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EFFECT OF SEX HORMONES ON THE EXCRETION OF FREE HISTAMINE BY MALE AND FEMALE RATS

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The excretion of free histamine by male rats was increased 10-fold by treatment with oestrogen or castration. That of female rats was reduced to 1/10 by treatment with testosterone. Ovariectomy further increased the histamine excretion by female rats, which was slightly reduced by treatment with oestrogen, and at oestrus. The low level of free histamine excretion by male rats was not increased by compound SKF 525-A, and inhibition of diamine oxidase by aminoguanidine caused the same proportionate increase in both sexes. It is concluded that the conjugation of histamine by male rats requires the presence of androgens, and some preliminary indication is given regarding the possible mechanisms involved.

Leitch, Debley & Haley (1956) and Gustafsson, Kahlson & Rosengren (1957) reported that male rats excrete about 1/10 as much free histamine in the urine as females. Westling (1958) confirmed these observations and, studying the origin of this difference using [^{14}C]histamine, concluded that the male rats had a larger capacity to methylate histamine. The object of the experiments described in the present paper was to determine in what way, if any, this phenomenon was associated with the sex hormones.

METHODS

Urine collection. Rats of the Wistar strain, of both sexes, were used. They were placed in metabolism cages for the collection of serial 24-hr urine specimens. The cages, of galvanized wire mesh, were circular and of 25 cm diameter. Each cage was supported over a large polythene funnel, at the neck of which the conventional pear-shaped glass globe was suspended to separate urine from faeces. In the earlier experiments, urine was collected into bottles containing 2 g solid trichloracetic acid, but subsequently 0.1 to 0.5 ml. concentrated hydrochloric acid was used, according to the number of rats in the cage. The acidified urine samples were stored in the refrigerator at 4° C.

Extraction and assay of histamine. The urine specimens preserved with trichloracetic acid were shaken with three portions each of 4 vol. ether to remove the trichloracetic acid. In later experiments, this time-consuming procedure was avoided by using hydrochloric acid as preservative, when all that was necessary was to neutralize with a few drops of 20% sodium hydroxide. Where the final dilution for assay was several 100-fold, the acidity was adequately diluted and buffered out by the Tyrode solution, without previous neutralization. The amount of free histamine in the urine was determined by 4-point assay on isolated guinea-pig ileum suspended in Tyrode solution containing atropine sulphate, 10^{-7} g/ml., in an automatic apparatus.

In a few specimens, hydrolysable histamine was determined by refluxing a 5 ml. aliquot of the urine with 1.25 ml. concentrated hydrochloric acid under an air condenser for 90 min.

thereafter proceeding according to Code's modification (1937) of the method of Barsoum & Gaddum (1935) for blood histamine.

Drugs. Stilboestrol, B.P. (B.D.H.) (solution of 1 mg/ml. in oil); injection of oestradiol monobenzoate, B.P. (B.D.H.) (2 mg/ml. in oil); injection of testosterone propionate, B.P. (B.D.H.) (5 mg/ml. in oil); SKF 525-A, research sample of β-diethylaminoethyl diphenylpropylacetate, supplied by Smith, Kline & French Laboratories; aminoguanidine bicarbonate (May & Baker).

RESULTS

Effect of oestrogen and castration on histamine excretion of male rats

Two groups each of 6 male rats, of 100 g wt., were housed in two metabolism cages, and serial 24-hr urine specimens were collected. After a 6-day control period, group A received stilboestrol, 50 μg/rat/day subcutaneously for 12 days; group B, arachis oil, 0.05 ml./rat/day subcutaneously. After 6 days' latent period, the histamine excretion of the oestrogen treated group rose steeply from under 5 μg/rat/day to a peak of 44 μg, which is in the range of excretion for female rats. When the stilboestrol was stopped on the 18th day, the histamine excretion fell again, reaching the original level about the 38th day. The histamine excretion of the control group receiving arachis oil remained at the low male level (Fig. 1).

