-Elvehjem homogenizer. The homogenate was centrifuged at 750 X g at 4°C for 10 min. After re-centrifugation at 750 X g for 10 min, the supernatant was transferred to fresh tubes and centrifuged at 7000 X g for 10 min. The supernatant was discarded, the pellet re-suspended in 0.25 M sucrose solution and re-centrifuged at 7000 X g for 10 min. The resulting pellet – consisting of an almost pure mitochondrial fraction, according to electron microscopy – was suspended in modified Krebs-Ringer bicarbonate (3.6 meq. K\(^+\) per litre) and distributed into 6 incubation flasks (final volume 6 ml/flask). The following substances were added to the incubation mixture: sodium fumarate, 10 mmol/l; NADP, 0.25 mmol/l; glucose-6-phosphate, 4.5 mmol/l; glucose-6-phosphate dehydrogenase, 0.5 U/ml (Boehringer). Tritiated precursor steroids were added in ethanol (0.06 ml/flask). The incubation conditions were identical to those used for whole adrenal tissue.

The protein content of the mitochondrial suspension was determined – before the addition of glucose-6-phosphate dehydrogenase – by the method of Lowry et al. (1951).

**Paper chromatography**

The following solvent systems were used for paper chromatography of labelled precursor steroids and conversion products:

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1. mean ± standard deviation, N = 6. Blood was obtained by aortic puncture under ether anaesthesia.

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A: cyclohexane-benzene-methanol-water (5:10:10:2)
B: cyclohexane-benzene-methanol-water (20:8:20:5)
C: cyclohexane-benzene-methanol-water (4:3:4:1)
D: cyclohexane-dioxane-methanol-water (4:3:2:1)
E: cyclohexane-dioxane-water (4:4:1)
F: iso-octane-tertiary butanol-methanol-water (4:3:4:2)
G: trimethylbenzene/methanol-water (3:1), reverse phase system.

Labelled steroids

[4-14C] Corticosterone (56.7 Ci/mol)[2] and [1,2-3H] corticosterone (40 Ci/mmol)[2] were purified by paper chromatography in systems D and C, [4-14C] aldosterone (56.7 Ci/mol)[2] in systems A and E, [4-14C] deoxycorticosterone (54.3 Ci/mol)[3] and [1,2-3H] deoxycorticosterone (30 Ci/mmol)[3] in systems B and F. The desired specific activity of labelled precursor steroids was obtained by adding appropriate amounts of unlabelled steroids.

[4-14C] 18-Hydroxycortico¬sterone was prepared by incubating [4-14C] corticosterone with capsular adrenals of rats which had been kept on a sodium-deficient diet (5 meq. Na+/kg, 230 meq. K+/kg) for two weeks. The labelled 18-hydroxycorticosterone was separated from corticosterone and aldosterone by chromatography in system A and was further purified by chromatography in system E. Unlabelled 18-hydroxycorticosterone was used as a chromatographic reference. The radiochemical purity was checked by mixing an aliquot with a trace amount of [1,2-3H] 18-hydroxycorticosterone. The isotope ratio remained stable throughout multiple chromatographies before and after periodic acid oxidation and chromic acid oxidation.

[4-14C] 18-Hydroxydeoxycorticosterone was prepared by incubating [4-14C] deoxycorticosterone with decapsulated rat adrenals. It was separated from deoxycorticosterone and corticosterone, respectively, by chromatography in systems D and A. Radiochemical purity was tested by mixing an aliquot with a trace amount of [1,2-3H] 18-hydroxydeoxycorticosterone. The isotope ratio remained constant throughout 4 chromatographies in different systems, two before and two after periodic acid oxidation.

Analysis of radioactive conversion products

Tritated steroids were measured by double isotope dilution, i.e. addition of a known amount of 14C-labelled steroid, and chromatographic purification to a constant isotope ratio. Trace amounts of 14C-labelled aldosterone, 18-hydroxycorticosterone, cortico-

1) The following trivial names and abbreviations are used in this paper:

aldosterone = ALDO = 11β,21-dihydroxy-3,20-dioxo-pregn-4-ene-18-al;
corticosterone = B = 11β,21-dihydroxy-pregn-4-ene-3,20-dione;
deoxycorticosterone = DOC = 21-hydroxy-pregn-4-ene-3,20-dione;
18-hydroxycorticosterone = 18-OH-B = 11β,18,21-trihydroxy-pregn-4-ene-3,20-dione;

