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# THE URINARY EXCRETION OF SUBCUTANEOUSLY INJECTED HISTAMINE

BY

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Histamine is excreted in the urine in a conjugated form, derived from the alimentary tract, and in a free form, derived mainly from endogenous sources (Anrep, Ayadi, Barsoum, Smith, and Talaat, 1944; Adam, 1950). When histamine is given by intravenous infusion to healthy human beings, about 1% of the dose appears in the urine as free histamine (Adam, 1950; Adam, Card, Riddell, Roberts, and Strong, 1954). These reports suggested that a similar technique might be used to determine how histamine entering the blood stream is dealt with under abnormal conditions. The intravenous infusion of histamine, however, presents practical disadvantages which limit its usefulness as a clinical test. Anrep and his colleagues (1944) showed that the subcutaneous injection of histamine in dogs resulted in the appearance of free histamine in the urine, and the present work was therefore undertaken to gain more information about the excretion of histamine administered to human beings by subcutaneous injection.

## METHODS

Free histamine was extracted from urine by the method of Roberts and Adam (1950). The dried, acidified extracts were taken up in 5 ml. of warm Tyrode solution, neutralized, and assayed on a strip of guinea-pig ileum suspended in a bath of Tyrode solution containing atropine (0.1 $\mu$g./ml.), using a standard solution of histamine acid phosphate for comparison. A series of 10 recovery experiments, in which histamine was added to urine before extraction at the rate of 1 $\mu$g./50 ml. (5 tests) or 5 $\mu$g./50 ml. (5 tests), gave a mean recovery of 63.4% with a standard error of 2.8, which is not significantly different ($P > 0.3$) from the mean recovery of 66.5% $\pm$ 1.8 reported previously (Mitchell and Code, 1954a). The figure of 63.4% has been applied as a correction factor in the present work, but all values for histamine are given uncorrected in terms of histamine base, unless stated otherwise.

Evidence that the activity measured was due to histamine was obtained by frequent tests with mepyramine maleate (Roberts and Adam, 1950), and occasional parallel assays on the cat's blood pressure, when satisfactory agreement was always obtained.

Basiphil and eosinophil leucocytes in capillary blood were enumerated in the same counting chamber according to the method of Moore and James (1953).

The experiments were carried out on healthy men aged between 26 and 34 years. In a preliminary experiment, a meal of 500 g. of beef steak was given to a man who had fasted for 24 hr., and the urinary free histamine was estimated during each period of 2 hr. for 4 hr. before and 14 hr. after the meal.

Before each of the subsequent experiments, the subject fasted for at least 10 hr. At 5.30 a.m., urine was voided and discarded; thereafter urine was collected at the end of each 2-hr. period by voiding directly into a bottle containing 0.5 ml. of 2N-HCl. The experiment was completed at 1.30 p.m., giving four urine specimens for extraction and assay of histamine. The first and second gave values for the basal excretion of free histamine under fasting conditions; during the third collection period, histamine acid phosphate was injected subcutaneously, the total dose being injected into the thighs in four equal parts, at 9.30, 10, 10.30 and 11 a.m. Throughout the 8 hr. of the experiment, the subject continued to fast but drank 120 ml. of water each time he voided urine, to ensure an adequate output of urine.

The eosinophils and basiphils in the blood were counted before, during and after the 2-hr. period during which the histamine was given, at 9.25, 10.30 and 11.35 a.m.

At least four weeks elapsed between successive experiments on any individual. Three experiments were performed on each of four subjects (A, B, C, and D). In the first experiment of each series, a total dose of 5 $\mu$g. of histamine acid phosphate for each kg. of body weight was injected, in the second 10 $\mu$g./kg., and in the third 20 $\mu$g./kg.

One experiment was carried out (on subject A) without any change in the above details except for the substitution of sterile normal saline for histamine in the injections.

