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Alterations of histamine metabolism after injections of sex hormones in mice

S. S. G. HENNINGSSON AND ELSA ROSENGREN

Institute of Physiology, University of Lund, Sweden

Summary

1. Urinary excretion of histamine in female mice was determined after the injection of oestradiol, progesterone or testosterone. Histamine excretion was increased by oestradiol but was not affected by progesterone. Testosterone administration, by contrast, effected a striking reduction of histamine excretion.

2. After injection of oestradiol, the kidney histamine forming capacity was greatly elevated, but in the other tissues investigated no significant change occurred.

3. Testosterone administered *in vivo* but not *in vitro* reduced the histidine decarboxylase activity of female mouse kidney to a small fraction of normal.

4. On thin-layer chromatography, after extraction and coupling of the amines to 2,4-dinitrofluorobenzene, the amount of histamine and to a lesser extent, methyl histamine in urine was reduced after testosterone administration. On the chromatogram of urine from untreated mice, an unidentified yellow spot appeared and the quantity of this spot was increased during testosterone treatment.

Introduction

The metabolism of histamine is changed by sex hormones as well as by the secretion of various endocrine glands. The female rat excretes larger amounts of free histamine than the male (Leitch, Debley & Haley, 1956; Gustafsson, Kahlson & Rosengren, 1957). This sex difference is ascribed to a more efficient inactivation of histamine in the male in which methylation is the principal catabolic pathway (Westling, 1958). Testosterone increases the proportion of methylhistamine in the urine of castrated male and female rats (Westling & Wetterqvist, 1962). In mice also, a sex difference in histamine metabolism has been disclosed in that the kidney of the female, even in non-pregnancy, is endowed with a much higher histamine forming capacity than that of the male (Rosengren & Steinhardt, 1961).

In the pregnant mouse, a striking elevation of kidney histidine decarboxylase activity prevails (Rosengren, 1963). It thus appeared important to investigate whether exposure of the non-pregnant mouse to oestrogens and progestins would produce an increase in urinary histamine and histamine formation of the kidney on the scale noted in pregnancy. The pertinent effects of testosterone were also studied.

Methods

Most of the experiments were done on female mice of the NMRI strain (Naval Medical Research Institute, Bethesda, USA). In some experiments female mice

of an inbred strain were used. They were fed a standard pellet diet and water *ad libitum* except for the animals in which urine was collected, which were given 4 g daily of a partly synthetic diet containing less than 0·02 μg histamine/g. For urine collection the animals were kept in metabolism cages. The 24 h samples were collected in vessels containing hydrochloric acid. The content of free histamine was assayed on the guinea-pig ileum as described by Gustafsson *et al.* (1957).

Ovariectomy was carried out under ether anaesthesia. The steroid hormones used were oestradiol monobenzoate, oestradiol-17β, progesterone and testosterone (AB, Leo, Helsingborg); they were suspended in arachis oil and administered subcutaneously. Controls were injected with arachis oil only.

Determination of histamine forming capacity

The rate of histamine formation, that is the histidine decarboxylase activity, was determined by procedures originally devised by Schayer, and adapted for use in our laboratory (Kahlson, Rosengren & Thunberg, 1963). The method involved the following procedures: minced tissue samples were incubated for 3 h at 37° C under nitrogen in beakers containing 100–200 mg of tissue, 40 μg of 2-ring-[^{14}C] L-histidine (base), 10^{-4}M aminoguanidine sulphate, 10^{-1}M sodium phosphate buffer of pH 7·4 and 0·2% (w/v) glucose, the total finally made up to a volume of 3·2 ml. On completing the incubation, carrier histamine and perchloric acid were added. After filtration, radioactive histidine was separated from radioactive histamine on an ion exchange resin (Dowex 50 W-X4, 100–200 mesh) and after converting the histamine to pipsyl histamine the radioactivity of the histamine formed was determined at infinite thickness in a flow counter. The pipsyl samples were repeatedly recrystallized from acetone until they displayed constant radioactivity. With the [^{14}C] histidine and the measuring equipment used, 1 μg [^{14}C] histamine formed per g tissue in 3 h corresponded to 5,000 c.p.m. Activity is expressed in μg [^{14}C] histamine formed per g tissue in 3 hours. The rate of histamine formation determined by this method is referred to as histamine forming capacity.