2) Radiochemical Centre, Amersham, England.
3) New England Nuclear Corporation, Boston, Mass., USA.
4) Gift from Dr. J. Schmidlin, CIBA-GEIGY Limited, Basle.
5) Gift from Dr. S. Ulick, Bronx, N. Y.
sterone and 18-hydroxydeoxycorticosterone were added to 5 ml aliquots of the incubation medium. The medium was extracted with 35 ml of dichloromethane. The extract was washed with 3 ml of 0.1 M NaOH and with 3 ml of 0.1 M acetic acid. After the addition of 40 µg of unlabelled aldosterone, prednisolone and corticosterone as chromatographic markers, the dried extract was chromatographed in system A, which clearly separates 18-hydroxycorticosterone (running with prednisolone), aldosterone, 18-hydroxydeoxycorticosterone and corticosterone.

Aldosterone was further purified by acetylation with unlabelled acetic anhydride (Kliman & Peterson 1960). The diacetate was chromatographed in systems B and D. Following treatment with chronic acid (Kliman & Peterson 1960), the oxidation product was chromatographed in system C.

18-Hydroxycorticosterone was converted to its γ-lactone derivative by oxidation with periodic acid according to Schmidlin et al. (1957) and Nicolis & Ulick (1965). This compound was chromatographed in systems C (marker: cortisol-21-acetate) and D (marker: corticosterone). It was further oxidized with chronic acid according to Kliman & Peterson (1960). The resulting 11-dehydro derivative was chromatographed in system C, where it ran between cortisone-21-acetate and 11β-hydroxy-androst-4-ene-3,17-dione.

Corticosterone was acetylated with unlabelled acetic anhydride. Corticosterone-21-acetate was chromatographed in systems B and D and oxidized with chronic acid. The resulting 11-dehydro derivative was chromatographed in system F.

18-Hydroxydeoxycorticosterone was converted to its γ-lactone derivative by treatment with periodic acid and was then chromatographed in systems B (marker: 17α-hydroxyprogesterone) and D (marker: 11β-hydroxyandrost-4-ene-3,17-dione).

In some experiments 14C-labelled corticosterone was incubated with adrenal tissue. At the end of the incubation, radioactive corticosterone and aldosterone were isolated by extraction and chromatography in system A. Their specific activities were determined by double isotope dilution derivative assays according to Kliman & Peterson (1960). Following acetylation with tritiated acetic anhydride, aldosterone diacetate was purified by chromatography in systems B, D and – after chronic acid oxidation – C. Corticosterone-21-acetate was chromatographed in systems B, D and G.

Tritium and carbon-14 were measured in a liquid scintillation counter (Mark I, Nuclear Chicago).

**RESULTS**

_Evaluation of assay procedure_

*Radiochemical purity of conversion products.* – In order to check the efficacy and specificity of the isolation procedures used in the double isotope dilution assays of the tritiated conversion products, aliquots of four samples were taken and counted at different stages of purification. As shown on Table 1, 3H/14C ratios became constant after two or three chromatographies and a chemical modification of the steroid molecule. Satisfactory radiochemical purity of the isolated tritiated steroids could thus be assumed.

*Effect of end-products.* – The addition of unlabelled end-products, i.e. aldosterone or 18-hydroxycorticosterone, in substrate amounts to the medium before incubation did not affect the conversion of tritiated corticosterone to
Table 1.
Evaluation of purification procedure used in double isotope dilution assay of tritiated conversion products.

<table>
<thead>
<tr>
<th>Purification step</th>
<th>$^3$H/$^{14}$C ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sample No.</td>
</tr>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>$^3$H-Aldosterone-assay</td>
<td>6.7</td>
</tr>
<tr>
<td>chromatogr. A</td>
<td></td>
</tr>
<tr>
<td>acetylation, chromatogr. B</td>
<td>3.7</td>
</tr>
<tr>
<td>chromatogr. D</td>
<td>3.3</td>
</tr>
<tr>
<td>CrO$_3$ oxidation, chromatogr. C</td>
<td>3.3</td>
</tr>
<tr>
<td>$^3$H-18-Hydroxycorticosterone-assay</td>
<td></td>
</tr>
<tr>
<td>chromatogr. A</td>
<td>7.4</td>
</tr>
<tr>
<td>HIO$_4$ oxidation, chromatogr. C</td>
<td>4.8</td>
</tr>
<tr>
<td>chromatogr. D</td>
<td>4.7</td>
</tr>
<tr>
<td>CrO$_3$ oxidation, chromatogr. C</td>
<td>4.4</td>
</tr>
<tr>
<td>$^3$H-18-Hydroxydeoxycorticosterone-assay</td>
<td>76</td>
</tr>
<tr>
<td>chromatogr.A</td>
<td></td>
</tr>
<tr>
<td>HIO$_4$ oxidation, chromatogr. B</td>
<td>42</td>
</tr>
<tr>
<td>chromatogr. D</td>
<td>41</td>
</tr>
</tbody>
</table>