In a further experiment, a healthy man was given 200 mg. of cortisone acetate by mouth, and the basiphils and eosinophils in the blood were counted at hourly intervals for 2 hr. before and 11 hr. after administration. Two-hourly specimens of urine were

TABLE I

THE EXCRETION OF FREE HISTAMINE IN THE URINE OF FOUR HEALTHY MEN BEFORE, DURING AND AFTER THE SUBCUTANEOUS INJECTION OF HISTAMINE ACID PHOSPHATE
(Given in four doses over 2 hr.)

| Total Dose (μg./kg.) of Histamine Acid Phosphate Injected During Period III | Subject and Body Weight (kg.) | Free Histamine in Urine in μg. Histamine Base/2-hr. Period | | | | Extra Histamine Excreted in Period III as % of Total Dose Injected |
|---|---|---|---|---|---|---|
| | | I 5.30–7.30 | II 7.30–9.30 | III 9.30–11.30 | IV 11.30–1.30 | |
| 5 | A. 70 | 0·43 | 0·37 | 0·83 | 0·54 | 0·54 |
| | B. 70 | 0·77 | 1·07 | 1·28 | 1·06 | 0·45 |
| | C. 84 | 0·96 | —* | 1·62 | 0·68 | 0·69 |
| | D. 68 | 0·77 | 0·68 | 1·05 | 0·79 | 0·42 |
| | Mean | 0·73 | 0·71 | 1·20 | 0·77 | 0·53 |
| 10 | A. 70 | 0·60 | 0·53 | 2·68 | 0·74 | 1·32 |
| | B. 70 | 0·62 | 0·72 | 2·48 | 1·12 | 1·13 |
| | C. 84 | 1·03 | 0·99 | 3·04 | 1·14 | 1·05 |
| | D. 70 | 0·75 | 0·84 | 2·33 | 0·47 | 0·96 |
| | Mean | 0·75 | 0·77 | 2·63 | 0·87 | 1·12 |
| 20 | A. 70 | 0·67 | 0·39 | 5·06 | 0·88 | 1·41 |
| | B. 70 | 0·78 | 0·63 | 3·94 | 0·90 | 1·01 |
| | C. 84 | 1·10 | 0·95 | 5·04 | 1·72 | 1·17 |
| | D. 75 | 0·43 | 0·56 | 5·04 | 0·61 | 1·32 |
| | Mean | 0·75 | 0·63 | 4·77 | 1·03 | 1·23 |

* Sample lost.
(Note: The weight of subject D increased in the several weeks between each experiment.)

collected for free histamine determination for 2 hr. before and 8 hr. after giving the hormone. The man fasted for 24 hr. before the experiment and throughout its duration. This experiment was repeated on the same subject using 50 mg. of corticotrophin in saline solution given by intramuscular injection, instead of cortisone.

In all the experiments, the extraction of histamine was started within a few hours of completing the final collection of urine.

## RESULTS

In the preliminary experiment the amount of free histamine in the urine started to increase