Determination of histamine and methylhistamine by reaction with 2,4-dinitrofluorobenzene (DNFB)

Besides the bioassay, the amount of histamine and methylhistamine [1-methyl-4 (β-aminoethyl)-imidazole] in the urine was simultaneously determined by reaction with 2,4-dinitrofluorobenzene (DNFB). This assay was carried out as described by White (1966) and the procedure involved the following steps: (a) purification of urinary histamine and methylhistamine on a column of Dowex 50 W-X4 and subsequent elution of the histamine and methylhistamine absorbed, (b) coupling of the amines to DNFB, (c) development of thin layer chromatograms of the DNFB compounds, (d) spectrophotometric quantitation of the products on the basis of their light absorption. In addition to DNFB-histamine and DNFB-methylhistamine, a yellow DNFB-product appeared on the chromatograms the chemical nature of which has as yet not been established. The amount of this unknown compound has provisionally been assessed in μg/24 h under the assumption that its DNFB products absorb the same amount of light per unit weight as DNFB-histamine and DNFB-methylhistamine.

Results

Effect of gonadal hormones on urinary excretion of free histamine

Urinary histamine was determined before, during and after injections of oestrogen, progesterone or testosterone. Before treatment with hormones the excretion of free histamine was low, mostly 1–2 μg/24 hours. In a few animals the excretion was as large as 20 μg/24 hours. The daily fluctuations in one and the same mouse were not particularly great.

Oestrogens

Mice were ovariectomized at the age of 10 weeks and urine collection began 5 weeks later. This was done by placing the mice individually in metabolism cages every second day and urine was collected in 24 h periods. Three mice were given 10 μg oestradiol monobenzoate daily for 10 days and another three received 100 μg oestradiol monobenzoate daily for 2 days. A progressive increase in histamine excretion occurred in both groups with a maximum 2–3 weeks after the first injection (Fig. 1). In the group injected with the larger dose a peak of 10 times the pretreatment level was attained. Injection for 10 days with the smaller dose produced a peak 50 times the control level. The high histamine excretion after

