them were beginning to change into fibrous tissue and were occasionally calcified. Sections stained with Oil Red O and Sudan B black showed an increase in the positively staining lipoid material. No abnormalities were observed in the medullae.

The adrenal changes differed strikingly from those usually seen in the course of severe nonspecific stress. They closely resembled those which PRADER and GURTNER[2], and others[3], described as congenital-lipoid-adrenal hyperplasia in new-born infants, and were also similar to those found in humans and experimental animals following treatment with aminoglutethimide[4,5]. Under these conditions there is an inhibition of the enzymatic conversion of cholesterol to Δ^5-pregnenolone due to interference with 20-hydroxylation of the cholesterol side-chain and with the desmolase complex activity. As a result of these morphological similarities it can be supposed that a block also occurs in corticosteroidogenesis of aniline-treated rats. However, biochemical investigations are required to prove this hypothesis.

The aniline-induced adrenal changes can be prevented by simultaneous treatment with glucocorticoids which suggests that increased release of ACTH plays a decisive role in eliciting the adrenal lesion. This finding is compatible with the view that aniline, by inhibiting steroid synthesis, primarily affects the adrenal cortex, and the subsequent lack of circulating corticoids leads to a compensatory hypersecretion of ACTH. The importance of the local factor, however, cannot be neglected as the adrenal alterations elicited by aniline considerably differ from those due to the administration of ACTH.

Since aniline is metabolized to various substances mainly by the liver microsomes[6], the question arises whether aniline itself or one of its metabolites is responsible for the adrenal effect. There is no satisfactory answer at present to this question. However, it is worth mentioning that adrenal enlargement and lipoid accumulation in aniline-treated rats are not prevented by SKF 525-A, a potent inhibitor of a variety of microsomal enzymes[7].

It is interesting that both amphenone and aminoglutethimide, the well-known steroid synthesis inhibitors, contain aniline residues in their molecules. Further study is needed to verify whether aniline is the active principle in blocking steroidogenesis when these compounds are used[8].

Zusammenfassung. Subkutan injiziertes Anilin verursacht bei der Ratte eine Vergrösserung der Nebennierenrinde mit beachtlicher Ansammlung von Lipoiden. Morphologisch unterscheiden sich diese Veränderungen wesentlich von denen, die nach schwerer unspezifischer Stresswirkung entstehen, ähneln aber den durch Aminoglutethimid hervorgerufenen Läsionen. Es scheint, dass die Corticoidsynthese durch Anilin gestört wird und dass die Gewichtssteigerung der Nebennieren auf die ausgleichende Ausschüttung von ACTH zurückzuführen ist.

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Group	Duration of treatment	No. of rats	Final body weight (g)	Adrenal weight (mg/100 g body wt.)	
Control	–	10	106 ± 3.2[a]	33.3 ± 0.9	$p < 0.001$
Aniline	7 days	10	102 ± 2.7	56.2 ± 2.3	
Control	–	10	118 ± 1.6	27.3 ± 0.9	$p < 0.001$
Aniline	14 days	10	120 ± 4.1	64.3 ± 5.2	

[a] Mean ± S.E.

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In vivo Conversion of Corticosterone into Aldosterone in Rats Treated with ACTH or Submitted to Stress

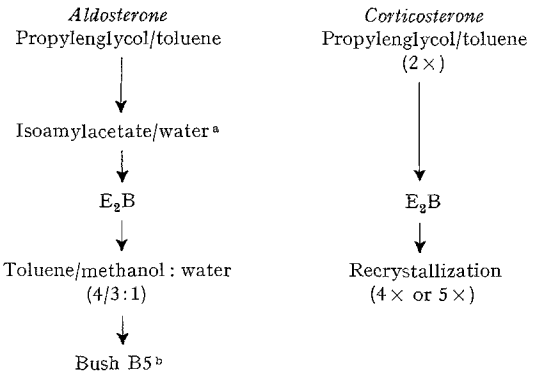
The functional and morphological alterations of the adrenals in the resistance stage of the general adaptation syndrome (GAS) are generally attributed to an increased secretion of endogenous ACTH[1]. However, considerable differences in the pattern of adrenal cortical hormones have been demonstrated in rats treated with ACTH and rats injected with formaldehyde (a typical 'stressor' eliciting the GAS). After repeated administration of ACTH, the production of aldosterone was decreased; after formaldehyde treatment it was increased[2–5]. However, little is known of the biochemical background of this difference. The experiments described here were undertaken in order to obtain information on the in vivo conversion of corticosterone into aldosterone under different conditions.

Material and methods. Male white rats of 180–280 g body weight were anaesthetized with Nembutal (40 mg/kg i.p.). After i.v. injection of 1-2-H^3-corticosterone the adrenal venous blood was collected for 1.5 h according to the method described by VOGT[6]. The blood taken from the adrenal was permanently replaced by using an automatic device (for details see [7]).

The collected blood was haemolyzed and extracted with dichlormethane. The radioactive fractions of aldosterone and corticosterone were isolated by repeated chromatography and – in the case of corticosterone – by recrystallization (Figure 1). Their radioactivities were measured in a liquid scintillation spectrometer (Packard, type 3003).

