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# THE EFFECT OF INTRAVENOUS INFUSIONS OF HISTAMINE ON THE URINARY HISTAMINE AND ON GASTRIC SECRETION IN MAN

BY

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When histamine is infused intravenously in man at a rate that produces distinct pharmacological effects, it can be easily detected in the urine but not in the venous plasma (Adam, 1950). This author estimated the urinary histamine by a simplified method based on that described by Anrep, Ayadi, Barsoum, Smith, and Talaat (1944). It was possible by this method to follow the excretion when 3.3 mg. of histamine base was infused intravenously, but not the normal or basal excretion of free histamine in the urine. Roberts and Adam (1950) have since shown by a new method that free histamine appears continuously and in measurable amounts in the urine of healthy men, and suggested that the immediate source of this histamine was the blood plasma. The aim of the present work was to obtain further evidence in support of this assumption by following the effect of intravenous infusions of histamine on the urinary excretion of free histamine and on the acid gastric secretion. It was possible in this way to measure the proportion of the drug excreted during the infusion of small doses and to relate the amount excreted to the effect of the infusion on the acid gastric secretion. Since it was not possible to detect free histamine in the plasma by direct methods, the acid gastric secretion was used as a measure of the concentration of free histamine in the circulating blood (Ivy and Javois, 1924 ; Kalk, 1929 ; Ungar, 1935 ; Feldberg and Holmes, 1941 ; Emmelin, Kahlson, and Wicksell, 1941 ; Grob, Lilienthal, and Harvey, 1947).

Incidental observations were made on the histamine content of the gastric juice. The dose-response relationship of histamine to the acid secretion of the stomach was also investigated, but the results will be reported elsewhere.

Three healthy men, designated as W.I.C., J.A.S., and A.A.G., each received a series of histamine infusions, and the free histamine was estimated in samples of the urine, venous blood plasma and gastric juice. The doses infused were in the range that produced graded effects on the acid gastric secretion. Infusions with the lower doses were symptomless, yet they produced a distinct stimulation of the acid secretion and a measurable increase in the excretion of histamine in the urine.

## METHODS

### *Pharmacological Methods*

*Extraction of Free Histamine.*—The method of Roberts and Adam (1950) was used, and their paper should be consulted for the precise details. This method consists in the separation of free histamine from conjugated histamine and other substances in 50 to 100 ml. of urine by absorption on a column of the cationic exchanger Decalso F (The Permutit Co., London). The histamine is then eluted by treating the column with concentrated ammonium hydroxide (A.R.) followed by chloroform saturated with ammonia gas. After evaporation of the eluent, the extract is taken up in 5 to 10 ml. 0.9% NaCl and neutralized. In this way it is possible to concentrate the free histamine to the extent required for the biological assay. The mean recovery of 116 experiments in which histamine was added to various body fluids in the range of 0.5 to 5 $\mu$g. was 67% $\pm$1.1 (S.E. of mean). This fraction is a characteristic of the Decalso column, and a correction can be applied to the results ; the results presented in this paper are uncorrected. The same method, with slight modifications, was used for the extraction of histamine from venous blood plasma and gastric juice. Some of the 25-ml. plasma samples were divided and 10 ml. extracted in parallel by Code's modification (1937) of the method of Barsoum and Gaddum (1935). The Decalso method does not require precipitation of the plasma proteins, but, unlike the urine, the plasma extract must be boiled in strong acid to destroy other gut-contracting substances. The plasma samples were concentrated three- to five-fold by taking up the extracts in 5 ml. 0.9% NaCl. An aliquot of 25 ml. from a pooled sample of gastric juice was filtered through glass wool, and mucus in the filtrate precipitated at *p*H 4 by the addition of an equal volume

of acetone. After removal of the precipitate by centrifuging, the supernatant fluid was brought to *p*H 8, recentrifuged and applied to the column. Like the plasma, the gastric juice extract was boiled in strong HCl to destroy other substances which interfere with the assay. The final extract was taken up in 5 ml. 0.9% NaCl.