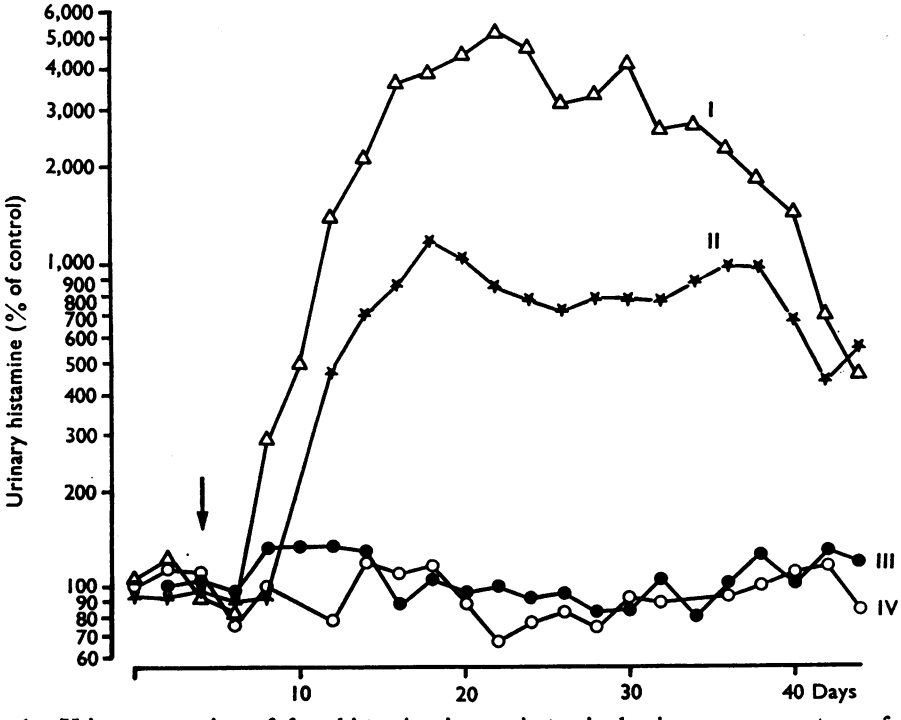

FIG. 1. Urinary excretion of free histamine in ovariectomized mice as a percentage of the value obtained on the first day of the observation period. Group I (mean of values from three mice) were injected with 10 μg oestradiol daily for 10 days, group II (three mice) with 100 μg oestradiol for 2 days, group III (two mice) with arachis oil, group IV (three mice) with 1 mg progesterone daily for 10 days. Injections started at the arrow. Note logarithmic scale of the ordinate.

oestrogen injections persisted during the whole period of observation, that is for up to 4 weeks after the last injection.

Even in normal (not ovariectomized) female mice, oestrogen injections increased the histamine excretion, as shown in Fig. 2. Group I and group II of this figure had the same treatment, 10 μg oestradiol monobenzoate daily for 10 days. Group I was of an inbred Institute strain, age 7 months at the beginning of the experiments, group II was of the NMRI strain, age 3 months. Oestrogen increased the histamine excretion in both groups, more so in the first group. It was not investigated whether the observed quantitative difference in the effect of oestrogen is due to strain, age or some other factor(s).

The dose of oestrogens required to cause an increase in histamine excretion is rather large. No significant change was seen with 2 μg oestradiol-17β injected for 2 days and the urine thereafter examined for a fortnight. With 5 μg oestradiol administered daily for 10 days in six mice a gradual increase in urinary free

