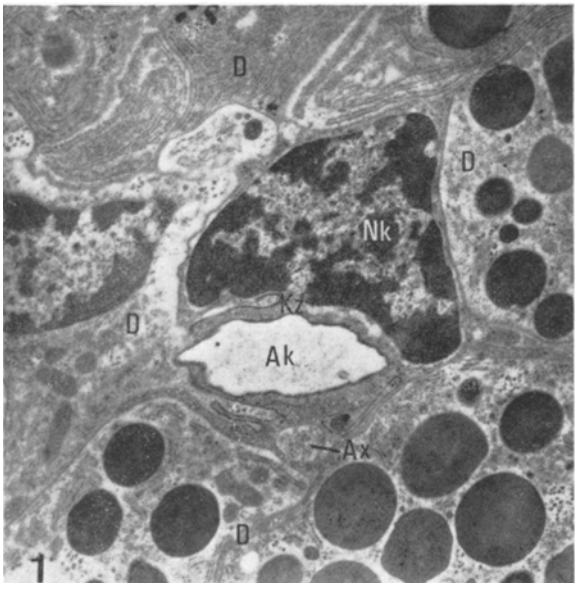


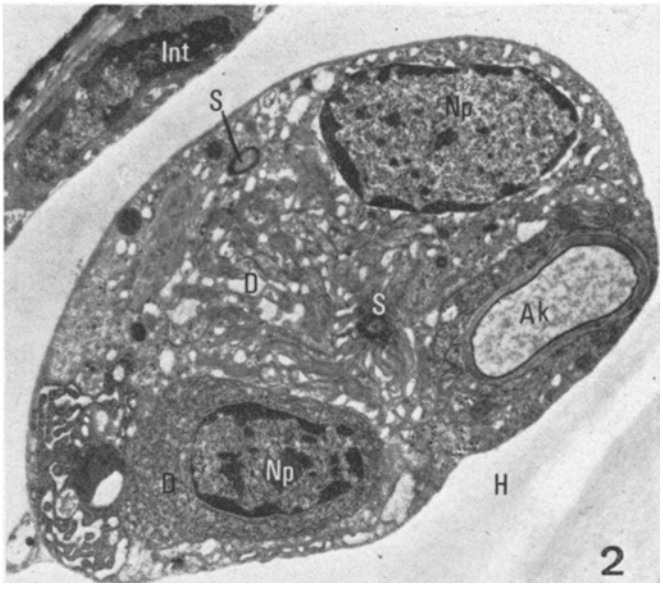


Fig. 1. Schnitt durch die Glandula mandibularis. Ak, Hauptausführkanal; Ax, Neurosekretorisches Axon; D, Drüsenparenchymzellen mit zahlreichen Sekretvakuolen, granulärem ER und Mitochondrien; Kz, Kanalwandzelle; Nk, Kern der Kanalwandzelle. ×13800.
Fig. 2. Schnitt durch die Glandula maxillaris II. Ak, Hauptausführkanal; D, Drüsenparenchymzellen unterschiedlicher Funktionsphasen; H, Haemocoel; Int, Integument; Np, Kerne von Parenchymzellen; S, quergeschnittene Seitenkanäle. ×7500.

Beide Drüsenpaare waren von FAHLANDER[3] als exokrine Drüsen beschrieben worden. Die Funktion der kleinen Gl. maxillares II musste aber unklar bleiben, weil ihre Ausführgänge caudal von den Coxen der 2. Maxillen nach aussen, also nicht in den Praeoralraum münden sollen.

Die Ultrastruktur kann sichere Auskunft geben, ob eine exokrine oder eine endokrine Drüse vorliegt[4]. Bei den fraglichen Organen handelt es sich eindeutig um exokrine Drüsen (Figuren 1 und 2). Neben den Sekret produzierenden Parenchymzellen (D), deren Kerne (Np) sich durch gleichmässig verteiltes Chromatin auszeichnen, findet man typische Kanalwandzellen (Kz). Letztere bilden ein System aus zuführenden Seitenkanälen (S) und einem Hauptausführkanal (Ak). Alle Kanäle sind mit einer kutikularen Intima ausgekleidet. Von den Drüsenparenchymzellen abgegebenes Sekret wird in relativ enge Interzellularspalten ausgeschleust, die Anschluss an das ausführende Kanalsystem finden.