*Estimation and Identification of Histamine.*—The histamine values are all calculated in terms of the base, on the assumption that this represents 36.16% of the weight of the phosphate. The extracts were tested on a strip of guinea-pig ileum suspended in 2 ml. Tyrode's solution containing atropine (0.1 μg./ml.) and were compared with a standard solution of histamine acid phosphate (British Drug Houses Ltd.). Mepyramine maleate (May and Baker Ltd.) was used as a test for histamine in some of the extracts (Reuse, 1948).

### *Clinical Methods*

Most of the infusions were given at weekly intervals. A preliminary infusion of 0.9% NaCl preceded each infusion of histamine. The histamine infusion itself lasted about three hours, and during this time the drug entered the circulation at a constant rate. Samples of urine, venous blood, and gastric juice were collected before and during the infusion; urine was also collected after the infusion. In a separate experiment each subject received a control infusion of physiological saline only; this was also maintained for three hours.

*Collection of Samples. Urine.*—This was collected in chemically clean stoppered flasks of 750 ml. capacity which contained 2 to 5 ml. 2N HCl to reduce the *p*H of the urine to less than 4. The samples were stored at 0° C. pending extraction of the histamine two to three hours later.

At a definite time before the preliminary infusion of saline was due to start the subject emptied his bladder and discarded the urine. Immediately before the infusion of histamine was begun he emptied it again to provide the first control sample. At the end of the infusion he again passed urine: this middle sample contained all urine formed during the infusion plus a small amount that was formed after the infusion was stopped. There was always some delay in collecting this sample because it was essential to free the subject from the apparatus. The average delay was 14 min. (range (18) 8–35 min.). A second control sample was collected in the course of the next three hours.

A correction for the basal excretion of free histamine was applied to the values obtained from urine samples formed during the infusion. It was assumed, on reasonable evidence (see Table II), that excretion of the dose in the urine ceased soon after the infusion was ended. This simplified the calculation, which was done in the following way. The sum of the histamine values for the control samples was divided by the total time in minutes required for the collection of these samples. The result gave the mean rate of excretion in μg./min. during the control periods. The product of this rate, and the time in minutes over which the middle sample was collected, provided an estimate of the basal excretion during the infusion. The difference between the amount of free histamine in the sample formed during the infusion and the calculated basal excretion was taken to represent the amount of administered histamine that appeared in the urine; this amount was expressed as a percentage of the dose infused.

*Venous Blood.*—Two samples were withdrawn from each subject at the time he received the largest dose of histamine: the first sample was obtained during the preliminary saline infusion, and the second towards the end of the histamine infusion. A needle (15 S.W.G.) with rubber tubing attached was inserted into the antecubital vein and 50 ml. of blood allowed to run into a glass tube containing 500 units of heparin (Liquemin, Roche). The sample was kept at 2–4° C. for about an hour and then centrifuged at 3,000 r.p.m. for 15 min. The plasma was separated and applied to the column without delay.

*Gastric Juice.*—The subject omitted breakfast, and, about two hours before the infusion, a Ryle's tube was passed into the stomach and its position verified radiologically. He then lay in a semi-recumbent position and the tube was connected to a source of continuous suction. The collection of 10-min. samples was begun 40 to 120 min. before, and continued throughout, the infusion. The free and total acid were determined in each sample by titration with N/10 sodium hydroxide, first to Töpfer's reagent and then to phenolphthalein as indicator. The summed values for the total acid output* only are given in this paper, and are expressed in milli-equivalents of HCl. The samples collected during the control and infusion periods were pooled independently. An aliquot of 25 ml. was taken from each pooled sample for estimation of the free histamine. Estimates were calculated for the basal amounts of histamine and total acid in the gastric juice, and a correction was applied to the results in the manner already described for the urine.

*Intravenous Infusion of Histamine.*—A sterile stock solution was prepared on the day before infusion, from which a dilution with 0.9% NaCl was made for infusion. A 50-ml. glass syringe was filled with this solution and fixed horizontally in an apparatus which drove the piston forward at a constant rate. The mean volume flow from the needle connected to the syringe as for infusion was $32.2 \pm 0.3$ ml. per hour. The syringe did not usually require to be refilled more than once during the infusion, and the delay involved was less than two minutes.