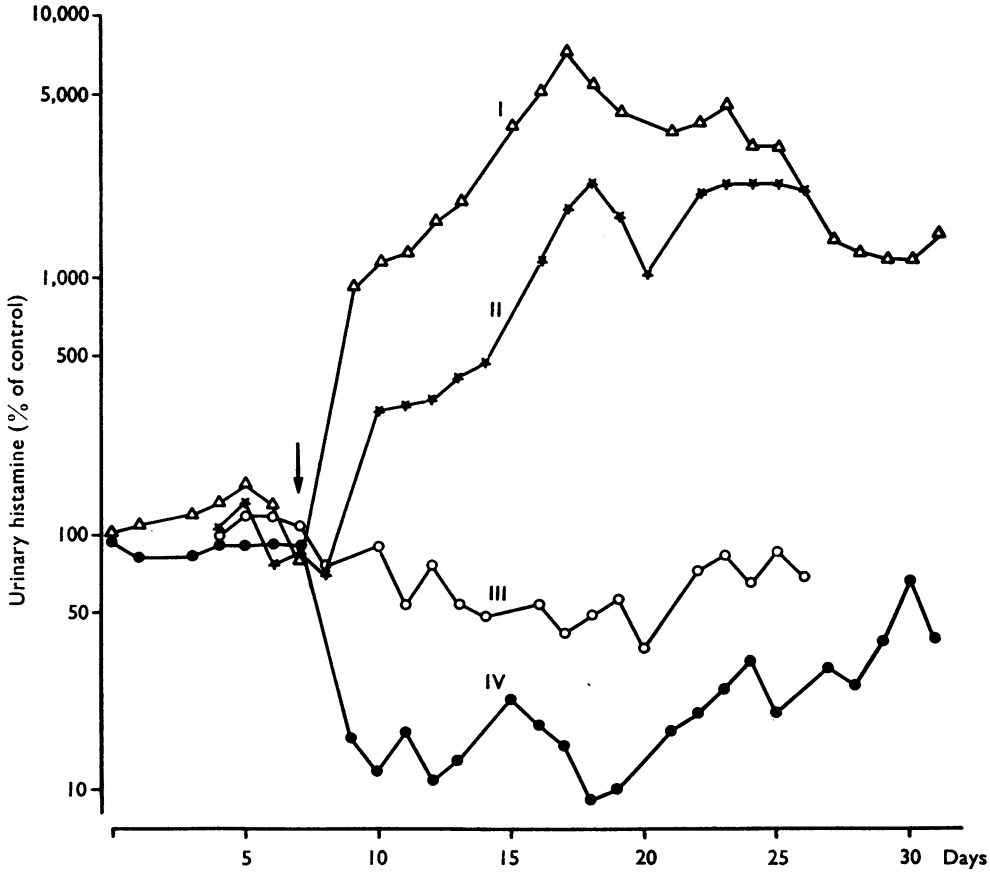

FIG. 2. Urinary excretion of free histamine in intact female mice as a percentage of the value obtained on the first day of the observation period. Group I (two mice) and group II (three mice) were injected with 10 μg oestradiol daily for 10 days, group III (three mice) and IV (two mice) with 0·5 mg testosterone for 10 days. Injections started at the arrow. Groups I and IV were of the inbred Institute strain, groups II and III of the NMRI strain. Note logarithmic scale of the ordinate.

histamine was observed with a maximum 2–3 weeks after the onset of treatment. In two of the mice the increase was 10-fold.

Progestins

Urinary excretion of free histamine was followed before, during and after injections of progesterone, 1 mg daily for 10 days. Results from three ovariectomized mice are given in Fig. 1 which shows that progesterone did not detectably affect the urinary histamine.

Testosterone

The urinary excretion of free histamine in response to testosterone was followed in five intact female mice. Two of these, group IV in Fig. 2, were of the inbred Institute strain, age 7 months. The other three, group III in Fig. 2, were of the NMRI strain, age 3 months. The two groups received injections of 0·5 mg testosterone daily for 10 days. A striking reduction of urinary histamine excretion occurred in both groups. As with oestrogens the effect was strongest in mice of the Institute strain in which the urinary histamine fell to 10% of the values obtained in the control period before injections.

Histidine decarboxylase activity in the kidney and other tissues after hormone injections

Oestrogens

In order to identify the site(s) of increased histamine formation resulting from oestradiol treatment, ovariectomized mice received oestradiol monobenzoate, 100 μg daily, on 2 successive days. Various tissues were excised 3 weeks after the first injection, that is about the day when the histamine forming capacity as reflected in histamine excretion was known to be at peak level. The results are summarized in Table 1. A conspicuous elevation of kidney histamine forming capacity was noted, but in the other tissues investigated no change on the scale seen in the kidney was found.

Testosterone

Only the kidney was examined for histamine forming capacity after testosterone administration. Ovariectomized mice were injected with 0·5 mg testosterone daily

TABLE 1. *Histamine forming capacity (ng/g) of various tissues of adult ovariectomized mice*

	Oestradiol			Controls		
	Mean	Range	No. animals	Mean	Range	No. animals
Skin	70	30–100	4	50	0–100	4
Lung	150	110–190	3	80	30–130	3
Liver	20	20–30	4	10	5–15	4
Spleen	320	180–530	3	150	100–190	3
Stomach	650	360–830	3	530	160–850	3
Intestine	0	±5	3	0	±5	3
Kidney	14,000	5,500–28,000	6	810	30–1,900	5
Uterus	30	0–60	4	110	0–260	4

Oestradiol; injection of 100 μg oestradiol monobenzoate daily on two successive days. Controls; arachis oil only. Various tissues were excised 3 weeks after injections.

for 10 days. Controls received arachis oil only. It should be mentioned that mice treated with 0·5 mg testosterone daily for 10 days showed an increase in kidney weight of about 35%. The kidneys were removed one day after the last injection and their histamine forming capacity was determined. Less than 3% of the kidney histamine forming capacity persisted after testosterone administration as shown in Table 2. The addition of testosterone to minced kidney *in vitro* had no effect on histamine forming capacity.