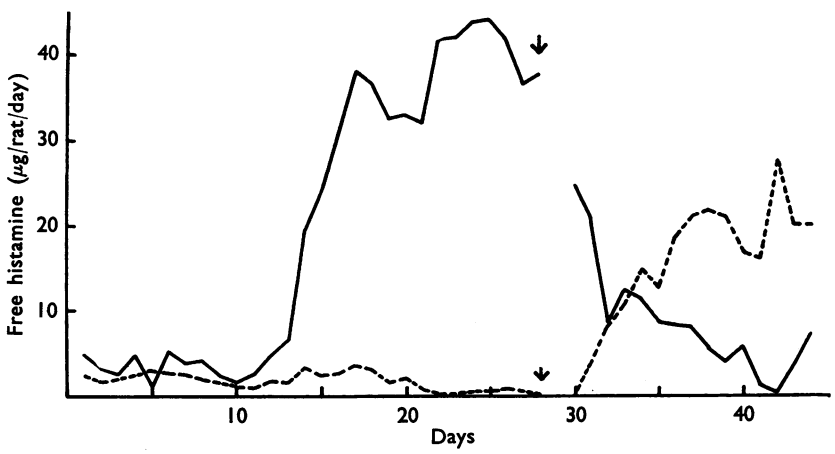

Fig. 1. Effect of stilboestrol and castration on the histamine excretion of male rats. Group A (continuous line): 6 rats treated with stilboestrol, 50 μg/rat/day subcutaneously, 7th to 19th day; "sham" operated 28th day (↓). Group B (broken line): 6 rats treated with arachis oil, 0.05 ml./rat/day subcutaneously, 7th to 19th day; castrated 28th day (↓).

On the 28th day, the rats in group B were castrated, under ether anaesthesia, by the method described by Burn (1950a). Those in group A were "sham" operated, that is, they received the anaesthetic and surgical treatment associated with the castration procedure, but without the testes being removed. Both groups were allowed 24 hr recovery period in ordinary cages on cotton wool before returning to the metabolism cages, and hence missed one 24-hr collection of urine. Immediately after castration, the histamine excretion of group B rose sharply, reaching a peak of 28 μg/rat/day 12 days after operation, and thereafter maintaining the high female histamine output indefinitely (Fig. 1).

Hydrolysable histamine in urine from male rats

In the previous experiment, aliquots from a few urine samples were subjected to hydrolysis, as described under "methods," before assay of histamine. The results are shown in Table 1. It will be seen that, in group A when the free histamine excretion was low, the amount of biologically detectable histamine was

TABLE 1

EFFECT OF HYDROLYSIS ON THE HISTAMINE CONTENT OF URINE FROM MALE RATS (FIG. 1)

Values for histamine expressed as μg/rat/day

		Group A			Group B		
Day		Free	Hydrolysed (total)	% hydrolysed free	Free	Hydrolysed (total)	% hydrolysed free
7		3·9	12·2	314			
8		4·2	16·7	397			
15	Stilboestrol	24·0	25·5	106	2·2	10·5	475
17		38·2	29·5	77	3·4	11·1	328
19		32·4	26·0	80	1·6	5·0	310
25		44·0	46·3	105	0·7	6·5	980
39		4·0	18·0	450	21·0	28·3	135
45		4·0	7·2	180	25·2	25·2	100
56		3·0	9·1	303	32·3	29·2	90
64		4·0	16·7	417	23·5	26·5	113

Castrated

increased by 3 to 5-fold by acid hydrolysis of the urine, whereas during treatment with stilboestrol, when the free histamine level was raised, hydrolysis produced little or no increase in assayable histamine. The same decrease in proportion of hydrolysable histamine was seen in group B when the free histamine excretion rose after castration.

Effect of androgen, oestrogen and ovariectomy on the histamine excretion of female rats

The experiment was carried out in the same time sequence as the previous one, using female rats of 100 g. Group A received testosterone, 0.5 mg/rat/day subcutaneously, the control group B arachis oil, 0.1 ml./rat/day. One day after beginning this treatment, the histamine excretion of the testosterone treated rats fell sharply, reaching the low male level of less than 5 μg/rat/day, subsequently returning to the original level when treatment was withdrawn (Fig. 2).

On the 28th day, the rats in group B were ovariectomized, under ether anaesthesia, by the method described by Burn (1950b), and those in group A were "sham" operated. The histamine excretion of the ovariectomized rats did not fall, as expected, but rose higher, reaching a peak of 76 μg/rat/day (Fig. 2).