aldosterone and 18-hydroxycorticosterone by capsular adrenals of potassium-replete rats (Table 2).

Incubation time. – When capsular adrenals were incubated with tritiated corticosterone for 15, 30 and 60 min the relation between time and the con-

Table 2.
Conversion of tritiated corticosterone to aldosterone and 18-hydroxycorticosterone by capsular adrenals of potassium-replete rats in the absence and presence of unlabelled aldosterone or 18-hydroxycorticosterone: Substrate: [1,2-$^3$H] corticosterone; 300 nmol/flask. Mean values of two incubations ± range.

<table>
<thead>
<tr>
<th>Steroid added before incubation (55 nmol/flask)</th>
<th>Conversion (nmol per mg tissue) of $^3$H-corticosterone to aldosterone</th>
<th>18-hydroxycorticosterone</th>
</tr>
</thead>
<tbody>
<tr>
<td>none</td>
<td>0.25 ± 0.03</td>
<td>0.49 ± 0.00</td>
</tr>
<tr>
<td>aldosterone</td>
<td>0.26 ± 0.04</td>
<td>0.46 ± 0.06</td>
</tr>
<tr>
<td>none</td>
<td>0.31 ± 0.07</td>
<td>0.57 ± 0.10</td>
</tr>
<tr>
<td>18-hydroxycorticosterone</td>
<td>0.29 ± 0.03</td>
<td>0.53 ± 0.05</td>
</tr>
</tbody>
</table>

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version to aldosterone was approximately linear (Fig. 1). During the second hour of incubation the conversion rate decreased to a small extent, i.e. by 18% (potassium-replete) and 26% (potassium-deficient), respectively.

Effects of alterations in potassium balance

Zone specificity. – Capsular adrenals (»zona glomerulosa«) of potassium-deficient rats incorporated approximately 20 times less tritiated corticosterone into aldosterone and 18-hydroxycorticosterone than the capsular adrenals of normal rats and 30 times less than the capsular adrenals of potassium-replete rats, i.e. of animals which had been kept on the potassium-deficient diet for two weeks and had been drinking KCl-solution for two days (Table 3). In contrast, the same changes in potassium balance did not alter the conversion of tritiated deoxycorticosterone to 18-hydroxydeoxycorticosterone and 18-hydroxycorticosterone by decapsulated adrenals (»zona fasciculata-reticularis«).

Substrate concentration. – The marked difference between capsular adrenals of potassium-deficient and potassium-replete rats in their capacity to convert labelled corticosterone to aldosterone and 18-hydroxycorticosterone was demonstrable over a large range of substrate concentrations (Fig. 2). When capsular adrenals of potassium-replete rats were incubated with tritiated corticosterone added in four different concentrations, the substrate/conversion curves

![Conversion curves](image)

**Fig. 1.**
Influence of incubation time on the conversion of tritiated corticosterone (3H-B, 100 nmol per flask) to aldosterone (ALDO) by capsular adrenals of potassium-replete and potassium-deficient rats. Dots denote single values of one experiment.
Table 3.
Effect of alterations in potassium balance on corticosteroid biosynthesis in separate zones of rat adrenals. Mean values of two experiments (N = 4) ± standard deviation. *P* values of significance were calculated by *t*-tests and refer to differences between adjacent lines. NS: not significant at the *P* = 0.1 level. Conversion expressed in nmol per mg tissue.