Urinary histamine excretion was reduced within a day or two of the testosterone injections. We established the time course of disappearance of histidine decarboxylase in mouse kidney after testosterone administration. The mean value of kidney histamine forming capacity of eleven controls is given as 100% in Fig. 3. Ten mice were injected with 0·5 mg testosterone and killed 12 h later. The kidney histamine forming capacity of this group was reduced to half of normal. Another twelve mice, divided into four groups, were given 0·5 mg testosterone twice at an interval of 24 h and killed 48 h, 72 h, 6 days or 10 days after the first injection. A maximum decrease in kidney histamine forming capacity was observed at 48 h when

TABLE 2. *Histamine formation (ng/g) of mouse kidney after subcutaneous injections of* 0·5 *mg testosterone daily for* 10 *days*

	Control	Testosterone
	110	12
	300	4
	470	2
	1,100	16
	500	16
	520	25
Mean±S.D.	500±332·6	12·5±8·54

The mice were ovariectomized 5 weeks before injections. The means ± S.D. are also given.

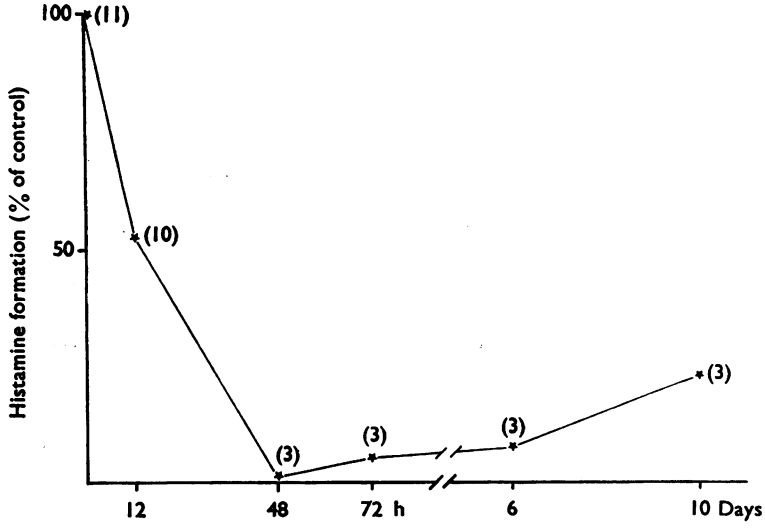

FIG. 3. Inhibition of histamine formation in mouse kidney after testosterone administration. The mice were given 0·5 mg testosterone twice, at an interval of 24 h, and killed at different times after the first injection as indicated. The group killed at 12 h was given one injection only. Controls were given arachis oil. Figures in parentheses are the number of observations.

histamine formation was lowered to 1·4% of the value of the controls. The kidney histamine forming capacity was restored very slowly; 9 days after the last injection the enzyme activity was only 22% of normal.

Excretion of histamine and methylhistamine determined by reaction with DNFB

A reduction of urinary free histamine can result from decreased formation and mobilization of histamine or an increase in the rate of histamine catabolism or a