Fig. 3 is a continuation of the same experiment, and shows that the histamine excretion of both ovariectomized and "sham" operated rats was reduced by oestrogen, in this experiment, oestradiol benzoate. The ovariectomized rats were, however, more sensitive to oestrogen than the normals, since 50 μg/rat/day produced a larger fall of histamine excretion in the former than 100 μg/rat/day in the latter.

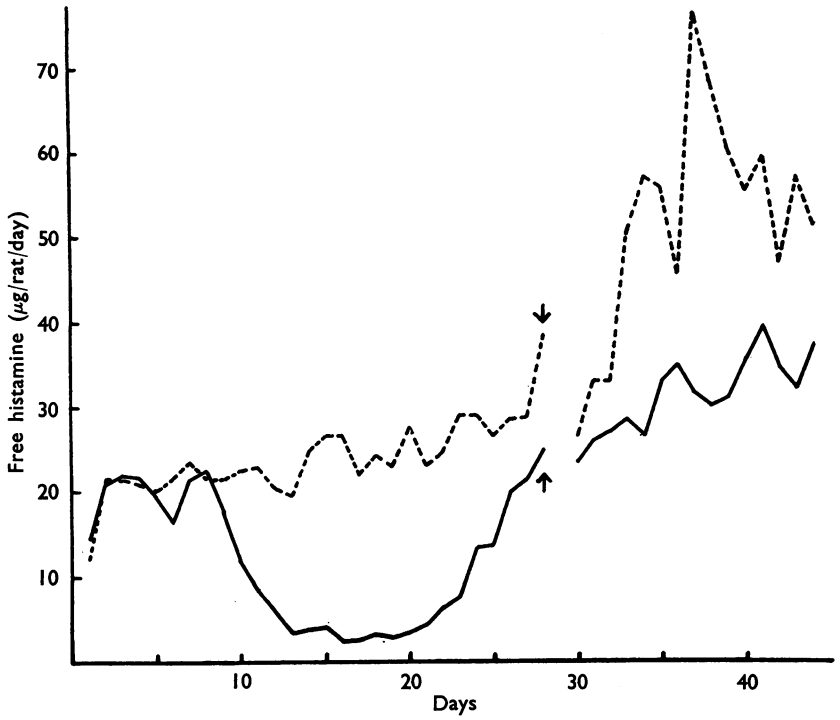

Fig. 2. Effect of testosterone and ovariectomy on the histamine excretion of female rats. Group A (continuous line): 6 rats treated with testosterone, 0.5 mg/rat/day subcutaneously, 7th to 19th day; " sham " operated 28th day (↑). Group B (broken line): 6 rats treated with arachis oil, 0.1 ml./rat/day subcutaneously, 7th to 19th day; ovariectomized 28th day (↓).

From these results, it appears that the ability of male rats to conjugate histamine is dependent upon the presence of androgen.

Histamine excretion and oestrus cycle in female rats

In view of the effects of ovariectomy and of oestrogens on histamine excretion already observed (Figs. 2 and 3), it seemed probable that there might be a fluctuation of histamine excretion in female rats in parallel with the oestrus cycle. To investigate this possibility, it was necessary to collect 24-hr urine specimens from one female rat, and Fig. 4 shows the results of such an experiment. The arrow shows the incidence of oestrus as indicated by cornified epithelial cells in the vaginal smear. Daily urine volumes are also recorded. It will be seen that there are well-defined troughs in the histamine excretion graph coincident with oestrus, which is in agreement with the previously observed depressant action of injected oestrogen. It was also noticed that the volume of urine excreted was depressed at oestrus. This may be related to the mild action of oestrogens in promoting sodium and water retention, first reported by Thorn & Engel (1938). Daily injections of 50 μg stilboestrol for 5 days produced a continuous condition of oestrus and a continuous depression of both urine and histamine excretion. When oestrogen was stopped,