<table>
<thead>
<tr>
<th>Rats</th>
<th>Capsular adrenals</th>
<th>Decapsulated adrenals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conversion of</td>
<td>Conversion of</td>
</tr>
<tr>
<td></td>
<td><em>³H-B</em>¹ to</td>
<td><em>³H-Doc</em>² to</td>
</tr>
<tr>
<td></td>
<td>Aldosterone</td>
<td>18-OH-DOC*³ to</td>
</tr>
<tr>
<td>normal</td>
<td>0.214 ± 0.050</td>
<td>1.76 ± 0.32</td>
</tr>
<tr>
<td></td>
<td>(NS)</td>
<td>(NS)</td>
</tr>
<tr>
<td>potassium-replete</td>
<td>0.333 ± 0.063</td>
<td>2.02 ± 0.23</td>
</tr>
<tr>
<td></td>
<td>(P &lt; 0.05)</td>
<td>(P &lt; 0.001)</td>
</tr>
<tr>
<td>potassium-deficient</td>
<td>0.011 ± 0.007</td>
<td>1.87 ± 0.29</td>
</tr>
<tr>
<td></td>
<td>(NS)</td>
<td>(NS)</td>
</tr>
</tbody>
</table>

1) [1,2-*³H*] corticosterone, 390 nmol per flask.
2) [1,2-*³H*] deoxycorticosterone, 1 µmol per flask.
3) 18-hydroxycorticosterone.
4) 18-hydroxydeoxycorticosterone.

Fig. 2.
Effect of increasing substrate concentration on the conversion of tritiated corticosterone (*³H-B*) to 18-hydroxycorticosterone (18-OH-B) and aldosterone (ALDO) by capsular adrenals of potassium-replete and potassium-deficient rats. Dots represent mean values of three experiments (N = 3). Brackets denote ± standard deviation.
were hyperbolic with a plateau between the two highest substrate levels. From Lineweaver-Burk plots of the mean values of conversion the following parameters of apparent enzyme activities were calculated (Fig. 3):

- conversion of $^3$H-corticosterone to aldosterone:
  
  potassium-replete: $K_m = 58.2 \times 10^{-6}$ M, $V_{\text{max}} = 0.76$ nmol/mg $\cdot$ 2 h;
  
  potassium-deficient: $K_m = 16.3 \times 10^{-6}$ M, $V_{\text{max}} = 0.01$ nmol/mg $\cdot$ 2 h;

- conversion of $^3$H-corticosterone to 18-hydroxycorticosterone:
  
  potassium-replete: $K_m = 39.9 \times 10^{-6}$ M, $V_{\text{max}} = 1.34$ nmol/mg $\cdot$ 2 h;
  
  potassium-deficient: $K_m = 19.3 \times 10^{-6}$ M, $V_{\text{max}} = 0.04$ nmol/mg $\cdot$ 2 h.

**Mitochondria.** – Isolated mitochondria of capsular adrenals of potassium-replete rats converted 25 times more $^3$H-corticosterone to aldosterone and 38 times more $^3$H-corticosterone to 18-hydroxycorticosterone than the capsular adrenal mitochondria of potassium-deficient rats (Table 4).

**Time of resumed potassium intake.** – When potassium-deficient rats received a KCl solution as drinking fluid for increasing periods of time, a small effect

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**Fig. 3.**

Lineweaver-Burk plot of the mean values of onversion of tritiated corticosterone (B) to aldosterone (ALDO) and 18-hydroxycorticosterone (18-OH-B) by capsular adrenals of potassium-replete and potassium-deficient rats (see Fig. 2). Lines were computed by least-squares fit.
Conversion of [1,2-3H] corticosterone, 60 nmol/flask, to aldosterone and 18-hydroxycorticosterone by capsular adrenal mitochondria. Mean values of two experiments (N = 6) in nmol per 100 μg of mitochondrial protein. P values of significance were calculated by t-tests and refer to differences between adjacent lines.

<table>
<thead>
<tr>
<th>Rats</th>
<th>Conversion to Aldosterone</th>
<th>18-Hydroxycorticosterone</th>
</tr>
</thead>
<tbody>
<tr>
<td>potassium-deficient</td>
<td>0.012 ± 0.003</td>
<td>0.044 ± 0.006</td>
</tr>
<tr>
<td></td>
<td>(P &lt; 0.001)</td>
<td>(P &lt; 0.001)</td>
</tr>
<tr>
<td>potassium-replete</td>
<td>0.299 ± 0.085</td>
<td>1.689 ± 0.372</td>
</tr>
</tbody>
</table>

on capsular adrenal conversion of tritiated corticosterone to aldosterone and 18-hydroxycorticosterone was observed within 4 hours (Fig. 4). After 12 hours