Eine eingehende Beschreibung der Ultrastruktur soll zu einem späteren Zeitpunkt gegeben werden[5]. Hier sei nur der Nachweis erbracht, dass die erwähnten Drüsen in der Tat exokrin sind, und dass nach unserem bisherigen Wissen demnach allein die Gl.ecdysalis als Häutungsdrüse von *Lithobius* zu gelten hat.

*Summary.* Electronmicroscopic investigations of the glandula mandibularis and gl.maxillaris II of *Lithobius forficatus* demonstrate the exocrine character of these glands. Therefore, gl.ecdysalis remains the sole ecdysial gland of this centipede.

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## Activities of Kidney Decarboxylases of Histidine and Ornithine in Adrenalectomized Mice Substituted with Cortisone

Formation of putrescine by decarboxylation of ornithine occurs, like histamine formation by the agency of histidine decarboxylase, in all mammalian tissues.

Little is known of the control of the relevant decarboxylase activities in physiological conditions. In suitable experiments, the activities of these enzymes can be greatly enhanced or lowered. Glucocorticoid hormones have tentatively been suggested as controlling these enzymic activities. Increase of ornithine decarboxylase activity is brought about in animals subjected to strain[1]. A stabilizing influence of the adrenal gland is suggested by the fact that adrenal steroids released in 'stress' oppose some untoward effects of histamine. Cortisone is reported to protect adrenalectomized rats from traumatic shock[2].

In earlier work from this laboratory, the kidney of the female mouse was shown to be a suitable target organ in

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exploring steroid regulation of histamine and putrescine metabolism[3]. The present study is a continuation of the former work.

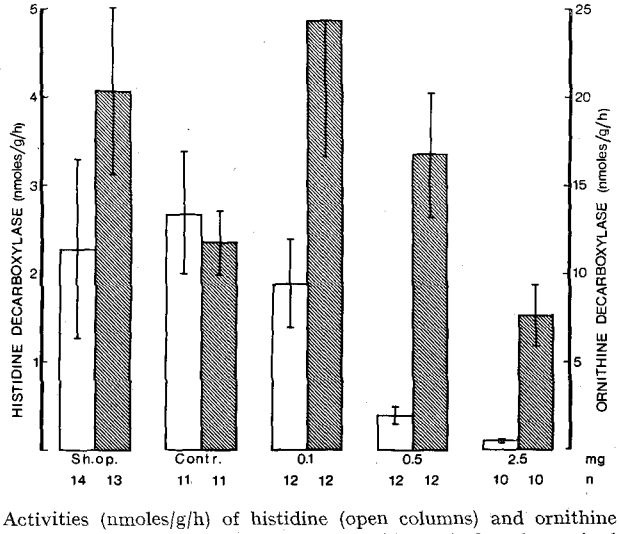
*Methods.* White female mice, NMRI strain, 3–5 months old, were used. They were fed a standard pellet diet with tap water ad libitum. Adrenalectomy, as well as sham-operation, was performed under ether anaesthesia. Adrenalectomized mice had free access to 0.9% NaCl solution (saline). Cortisone acetate suspended in 0.1 ml saline was injected i.p. and controls were injected with an equivalent volume of saline. The mice were stunned and exsanguinated 15 h after the last injection, the kidneys were promptly removed and kept cold. The pertinent tissue was gently homogenized (25 strokes with the pestle) in 1.7 ml cold 0.1 *M* sodium phosphate buffer (pH 6.9) containing $10^{-4}$ *M* EDTA, $5 \times 10^{-4}$ *M* dithiothreitol and 0.2% glucose. The homogenate was centrifuged at 20,000 *g* for 20 min at 4 °C. The supernatant was decanted and aliquots were used for determining the activities of histidine and ornithine decarboxylases. For determining histidine decarboxylase activity, the $^{14}CO_2$ evolved on decarboxylation of $^{14}$C-carboxyl-labelled histidine was measured. Earlier experiments had shown that in the kidney of adult female mice histamine formation is high and can be measured safely by this method, the virtue and limitation of which have been described[4]. Ornithine decarboxylase activity was measured as described by RUSSELL and SNYDER[5] with minor modifications[3].