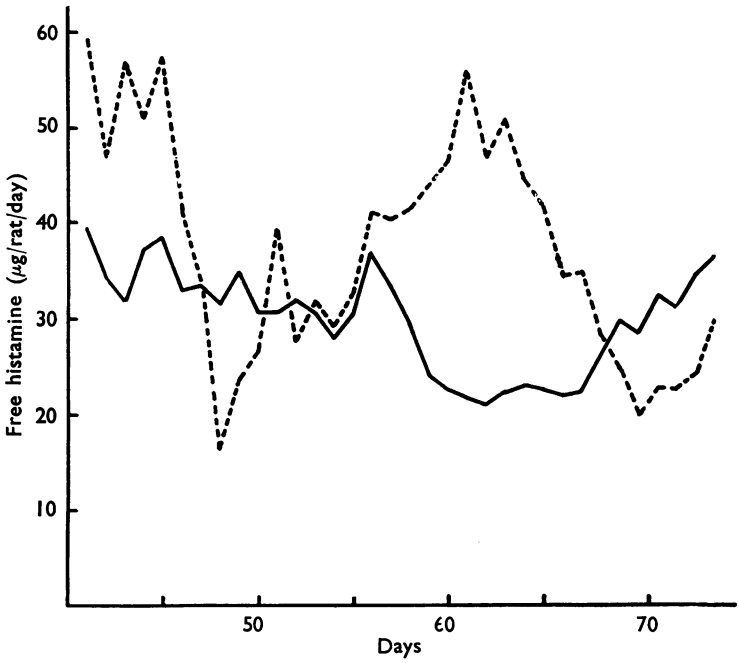

Fig. 3. Continuation of Fig. 2. Effect of oestradiol on the histamine excretion of female rats. Group A (continuous line): 6 "sham" operated rats treated with oestradiol, 100 μg/rat/day subcutaneously, 56th to 66th day. Group B (broken line): 6 ovariectomized rats treated with oestradiol, 50 μg/rat/day subcutaneously, 45th to 54th day.

histamine and urine excretion returned immediately to their original levels and cyclic fluctuations, though the oestrus cycle, as indicated by vaginal smears, was suppressed for 16 days, presumably by the depressant action of the exogenous oestrogen on the pituitary gland.

Development of sex difference in histamine excretion

To determine the age at which the histamine excretion by male and female rats becomes differentiated, 5 male rats and 5 females, litter-mates to the males, were placed in separate metabolism cages immediately after weaning at 20 days old. The histamine excretion of the two groups is shown in Fig. 5. The excretion curves begin to diverge after the 26th day of life. The males reached the characteristic low level by the 35th day, at about the time the testes descended. The females reached the high level of excretion at the 40th day of life, when the vaginae were opening. The first oestrus was recorded by vaginal smear on the 45th day, and 4 of the 5 rats had shown their first oestrus by the 48th day.

Site and mechanism of histamine conjugation in male rats

Conjugation mechanisms are enzymic processes and it should therefore be possible to prevent the conjugation of histamine by male rats by means of an