## RESULTS

These have been summarized for each subject and are set out in Table I. The data for the urine are given in Table II. The experiments are numbered in order of magnitude of the dose.

* Total acid output = concn. in m.equiv./l. × volume in litres.

TABLE I

HISTAMINE VALUES IN URINE, GASTRIC JUICE AND BLOOD PLASMA DURING INTRAVENOUS INFUSION OF HISTAMINE ACID PHOSPHATE, IN THREE SUBJECTS

(All the histamine values are expressed as the base.)

| Exp. | Infusion | | | Urine | | | | Gastric Juice | | | | | | Blood Plasma | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Duration min. | Dose (μg.) | Rate ng./kg./min. | Basal (calc.) μg. | Total (μg.) | Difference (μg.) | Difference Expressed as % of Dose | Total Acid Output (m.equiv. HCl) | Basal (calc.) μg. | Basal (calc.) μg./l. | Total μg. | Total μg./l. | Difference (Total/Basal) μg. | Control μg./l. | Infusion μg./l. |
| | | | | | | | *Subject W.I.C. ♂ 82 kg.* | | | | | | | | |
| 1 | 181 | 0 | 0 | 0·9 | 1·1 | 0·2 | | −1·2 | | | | | | | |
| 2 | 191 | 206 | 13·1 | 1·5 | 3·6 | 2·1 | 1·0 | 6·0 | 3·8 | 14 | 4·6 | 20 | 0·8 | | |
| 2a | 165 | 176 | 13·0 | 0·6 | 2·1 | 1·5 | 0·8 | | | | | | | | |
| 3 | 217 | 521 | 29·1 | 1·9 | 7·2 | 5·3 | 1·0 | 18·1 | 12·6 | 20 | 9·3 | 22 | −3·3 | | |
| 4 | 180 | 775 | 52·2 | 1·7 | 11·1 | 9·4 | 1·2 | 33·9 | 2·0 | 12 | 10·6 | 26 | 8·6 | | |
| 5 | 172 | 1,650 | 116·2 | 2·4 | 22·3 | 19·9 | 1·2 | 49·5 | 2·7 | 21 | 16·3 | 31 | 13·6 | <3 | <3 |
| | | | | | | | *Subject J.A.S. ♂ 75 kg.* | | | | | | | | |
| 1 | 170 | 0 | 0 | 1·8 | 1·5 | −0·3 | | 2·3 | 0·04 | 0·8 | 1·4 | 20 | 1·4 | | |
| 2 | 162 | 44 | 3·6 | 1·6 | 2·1 | 0·5 | 1·1 | 4·8 | 0·8 | 14·0 | 0·5 | 6 | −0·3 | | |
| 3 | 175 | 105 | 8·1 | 1·5 | 2·9 | 1·4 | 1·3 | 6·6 | 0·2 | 3·5 | 0·8 | 8 | 0·6 | | |
| 4 | 180 | 216 | 16·0 | 1·9 | 3·7 | 1·8 | 0·8 | 20·7 | 1·6 | 15·5 | 5·0 | 20 | 3·4 | | |
| 5 | 181 | 390 | 29·0 | 2·3 | 6·0 | 3·7 | 0·9 | 55·5 | — | — | — | — | — | | |
| 6 | 175 | 630 | 48·0 | 1·7 | 6·3 | 4·6 | 0·7 | 72·5 | 2·8 | 34·5 | 5·2 | 9 | 2·4 | | |
| 7 | 182 | 980 | 72·0 | 2·5 | 12·1 | 9·6 | 1·0 | 87·2 | 0·7 | 12·5 | 1·6 | 3 | 0·9 | <3 | <3 |
| | | | | | | | *Subject A.A.G. ♂ 73 kg.* | | | | | | | | |
| 1 | 177 | 0 | 0 | 1·1 | 1·2 | 0·1 | | 0·7 | | | | | | | |
| 2 | 183 | 98 | 7·4 | 1·3 | 3·2 | 1·9 | 1·9 | 5·9 | | | | | | | |
| 3 | 185 | 197 | 14·8 | 1·1 | 3·9 | 2·8 | 1·4 | 22·4 | | | | | | | |
| 4 | 185 | 395 | 29·6 | 1·7 | 6·4 | 4·7 | 1·2 | 29·1 | | | | | | | |
| 5 | 182 | 777 | 59·2 | 0·8 | 15·1 | 14·3 | 1·8 | 38·9 | | | | | | <3 | <3 |