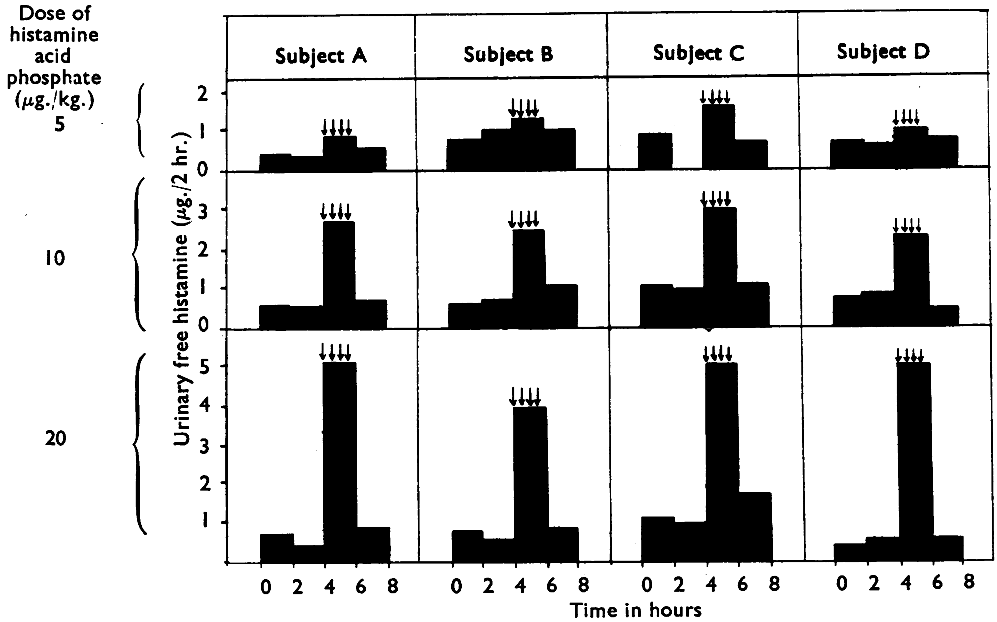


FIG. 1.—The effect of subcutaneously-injected histamine on the urinary excretion of free histamine by four healthy men. Total dose of histamine acid phosphate injected in four doses as indicated by the sets of four arrows.

within two hours of the meat meal, increased rapidly to a maximum of 2.0 $\mu$g. during the next two-hour period and returned to the fasting level of 0.5 $\mu$g./two hours at 10 hours after the meal. This pattern of free histamine excretion after a meat meal has also been found in dogs and in other human subjects (Irvine, 1956).

The changes in urinary free histamine following the subcutaneous injection of histamine are shown in Table I and Fig. 1. During the first and second two-hour periods the amount of free histamine excreted was small, ranging from 0.37 $\mu$g. to 1.10 $\mu$g./two-hour period, the mean value of 0.72 $\mu$g. being slightly less than the value of 1.05 $\mu$g. (range 0.44 to 1.67 $\mu$g.) calculated from the data of Adam and his colleagues (1954). When saline was injected during the third period, the rate of histamine excretion remained approximately constant, the amounts excreted in the four periods being 0.50, 0.40, 0.56, and 0.53 $\mu$g.

In all 12 experiments in which histamine was injected during the third two-hour period, the amount of free histamine in the urine increased above the fasting level during that period, and in every instance it decreased again in the fourth period, usually though not always to the fasting level (Table I). All, or almost all, of the extra free histamine was therefore contained in the third urine specimen, and in the calculations the small amounts occasionally excreted during the fourth period have been disregarded. Hence in each case the mean value for free histamine in the first two specimens was subtracted from the value in the third specimen to obtain the value for the extra histamine excreted. In the calculations, this value is multiplied by $\frac{100}{63.4}$ (the correction factor for the Decalso method) and then converted to the equivalent value for histamine acid phosphate, which is assumed to contain 36.16% of histamine base. The result is expressed as a percentage of the total dose of histamine acid phosphate injected.

The calculations show that when 5 $\mu$g. of histamine acid phosphate/kg. of body weight was injected subcutaneously during a period of two hours, 0.53% of the dose was excreted during the same period. This mean rate of excretion increased to 1.12% when the total dose was doubled to 10 $\mu$g./kg., and to 1.23% when the dose was doubled again to 20 $\mu$g./kg. (Table I).

Table II shows that there was no consistent change in the numbers of circulating basiphils or