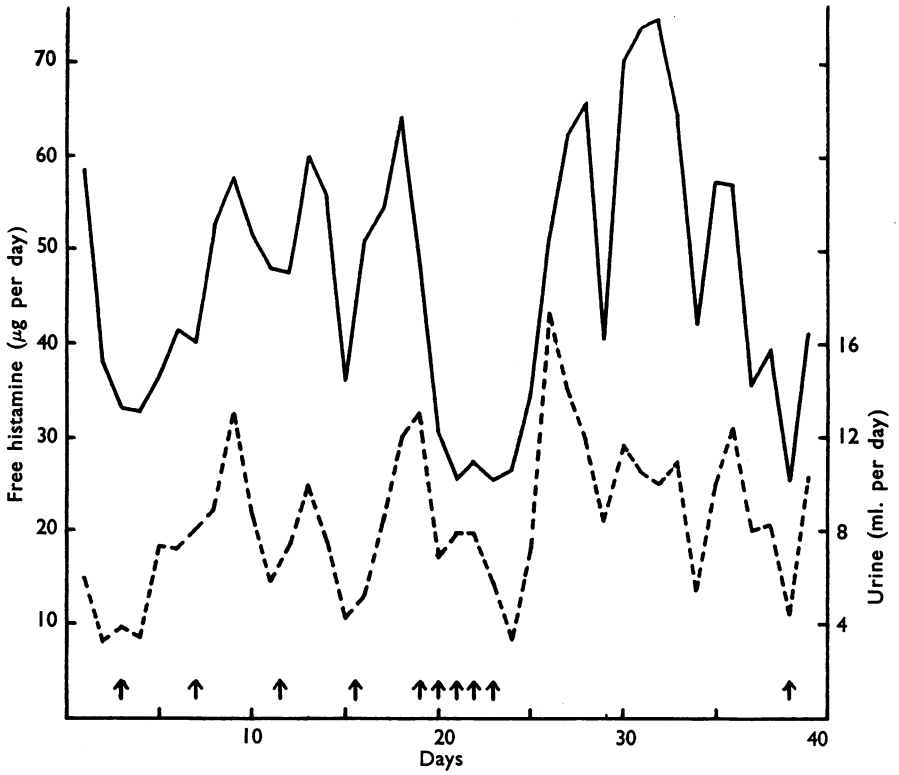

Fig. 4. Relationship between oestrus cycle and excretion of urine and histamine in one rat. Continuous line: histamine excretion; broken line: urine excretion. Arrows indicate incidence of oestrus. Stilboestrol, 50 μg/day from 18th to 23rd day.

appropriate enzyme inhibitor. Compound SKF 525-A is an inhibitor of a variety of detoxicating and conjugating mechanisms in the liver (Cooper, Axelrod & Brodie, 1954). Fig. 6 shows that, when this compound was injected into groups of male and female rats, it had no effect on the histamine excretion of either sex. In the same experiment, aminoguanidine, an inhibitor of diamine oxidase, produced about a 4-fold increase in the free histamine excretion of both males and females. This increase was due only to the blockade of histamine metabolism and not to specific blockade of conjugation by male rats, since the increase was proportionally the same in both sexes.

DISCUSSION

The failure of ovariectomy to produce a lowering of free histamine excretion in female rats indicates that the ability of male rats to conjugate most of their excreted histamine is not due to the absence of oestrogen. On the other hand, when female rats are given testosterone, their free histamine excretion falls to the male level, and when androgen production in males is suppressed, either by oestrogen administration or by castration, free histamine excretion rises to the female level. These observations indicate that the presence of androgen is an essential factor for the

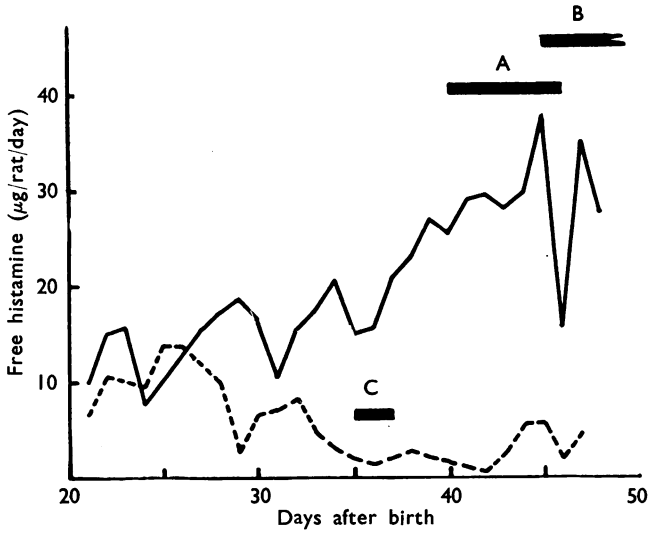

Fig. 5. Development of sex difference in histamine excretion in rats. Histamine excreted by 5 female rats (continuous line) and by 5 male rats (broken line). Bars A and B indicate the time of the opening of the vagina and the first oestrus respectively in the females and C the time of descent of the testes in the males.