To exclude an extra-adrenal conversion of corticosterone into aldosterone, blood samples of 7 adrenalectomized rats were collected from the femoral artery after the injection of 1-2-H^3-corticosterone. In all but one experiment, no radioactive aldosterone could be demonstrated in the femoral blood. In the 7th rat only a minimal amount of radioactive aldosterone (0.05% of the injected radioactivity/100) was obtained.

Fig. 1. Isolation of radioactive aldosterone and corticosterone



a Chromatographic system worked out in our laboratory, in which the Rf values of various corticosteroids are comparable with the system of formamide/aethylacetate : butylacetate : water[12]. b In several cases with sufficient aldosterone radioactivity aliquot parts of samples were also chromatographied in the system: benzene/heptan : methanol/water after acetylation. The specific radioactivity of the radioactive aldosterone remained unaltered after this chromatographic step. In all cases the identity and purity of the isolated aldosterone and corticosterone were proved by the unaltered specific radioactivity of the isolated steroids in the 2 or 3 last steps of the isolation.

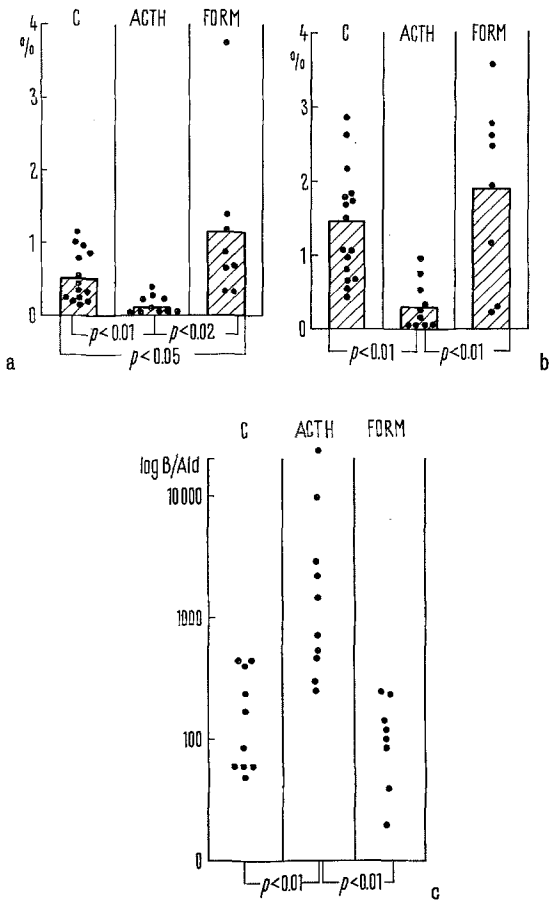


Fig. 2 a) Aldosterone radioactivity of adrenal venous blood samples expressed as % of the radioactivity of the dichlormethane extract of adrenal venous blood of control (C), ACTH- and formaldehyde (FORM)-treated rats. b) Aldosterone radioactivity of adrenal venous blood samples expressed as % of the injected corticosterone radioactivity/100. c) Logarithms of the B/Ald ratios.

Results. The adrenals of rats treated with ACTH[8] or formaldehyde[9] were hypertrophied as expected. The amount of radioactive aldosterone (Figure 2) can be expressed: a) as the percentage of the radioactivity of the dichlormethane extract, which contains corticosterone and intermediates which may be of significance in aldosterone biosynthesis; b) as the percentage of the injected precursor radioactivity; or c) as the ratio of corticosterone/aldosterone, where the corticosterone radioactivity found in adrenal venous blood represents the remaining radioactivity of the injected precursor.

ACTH pretreatment for 9–25 days caused a significant decrease in the conversion of radioactive corticosterone to aldosterone. This alteration of the biosynthesis may be responsible for the reduced aldosterone secretion in man found by NEWTON and LARAGH[10] after chronic administration of ACTH and by BIGLIERI[11] in the ectopic ACTH syndrome. A decrease in aldosterone production formed from endogenous precursors in rats was demonstrated by STARK et al.[4] and by MÜLLER[5].

In animals which received formaldehyde, only an insignificant or no increase in the conversion from corticosterone into aldosterone was found.

In rats submitted to repeated application of formaldehyde, the production of aldosterone from endogenous precursors is increased[2,3], whereas in ACTH-treated rats it is decreased[4,5]. Therefore, it may be assumed that chronic administration of formaldehyde, in addition to stimulating the release of endogenous ACTH, also affects the function of the adrenal cortex by means of other factors.

Studies on the in vivo conversion of corticosteroid precursors into cortical hormones may give more reliable information on biosynthetic pathways in the adrenal cortex than the technique of the in vitro incubation.

Zusammenfassung. Es wurde die in vivo Konversion von Corticosteron zu Aldosteron in den Nebennieren der Ratte nachgewiesen. Wiederholte ACTH-Gaben verminderten die Konversion, während diese nach chronischer «Stress»-Einwirkung (Formol-Behandlung) unverändert blieb. Es wird angenommen, dass die Vergrösserung und Funktionsänderung der Nebennieren unter Stress-Einwirkung nicht nur durch vermehrte Abgabe von ACTH vermittelt wird.

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