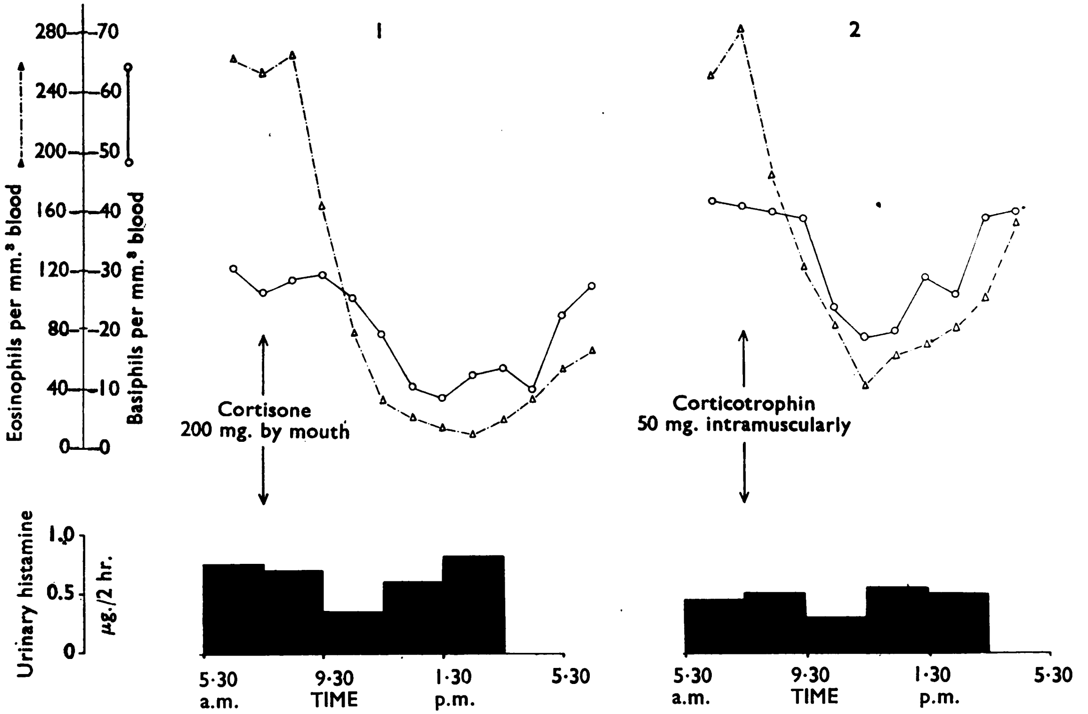


FIG. 2.—The effect of cortisone and of corticotrophin on the numbers of eosinophils and basiphils in the blood, and on the amount of free histamine in the urine of a fasting man (age 34 yr., wt. 75 kg.).

TABLE II

NUMBERS OF BASIPHIL AND EOSINOPHIL LEUCOCYTES IN THE BLOOD OF FOUR HEALTHY MEN BEFORE, DURING AND AFTER THE SUBCUTANEOUS INJECTION OF HISTAMINE ACID PHOSPHATE

(Given in four doses over 2 hr.)

| Dose of Histamine Acid Phosphate (μg./kg.) | Subject | No. of Basiphils per c.mm. of Capillary Blood | | | No. of Eosinophils per c.mm. of Capillary Blood | | |
|---|---|---|---|---|---|---|---|
| | | Before | During | After | Before | During | After |
| 5 | A | 22 | 20 | 19 | 72 | 81 | 69 |
| | B | 56 | 61 | 57 | 313 | 256 | 254 |
| | C | 41 | 40 | 36 | 300 | 344 | 276 |
| | D | 22 | 20 | 22 | 207 | 210 | 162 |
| | Mean | 35 | 35 | 34 | 223 | 223 | 190 |
| 10 | A | 19 | 26 | 14 | 141 | 85 | 62 |
| | B | 44 | 50 | 51 | 197 | 175 | 216 |
| | C | 46 | 38 | 43 | 416 | 448 | 429 |
| | D | 22 | 21 | 25 | 254 | 249 | 287 |
| | Mean | 33 | 34 | 33 | 252 | 239 | 249 |
| 20 | A | 20 | 16 | 17 | 78 | 51 | 72 |
| | B | 52 | 56 | 45 | 175 | 151 | 157 |
| | C | 40 | 46 | 44 | 471 | 535 | 532 |
| | D | 28 | 31 | 27 | 366 | 307 | 283 |
| | Mean | 35 | 37 | 33 | 273 | 261 | 261 |

eosinophils as a result of the injection of histamine and the mean counts remained approximately the same before, during and after the injections. The striking fall and subsequent rise in the numbers of these cells after a single dose of cortisone or corticotrophin are shown in Fig. 2. No increase in the amount of free histamine in the urine occurred during the period of action of either hormone.

When all the values for free histamine were plotted against the urine volume of the corresponding two-hour sample, there was no evidence of a relationship between the amount of free histamine excreted and the urine volume, thus confirming the findings of previous authors (Anrep *et al.*, 1944).