*Results.* 13 mice were adrenalectomized and 9 were sham-operated. They were sacrificed 6 to 8 days after the operation and histidine and ornithine decarboxylases of the kidney were determined. Following adrenalectomy, histidine decarboxylase activity was slightly increased, whereas ornithine decarboxylase activity was unchanged (Table I).

Table I. Activities of kidney histidine and ornithine decarboxylases (nmoles/g/h) in sham-operated and adrenalectomized mice

| | Sham-operated | | Adrenalectomized | |
|---|---|---|---|---|
| Histidine decarboxylase | 2.0 ± 0.65 | (9) | 3.4 ± 0.63 | (13) |
| Ornithine decarboxylase | 9.8 ± 1.41 | (9) | 9.0 ± 1.38 | (12) |

Mean values ± SEM are given: the figures in brackets stand for number of observations.




Activities (nmoles/g/h) of histidine (open columns) and ornithine (hatched columns) decarboxylases in the kidney of adrenalectomized mice substituted with cortisone acetate in the dose range 0–2.5 mg. Sh. op., sham-operated; contr., adrenalectomized; *n*, number of observations.

The effect of cortisone administration was studied in 45 adrenalectomized mice divided in 2 groups. In one group, the hormone treatment began on the first day of operation, the other group was given the first injection 7 days after adrenalectomy. In both groups cortisone acetate was given on 5 consecutive days in the doses: 0.1, 0.5 and 2.5 mg/mouse. Sham-operated and unsubstituted adrenalectomized animals (controls) were given saline. Activities of histidine and ornithine decarboxylase were determined in the kidney. Results obtained in both groups are combined in the Figure. Histidine decarboxylase activity in the kidney of adrenalectomized mice was higher than in that of sham-operated animals. Cortisone administration reduced histamine formation in a dose-related manner; with the dosages of 0.5 and 2.5 mg histidine decarboxylase activity was lowered to 15 and 5% respectively of the control value.

The dose-response curve for ornithine decarboxylase activity was different. Cortisone injection in a dosage of 0.1 mg for 5 days increased the enzyme level. On elevating the dose of cortisone further, ornithine decarboxylase activity decreased as seen in the Figure. Putrescine formation was higher in sham-operated mice than in adrenalectomized controls, a situation that is not entirely in accord with the results presented in Table I. This discrepancy should be seen against the fact that the results given in the Figure are from sham-operated animals injected daily i.p. with saline and that this procedure is likely to impose some degree of strain.

In the course of these experiments, it became apparent that cortisone injections in adrenalectomized mice increased the weight of the kidneys and that this effect was dose-dependent. There was no corresponding increase in the body weight as shown in Table II. It has been amply documented that di- and polyamine metabolism is markedly stimulated in various kinds of tissue growth; on the other hand, it has been reported that injections of hydrocortisone or growth hormone stimulate ornithine decarboxylase activity only temporarily, i.e. in the rat repeated injections of the hormones cannot sustain an elevated level of hepatic ornithine decarboxylase activity[6]. With the view to excluding an earlier change in ornithine decarboxylase, we estimated the kidney enzyme activity of adrenalectomized mice after an injection of 2.5 mg cortisone acetate and killed the animals 15 h later; and also administered 2 injections, 24 h apart, again sacrificing the animals 15 h after the last injection. In these experiments, no noteworthy change in enzyme activity was observed.