Fig. 4.
Influence of time of resumed potassium intake on the conversion of tritiated corticosterone (3H-B, 320 nmol per flask) to 18-hydroxycorticosterone (18-OH-B) and aldosterone (ALDO) by capsular adrenals. Rats were kept on a potassium-deficient diet for two weeks and then received a KCl-sucrose solution as drinking fluid for increasing periods of time.
of drinking KCl solution, the increments in the rates of conversion became statistically significant ($P < 0.001$ and $P < 0.005$, respectively). Within one day of resumed potassium intake, the conversion rates became as high as those observed in the adrenal tissue of normal rats (Table 3). Prolonged drinking of KCl solution for 48 and 96 hours induced only small additional increases in the conversion rates.

18-Hydroxydehydrogenation. – In view of the low rate of conversion of added 18-hydroxycorticosterone to aldosterone by incubated adrenal tissue which had been observed by Ulick et al. (1964) and Nicolis & Ulick (1965), we did not attempt to evaluate 18-hydroxydehydrogenase activity directly by measuring the conversion of added labelled 18-hydroxycorticosterone to aldosterone. Instead, we assessed the ultimate step of aldosterone biosynthesis indirectly by the relative conversion of tritiated corticosterone to 18-hydroxytocorticosterone and aldosterone. Assuming that all the tritiated aldosterone had been formed via 18-hydroxycorticosterone, we took the ratio of $^3$H-aldosterone to the sum of $^3$H-18-hydroxycorticosterone and $^3$H-aldosterone as an index of the fractional 18-hydroxydehydrogenation (Table 5). On an average, »fractional 18-hydroxydehydrogenation« was 18 % higher in the capsular adrenals of potassium-replete rats than in the tissue of potassium-deficient animals.

Specific activity of labelled aldosterone. – When the capsular adrenals of potassium-replete rats were incubated with 40 nmol/flask of added $^{14}$C-corticosterone, the aldosterone which was formed had a 35 % lower specific activity than the labelled corticosterone which was recovered from the medium after incubation (Table 6). At higher substrate concentrations, the specific activity

| Table 5. Effect of potassium intake on »fractional 18-hydroxydehydrogenation« by rat capsular adrenal tissue. Mean values of three experiments ($N = 3$) ± standard deviation. |
| [1,2-$^3$H] Corticosterone nmol/flask | »Fractional 18-hydroxydehydrogenation«* | |
| | Rats | Potassium-replete | Potassium-deficient |
| 40 | 0.30 ± 0.04 | 0.28 ± 0.03 |
| 145 | 0.35 ± 0.07 | 0.29 ± 0.01 |
| 530 | 0.34 ± 0.03 | 0.27 ± 0.03 |
| 1150 | 0.31 ± 0.05 | 0.24 ± 0.03 |
| mean ($N = 12$) | 0.32 ± 0.05** | 0.27 ± 0.03*** |

* $^3$H-aldosterone

$^3$H-18-hydroxycorticosterone + $^3$H-aldosterone

** Difference significant ($P < 0.005$) according to t-test.
Incubation of capsular rat adrenals with $^{14}$C-corticosterone. Determination of specific activities of labelled corticosterone and aldosterone at the end of incubation.

<table>
<thead>
<tr>
<th>[4-$^{14}$C] Corticosterone$^{a,**}$ incubated nmol/flask</th>
<th>Specific activities (cPM/nmol) at the end of incubation</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$^{14}$C-</td>
<td>$^{14}$C-</td>
<td>$^{14}$C-</td>
<td>$^{14}$C-</td>
</tr>
<tr>
<td>Potassium-replete rats</td>
<td>Potassium-deficient rats</td>
<td>Corticosterone</td>
<td>Aldosterone</td>
<td>Corticosterone</td>
</tr>
<tr>
<td>40</td>
<td>1640</td>
<td>1070</td>
<td>14420</td>
<td>3250</td>
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<tr>
<td>145</td>
<td>420</td>
<td>340</td>
<td>7560</td>
<td>3750</td>
</tr>
<tr>
<td>530</td>
<td>250</td>
<td>220</td>
<td>3690</td>
<td>2300</td>
</tr>
<tr>
<td>1150</td>
<td>170±10$^a$</td>
<td>140±20$^a$</td>
<td>2190±60$^a$</td>
<td>450±150$^a$</td>
</tr>
</tbody>
</table>

$^a$ mean ± standard deviation, N = 3.