## DISCUSSION

During starvation free histamine is excreted at a relatively constant rate, so that there is little variation in the amounts excreted during consecutive periods of two hours (Mitchell and Code, 1954a). The preliminary experiment was designed to determine the shortest period of fasting necessary to eliminate digestive free histamine from the urine, and the result indicates that a period of ten hours is sufficient. Urine was collected at intervals of two hours because more frequent collection may result in failure to obtain the minimum amount (50 ml.) required for the determination of free histamine. The histamine was injected in divided doses so that the total amount given during the period of two hours could be as large as possible. Little discomfort was felt during the first two experiments of each series, but headache and flushing were experienced when the total dose of histamine acid phosphate was 20 μg./kg.

These experiments confirm Adam's report (1950) that histamine administered parenterally to human beings is excreted in the free form in the urine, and indicate that the amount appearing in the urine is proportional to the dose administered. The results suggest that, as the dose of histamine injected subcutaneously is increased, the percentage excreted in the urine may also increase. However, the extra histamine excreted when 5 μg. of histamine acid phosphate/kg. was injected was so close to the fasting level of excretion that the possibility of error is greater than when 10 or 20 μg./kg. was injected, and the difference between the mean percentages excreted at the latter dosage levels is not significant ($P>0.3$). If the results of all 12 experiments are considered together, the mean percentage of the dose excreted is 0.96, a value similar to that of 1.15% found by Adam and his colleagues (1954) during the intravenous infusion of histamine, in doses comparable with those used in the present work. It seems probable therefore that within these dose ranges about 1% of the injected dose appears in the urine, presumably because these amounts are well below the saturation level of the enzyme systems destroying histamine.

There is some evidence that histamine stimulates the adrenal cortex by the release of corticotrophin (Nasmyth, 1951; Gray and Munson, 1951). The administration of cortisone to human beings over a period of days causes a slight increase in urinary free histamine (Mitchell and Code, 1954b); it might be argued, therefore, that the extra free histamine in the urine was not that originally injected, but was liberated as a result of pituitary-adrenal stimulation. However, cortisone also causes a fall in the numbers of basiphils and eosinophils in the blood (Code, Mitchell, and Kennedy, 1954) and no such fall occurred during the present experiments. Moreover, no increase in urinary free histamine followed the administration of a single dose of either cortisone or corticotrophin, although in each case a fall in the numbers of basiphils and eosinophils occurred (Fig. 2). It does not therefore appear likely that these hormones play any part in histamine excretion following its subcutaneous injection.

Histamine injected into the body is largely destroyed by enzymes, the small amount appearing free in the urine probably representing that fraction which escapes destruction. The close correspondence between the proportions of the dose excreted after subcutaneous and intravenous injections of histamine suggests that all, or nearly all, of the histamine injected subcutaneously reaches the blood stream unchanged, and that little is destroyed at the site of injection.

Histamine in human blood is normally carried in the basiphils and also, though less constantly, in the eosinophils (Code and Mitchell, 1957); very little histamine is present in human plasma (Lowry, Graham, Harris, Priebet, Marks, and Bregman, 1954). If the histamine were conveyed from the site of injection to the kidneys by the circulating basiphils and eosinophils, an increase in the numbers of these cells might have been expected during the experiments; the absence of significant change suggests that the histamine was conveyed in the plasma, possibly in a loosely bound form (Emmelin, 1945). Although Adam (1950) found no rise in the plasma histamine concentration during the intravenous infusion of histamine, he and his colleagues (1954) did find indirect evidence of such an increase by the effect of the infusion on the secretion of gastric juice.

The experimental routine described in this paper has been used as a histamine excretion test in clinical studies of histamine metabolism. When the total dose of histamine acid phosphate used is 10 μg./kg. no discomfort is caused to the patient, and the test is easily carried out under clinical conditions.

## Summary

1. When histamine acid phosphate was injected subcutaneously in healthy men in doses from 5 to 20 μg./kg. of body weight over 2 hr., about 1% of the dose was excreted as free histamine during the same 2-hr. period.

2. The extra free histamine excreted is thought to be that portion of the injected histamine which escaped enzymic destruction. It did not appear as the result of pituitary-adrenal stimulation or the trauma of the injection.

3. The histamine was probably not conveyed from the site of injection to the kidneys by the basiphil or eosinophil leucocytes, since the numbers of these cells in the blood did not alter significantly as a result of the histamine injections.

4. The excretion test described is readily applicable to the investigation of histamine metabolism under clinical conditions.

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