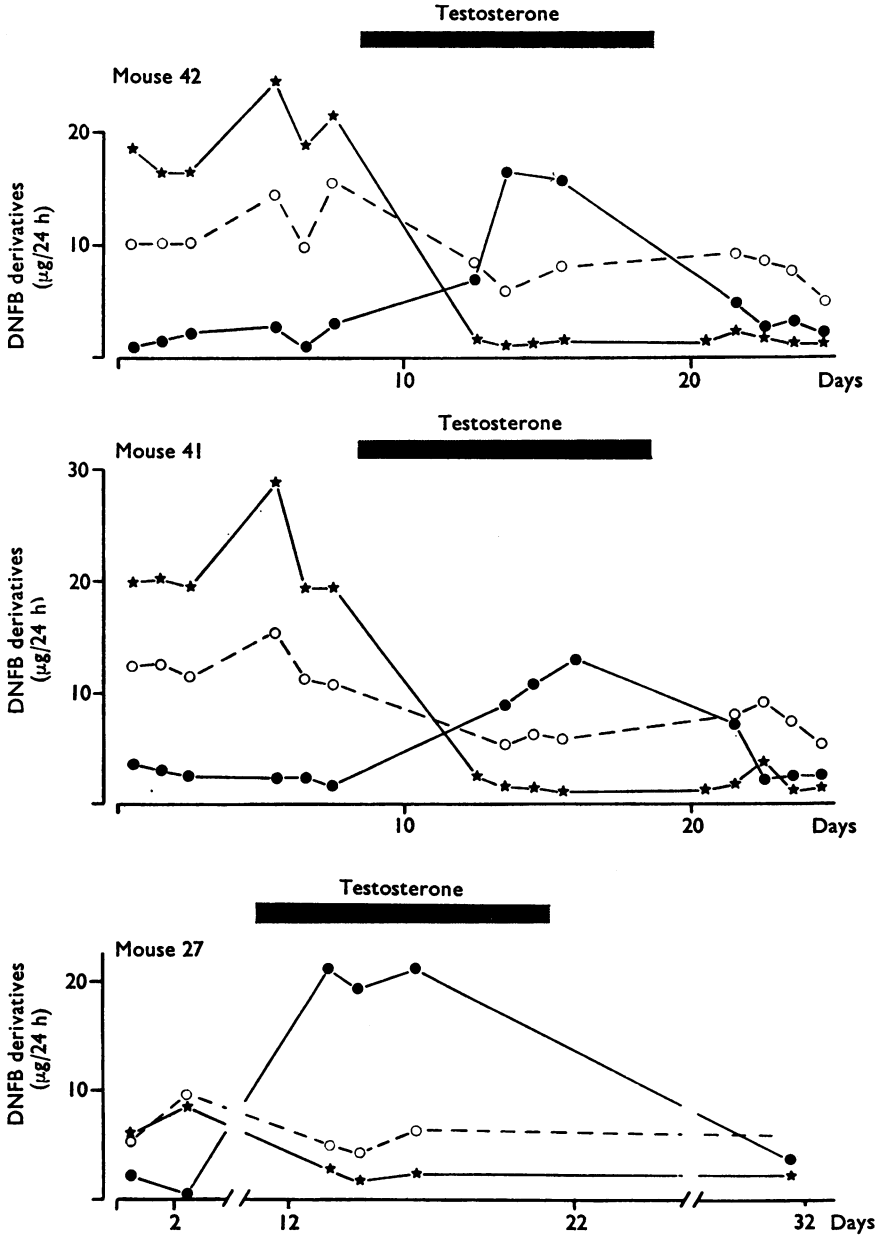

FIG. 4. Urinary excretion (μg/24 h) of histamine (*——*), methylhistamine (○– – –○), and an unidentified compound (●——●) determined by reaction with DNFB. Results from three mice, before, during and after testosterone administration (0·5 mg daily for 10 days).

combination of these changes. Methylation of endogenous histamine is a principal catabolic pathway for the amine in the mouse. If the reduced histamine excretion during testosterone administration were due to increased histamine catabolism, the amount of methylhistamine excreted should increase. Consequently, the amount of endogenous histamine and methylhistamine in the urine of mice treated with testosterone was determined by a method based on coupling of the amines to DNFB (White, 1966). With free histamine, a good agreement was found between the values obtained on bioassay and those obtained according to White. After injection of testosterone, excretion of histamine is strongly reduced and that of methylhistamine slightly reduced as can be seen from Fig. 4. Accordingly, an increased rate of conversion of histamine to methylhistamine does not account for the lowered excretion of free histamine during administration of androgenic hormones.

In addition to the yellow spots of DNFB-histamine and DNFB-methylhistamine, a third stable yellow compound was found in the thin-layer chromatograms. This spot appeared on the chromatograms of urine even in untreated mice; however, this spot increased in intensity during testosterone administration (Fig. 4). The amount of this unknown compound has been calculated on the assumption that the light absorption of its DNFB-derivatives agrees with those for DNFB-histamine and DNFB-methylhistamine. Preliminary results indicate that the compound is not a histamine metabolite. This assumption would agree with the observation that the effect of testosterone on urinary histamine persisted for weeks after discontinuing testosterone administration, while the amount of the unidentified compound fell as soon as testosterone injections ceased.

Discussion

The histamine forming capacity of tissues (that is the histidine decarboxylase activity), has been shown to be increased in pregnancy, first in the rat (Kahlson, Rosengren & Westling, 1958), then in the mouse and hamster (Rosengren, 1962, 1963, 1965). A strikingly high histamine forming capacity is a characteristic feature of some foetal tissues. Tissues other than embryonic have been recognized as target organs responsive to some unknown factor(s) conducive to elevation of histamine forming capacity; the pregnant mouse kidney and hamster placenta have been investigated in this respect.