$^{**}$ The specific activities of added [4-$^{14}$C] corticosterone correspond to those found at the end of incubation within the limits of predictable counting errors.

of the labelled aldosterone was 80–90% that of the corticosterone. These findings indicate that in the presence of high amounts of added corticosterone most of the aldosterone is formed via corticosterone, and are consistent with previous observations made on incubations of beef adrenal tissue by Ayres et al. (1960).

The results obtained in experiments with tissue of potassium-deficient rats are more ambiguous. The specific activity of aldosterone was 22, 50 and 62% that of corticosterone at the 40, 145 and 530 nmol/flask substrate levels and dropped to 20% when the substrate was increased to 1150 nmol per flask. These results are at least compatible with the possibility that under certain experimental conditions most of the aldosterone is produced by a biosynthetic pathway not involving corticosterone.

**DISCUSSION**

Since both sodium retention and potassium excretion are primary biological effects of aldosterone in the mammalian organism, adaptation of aldosterone secretion to alterations in sodium balance and adaptation to alterations in potassium balance appear to be of equal importance. Nevertheless, the influence of the total body potassium state on aldosterone production has not been as intensively investigated as the influence of the total body sodium state. According to the available evidence, alterations in potassium balance as well as alterations in sodium balance can influence aldosterone production by acting on at least two separate sites in the biosynthetic pathway (for a review see Müller 1971).

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Small increases in the potassium concentration of adrenal arterial plasma or the incubation medium acutely stimulated aldosterone production by the adrenal gland or by incubated adrenal tissue (Blair-West et al. 1962, 1970b; Davis et al. 1963; Kaplan 1965; Müller 1965; Burwell et al. 1969; Haning et al. 1970). Since stimulation of aldosterone production by potassium ions was found to be accompanied by increases in corticosterone output, it has been generally assumed that this effect occurred at a biosynthetic step preceding the formation of corticosterone. However, the exact site of action of potassium ions is still controversial. Whereas Burwell et al. (1969) have presented evidence that potassium ions act preferentially on 11β-hydroxylation, experiments carried out in this laboratory have indicated that the primary action of potassium ions is on the conversion of cholesterol to pregnenolone (Müller 1966) or at least at a biosynthetic step preceding the formation of deoxycorticosterone (Müller 1970, 1971).

The results of the present studies support our previously mentioned hypothesis that potassium deficiency impairs aldosterone biosynthesis by decreasing the activity of one or more of the enzymes involved in the conversion of deoxycorticosterone to aldosterone (Müller & Huber 1969; Müller 1970) and confirm and extend the finding of Boyd et al. (1971) that mitochondria of rats which have been subjected to oral potassium loading for two days, convert twice as much corticosterone to aldosterone as the adrenal mitochondria of control animals. We found that capsular adrenal tissue of potassium-deficient rats converted about twenty times less added corticosterone to aldosterone and 18-hydroxycorticosterone, than the capsular adrenals of normal rats, and that these decreased rates of conversion were restored to normal within one or two days of resumed potassium intake. These effects could not have been simply due merely to alterations in the zona glomerulosa size since they were far in excess of observed morphological changes. In our laboratory rats we have previously found that two weeks of potassium deficiency led to an approximately 30% decrease in the width of the zona glomerulosa (Müller & Huber 1969). The zona glomerulosa thickness of potassium-deficient rats did not noticeably increase within two days of resumed potassium intake (Baumann & Kistler, preliminary observations). When capsular adrenal mitochondria of potassium-deficient and potassium-replete rats were incubated with tritiated corticosterone, the observed differences in the conversion to aldosterone and 18-hydroxycorticosterone, respectively, were as marked as those seen when the intact capsular adrenal tissue was incubated. Thus, the decreased conversion of labelled corticosterone by tissue of potassium-deficient rats was due rather to a decreased enzyme activity than to the impaired transport of the added steroid substrate, differences in endogenous precursor pools or diminished coenzyme availability.