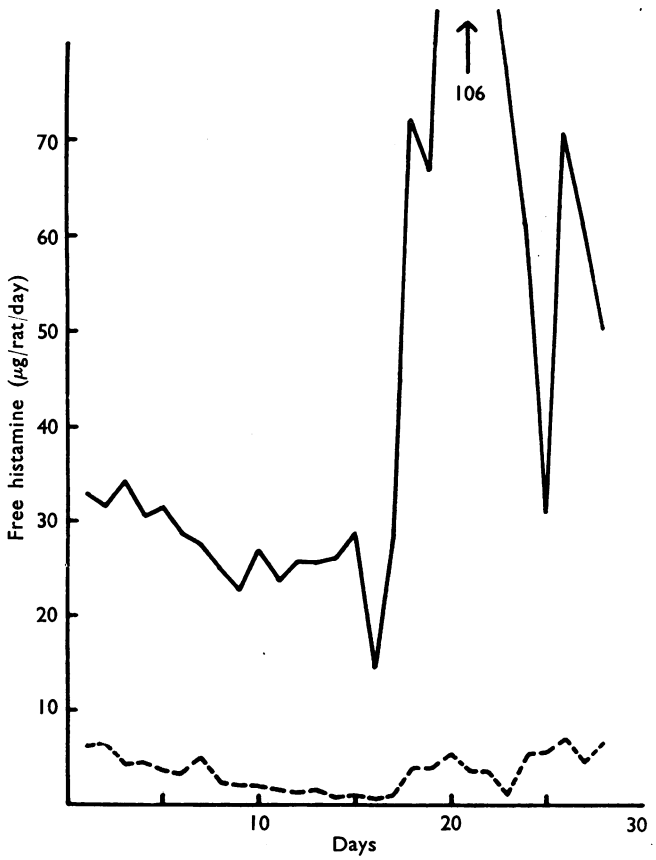

Fig. 6. Effect of compound SKF 525-A and aminoguanidine on the histamine excretion of male (broken line) and female rats (continuous line). All rats received compound SKF 525-A, 1.5 mg/rat/day, 10th to 15th day, and aminoguanidine, 1.0 mg/rat/day, 17th to 22nd day.

conjugation of histamine in rats. Leitch, Debley & Haley (1956) suspected that the difference in histamine excretion was linked with sex, but, in preliminary investigations into this phenomenon, were unable to demonstrate marked changes in histamine excretion after gonadectomy or administration of sex hormones.

The effect of oestrogens is curious, as they themselves produce moderate depression of free histamine excretion in female rats, both when injected and when released endogenously at oestrus. In individual rats this effect is seen to run parallel with a concurrent depression of urine volume at oestrus, and during treatment with oestrogen. It may be therefore that fluctuations of histamine excretion in female rats are simply related to clearance of histamine at varying rates of urine flow.

Association of the sex difference in histamine excretion with the sex hormones is further substantiated by the observations in young growing rats. At weaning the histamine output of both males and females is the same, and the levels only begin to diverge as puberty is approached.

The experiments using acid hydrolysis, though only preliminary, do give some indication of the nature of the conjugation of histamine in male rats. When male rat urine is hydrolysed, about one third of the free histamine excreted by females, or by males after castration or oestrogen treatment, is recovered. Since acetylhistamine is the only known conjugated compound of histamine hydrolysable by Code's method, it would seem that at least one-third of the histamine conjugated by male rats is acetylhistamine. This estimate may well be conservative since as much as 20% of the histamine may be lost during the hydrolysis and subsequent procedure, as indicated by the low " recoveries " of histamine after hydrolysis in some of the urines in which most of the histamine was in the free state. Westling (1958), on the other hand, found only 2 to 4% of acetylhistamine, most of the conjugated histamine being methylhistamine and imidazoleacetic acid. It should be emphasized, though, that the histamine measured in the present work was entirely endogenous, whereas Westling was measuring the excretion of injected histamine and its conjugates.

Further information, though negative, on the nature of the histamine conjugation was provided by the experiments with enzyme inhibitors. Since the effect of aminoguanidine was to increase the free histamine output of both males and females in the same proportion, its action must be entirely by blocking the oxidation of free histamine in both sexes and not by affecting the conjugation of histamine in the males. Compound SKF 525-A has been shown by Cooper, Axelrod & Brodie (1954) to inhibit many detoxication processes including sidechain oxidations, dealkylations, deaminations and ether cleavages, all of which take place in the liver microsomes and require oxygen and reduced triphosphopyridine nucleotide. The observation that compound SKF 525-A has no effect whatever on histamine excretion in either male or female rats eliminates these biochemical mechanisms from participation in the disappearance of free histamine in male rats and possibly also the liver as the site of such activity. Further investigation is therefore necessary to establish the site of histamine conjugation in male rats, to isolate the reaction *in vitro,* and then to study the way in which the mechanisms are influenced by androgens.

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