In the target organs mentioned, the state of pregnancy constitutes a prerequisite for high histamine forming capacity. It would thus appear that gestational hormones are involved in this particular process. The kidney of the non-pregnant or ovariectomized mouse lends itself well to the study of the relationship between hormones and histidine decarboxylase activity, because the mouse kidney in pregnancy has a consistently high histamine forming capacity. Oestrogen treatment increased histamine forming capacity in the kidney of intact non-pregnant as well as in ovariectomized mice. It thus appears reasonable to assume that the sex difference in kidney histamine forming capacity prevailing even in non-pregnancy is at least in part due to the secretion of small quantities of oestrogen.

Oestrogen treatment did not significantly alter the histamine forming capacity in any of the tissues investigated other than the kidney. This could be interpreted to mean that for a stimulus to be effective in generating histidine decarboxylase, the site of action must meet some unknown criteria, perhaps at the nuclear level, as

suggested by Kochakian (1965) for the stimulation of protein anabolism by androgens. The complexity of the mode of action of oestrogen in elevating kidney histamine forming capacity is also reflected in the fact that the elevation persisted for weeks after discontinuing oestrogen treatment. In the gastric mucosa, by contrast, the elevation of histamine forming capacity induced by feeding or gastrin injections fades away within a few hours (Kahlson, Rosengren, Svahn & Thunberg, 1964).

The urinary excretion of free histamine is very greatly increased on treatment with oestrogen and is accounted for by an increase in endogenous histamine formation. In the mouse a small part of endogenous histamine is excreted in the free form, the major portion is methylated. Further, in the pregnant mouse kidney, histidine decarboxylase resides mainly in the cortex (Henningsson & Rosengren, 1971), which represents only a portion of the total kidney mass. On the assumption that the distribution of histamine forming capacity after oestrogen treatment is the same as in pregnancy, the histidine decarboxylase activity at the specific site of action must be of a very high order.

In discussing the changes produced by testosterone treatment, it should be mentioned that it caused an increase in the weight of the kidney, a hypertrophy first described by Selye (1939) in the mouse, later confirmed by various workers. It is known that some rapidly growing tissues generate histidine decarboxylase activity at high rates. Obviously, the inhibition of kidney histamine forming capacity following testosterone administration cannot be correlated with growth (for full references to observations and views pertinent to the present report, see Kahlson & Rosengren, 1971).

The inhibition of kidney histidine decarboxylase obtainable *in vivo* by the injection of testosterone, is unequalled in its order of magnitude. In fact, the enzyme activity nearly disappears. A chemical compound, reserpine, has been reported to produce about 90% inhibition of the enzyme in the rat lung (Rosengren & Svensson, 1969). The mode of action of testosterone in inhibiting histidine decarboxylase is different from that of other *in vivo* inhibitors so far investigated as it failed to inhibit the enzyme *in vitro*. Furthermore, strong inhibition persisted *in vivo* for many days after discontinuing the testosterone treatment. At present there is no precise clue to an understanding of this unique effect of testosterone.

Enzymic changes related to protein synthesis have been extensively studied by Kochakian (1965) who presents an ingenious hypothesis for the mechanism of action of androgens in the kidney. Briefly, " androgens very likely act at an, as yet, unknown site at the nuclear level to stimulate the production of s-RNA, m-RNA and specially ms-RNA and possibly other factors with a resulting increase in protein biosynthesis ". The specific activity of some of the enzymes involved, for example alkaline phosphatase and arginase, increased on administration of physiological doses of androgen. Noteworthy in connexion with the present finding regarding testosterone and histidine decarboxylase is the observation of Kochakian that the addition of testosterone *in vitro* never produced a change in activity of even the most responsive enzymes. The discrepancy between changes produced *in vivo* and *in vitro* suggested to Kochakian that the primary action of the androgens was not mediated through any of the enzymes studied and that the androgens acted at a more basic level. The present report would indeed suggest that the interaction between testosterone and histidine decarboxylase takes place at a very basic level.

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