*Discussion.* Our observation that the kidney of the female mouse is a suitable target organ for studying the steroid regulation of histamine and putrescine metabolism was preceded by the discovery of FRIEDEN et al. that testosterone transitorily increases the rate of incorporation of L-$^{14}$C-glycine into mouse kidney slices[7] and that,

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Table II. Kidney and body weights of the same animals of which enzyme activities are presented in the Figure

| Treatment | Sham-operated | Cortisone acetate 0 mg | 0.1 mg | 0.5 mg | 2.5 mg |
|---|---|---|---|---|---|
| No of mice | 13 | 11 | 12 | 12 | 10 |
| Kidney weight (mg ± SEM) | 247 ± 11.8 | 222 ± 13.0 | 235 ± 8.2 | 259 ± 8.6 | 292 ± 13.2 |
| Body weight (g ± SEM) | 25.8 ± 1.00 | 24.9 ± 1.55 | 25.8 ± 0.97 | 25.0 ± 0.91 | 26.5 ± 1.48 |

The kidney weight of animals given 0.5 mg and 2.5 mg cortisone acetate was significantly increased.

conversely, administration of hydrocortisone resulted in a sustained decrease in incorporation rate[8].

In our experiments, removal of the adrenals, i.e. depriving the mouse of the normal level of circulating steroids, increased the activity of kidney histidine decarboxylase, suggesting that this hormone level exerts a restraining influence on this particular enzyme activity. This view is born out by the effects of cortisone administration, whereby histamine formation is reduced in a dose-related manner. Indeed, with the largest dose used, histidine decarboxylase activity was lowered to about 5% of the control value, a degree of inhibition that has previously been reported only by the injection of testosterone, under the influence of which the kidney enzyme activity nearly disappeared[9].

The alterations seen in ornithine decarboxylase activity were biphasic; a small dose elevated the activity, whereas on increasing the dose of cortisone the activity decreased. In cases in which the activities of decarboxylases of histidine and ornithine are altered, the levels have been reported to change in an inverse relationship, or merely either of the enzyme activities is involved[3,5].

The kidney weight deserves comment. Cortisone injections in adrenalectomized mice increased the weight of the kidney in a dose-dependent manner, whereas the body weight was not increased. The cortisone stimulated kidney became even larger than that of the sham-operated animals. We did not examine the kidney histologically or for specific indications of tissues growth. Nevertheless, it is noteworthy that the considerable increase in kidney weight was not accompanied by induction of any measurable rise in ornithine decarboxylase activity which otherwise is often seen in tissue hyperplasia.

*Summary.* In adrenalectomized mice, cortisone inhibited histidine decarboxylase of the kidney in a dose-related manner. The effect of cortisone on ornithine decarboxylase was diphasic: small doses elevated, high doses inhibited.

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## Effects of Oophorectomy and Sexual Hormones on Norepinephrine and Epinephrine Urinary Excretion

In previous papers[1,2] the existence of cyclic variations in the norepinephrine (NE) and epinephrine (E) urinary excretion during the sexual cycle of the female Wistar rat was proved. We attribute these variations to the changes in the secretion of the sexual hormones.

In order to verify this hypothesis, we will now proceed to the study of the effects of oophorectomy and the administration of sexual steroids: estradiol benzoate (EB), progesterone (P) and testosterone dipropionate (TD) on the catecholamines urinary excretion.

*Material and methods.* Female Wistar rats weighing 170–220 g were divided into the following groups: a) control rats in diestrus; b) oophorectomized; c) oophorectomized rats injected with EB, 4 µg daily; d) oophorectomized rats injected with P, 500 µg daily and e) oophorectomized animals injected with TD, 500 µg daily. After the surgical operations, steroid hormones were injected s.c. for 7 days and then, the experiments were performed. The animals were placed in metabolic cages and fed with a mixture of bread, milk and water ad libitum, for 12 h of light-darkness cycle. Samples of 24 h urine were collected on HCl 6 *N*. Urinary catecholamines were extracted by the chromatographic method of VON EULER and LISHAJKO[3] and evaluated by the fluorometric technique of COHEN and GOLDENBERG[4]. The results are given in µg/kg/24 h ± SEM and were analyzed statistically using Student's *t*-test. The urinary NE/E relationship was also studied in the different groups.

*Results.* Table I shows that the oophorectomy did not change the NE elimination as compared with the control animals, but it did increase E excretion ($p < 0.001$), with the consequent fall of the NE/E relationship. The

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