According to a majority of investigators, the conversion of corticosterone to
aldosterone is a two-step reaction, and 18-hydroxycorticosterone is an intermediary rather than a by-product of aldosterone biosynthesis. The results of the present studies are compatible with this assumption – which is mainly based on indirect evidence and analogy (Ullick et al. 1964; Nicolis & Ullick 1965; Greengard et al. 1967) –, but do not exclude the possibility that aldosterone and 18-hydroxycorticosterone are simultaneously formed by two different enzymes. If this were the case, potassium deficiency would appear to equally diminish the activity of both enzymes. On the other hand, if 18-hydroxycorticosterone were an intermediary of aldosterone production, our results would indicate that potassium balance has a definite influence on the 18-hydroxylase activity of the zona glomerulosa, but they offer no unequivocal information as to a simultaneous effect on 18-hydroxydehydrogenase activity. The activity of this enzyme is either not changed or it is decreased to the same extent as 18-hydroxylase activity or less. Since capsular adrenals of potassium-deficient rats convert tritiated corticosterone to 18-hydroxycorticosterone in trace amounts only, the further conversion of this intermediary to aldosterone may not be representative of 18-hydroxydehydrogenase activity. The small but statistically significant decrease observed in »fractional 18-hydroxydehydrogenation« by capsular adrenals of potassium-deficient rats can be explained by a marginal decrease in the affinity of the enzyme to its steroid substrate. However, it may also have been due to a relatively more active 18-hydroxylation due to contamination by zona fasciculata cells, which can convert corticosterone to 18-hydroxycorticosterone but not to aldosterone (Marusic & Mulrow 1967a; Baniukiewicz et al. 1968). As shown in Table 3, 18-hydroxylase activity of decapsulated adrenals, i.e. zona fasciculata-reticularis, was not affected by potassium deficiency.

Kinetic data – obtained from experiments in which the intact capsular adrenal tissue was incubated – indicate that the decrease in 18-hydroxylase activity induced by potassium deficiency is rather due to a decrease in enzyme content than to inactivation of available enzyme. Thus, the decreases in apparent $V_{\text{max}}$ were much larger than the decreases in apparent $K_{\text{m}}$. Moreover, the slow onset of enhanced conversion of corticosterone to aldosterone and 18-hydroxycorticosterone during resumed potassium intake could be indicative of de novo enzyme synthesis. A similar delay has been observed by Boyd et al. (1971) in their study on the effect of potassium loading on the conversion of corticosterone to aldosterone.

Increases in the 18-hydroxylase activity of the zona glomerulosa have as yet only been induced in vivo. Perhaps they are due to a long-term effect of altered blood electrolyte levels on the adrenal cortex, but they could also be mediated by an unknown extra-adrenal stimulator. Haning et al. (1970, 1971) have found that the fractional conversion of trace amounts of tritiated corticosterone to aldosterone by incubated rat adrenal glomerulosa cells or tissue can
be stimulated by unlabelled corticosterone added in small amounts to the incubation medium or by an increased output of endogenous corticosterone elicited by ACTH, serotonin or potassium ions. However, this direct in vitro effect of potassium ions on the conversion of corticosterone to aldosterone differs in at least two important respects from the effects induced in vivo by resumed or augmented potassium intake. Activation in vitro by added or endogenous corticosterone was limited to the conversion of corticosterone to aldosterone and did not include the conversion to 18-hydroxycorticosterone. A maximum in vitro effect was observed within 15 minutes after the addition of stimulators or corticosterone to the incubation medium, whereas in vivo a definite effect was observed only within a few hours and a maximum effect within days of increased potassium intake.

The results of the present investigations can only partially explain the previously observed alterations in endogenous corticosteroid output by capsular adrenal tissue induced by potassium deficiency (Müller 1970). Thus, under basal conditions of incubation, the tissue of potassium-deficient rats produced only about 30% less aldosterone than the tissue of normal rats. In view of the observed 20-fold decrease in the conversion of corticosterone to aldosterone, one might suspect that the potassium-deficient rat produces aldosterone mainly by a biosynthetic pathway not involving corticosterone. Some of the discrepancies in the specific activities recorded in Table 6 might be explained by such a hypothesis. If 18-hydroxylase activity only was decreased in otherwise normal glomerulosa cells, an increased corticosterone output would be expected particularly during stimulation with agents acting on the early steps of the biosynthetic pathway. Instead, corticosterone output was normal and deoxy-corticosterone output was increased. Since this finding was suggestive of an associated decrease in 11β-hydroxylase activity, parallel studies were performed in order to examine the effect of potassium balance on 11β-hydroxylation. The results of those experiments will be recorded in a forthcoming report (Baumann & Müller 1972).

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