In none of the three subjects did the control infusion of physiological saline produce an important rise in the urinary histamine, and the effect on the acid secretion was negligible. The values for the excretion before the histamine infusion show only small differences when compared with those after the infusion (Table II). In five out of seven infusions (Nos. 1, 2, 4, 5 and 7) J.A.S. excreted histamine at a slightly faster rate after the infusion than just before it: but it is improbable that he was still excreting infused histamine, since the effect did not increase with the dose, and also occurred after the infusion of saline alone. In two experiments on W.I.C. (Nos. 2 and 2a) exceptionally high values were obtained in samples collected after the infusion: we are unable to explain these particular results—which may have been due to some fault in technique—and have therefore disregarded them in our calculations. With these exceptions, it seems probable that the excretion of histamine given by infusion did not continue appreciably beyond the period of the infusion.

In many of the experiments the urine sample collected after the infusion was very concentrated. This was also seen in the control infusions with saline and was probably an effect of the prolonged fast and the continuous removal of gastric juice.

The calculated values for the basal excretion during the infusion were remarkably close in the different experiments on the same subject and even between subjects. It was thus possible to detect small increases when histamine was infused at low rates. The result obtained by infusing histamine at a rate of 3.6 ng./kg./min. (Table I, J.A.S., Expt. 2) is doubtful, but infusions at rates of 7.4 and 8.1 ng./kg./min. (Table I, A.A.G., Expt. 2; J.A.S., Expt. 3) about doubled the excretion of free histamine in the urine and also stimulated gastric secretion. In each of the three series of infusions, the excretion of histamine in the urine and the effect on the acid gastric secretion increased with the dose. The relation between the dose of histamine and these effects is also shown graphically (Fig. 1) for one series of infusions. In all these experiments the proportion of histamine excreted was approximately 1% of the dose (range (15) 0.7–1.9%) and this proportion was independent of the infusion rate.

Infusions up to a rate of 30 ng./kg./min. were indistinguishable in their subjective effects from the control saline infusion. Headache occurred neither during nor after these infusions and there was no flush or tachycardia; the only effect, apparently, was the stimulation of gastric secretion.

Even when the infusion rate was high (Table I, W.I.C., Expt. 5; J.A.S., Expt. 7; A.A.G., Expt. 5) histamine was not detected in samples of the venous blood plasma either by the Decalso or by Code's method. Since the Decalso method allowed of a three-fold concentration of any free histamine that might have been present in the plasma, it was possible to conclude from the assay that the hist-

TABLE II

DATA ON URINARY EXCRETION OF HISTAMINE BEFORE, DURING, AND AFTER INTRAVENOUS INFUSION, IN THREE SUBJECTS

Histamine is expressed as base

| Expt. | Sample | Collection Time (min.) | Vol. of Urine (ml.) | Histamine (μg.) |
|---|---|---|---|---|
| | | *Subject W.I.C.* | | |
| 1 | Before infusion | 153 | 104 | 0·83 |
| | During ,, | 192 | 184 | 1·10 |
| | After ,, | 170 | 54 | 0·72 |
| 2 | Before ,, | 140 | 207 | 1·0 |
| | During ,, | 207 | 200 | 3·6 |
| | After ,, | 148 | 36 | (8·3) |
| 2a | Before ,, | 156 | 102 | 0·51 |
| | During ,, | 190 | 303 | 2·14 |
| | After ,, | 204 | 101 | (7·7) |
| 3 | Before ,, | 227 | 554 | 2·2 |
| | During ,, | 230 | 180 | 7·2 |
| | After ,, | 215 | 740 | 1·5 |
| 4 | Before ,, | 135 | 86 | 1·2 |
| | During ,, | 200 | 238 | 11·1 |
| | After ,, | 185 | 73 | 1·5 |
| 5 | Before ,, | 150 | 150 | 1·4 |
| | During ,, | 210 | 290 | 22·3 |
| | After ,, | 160 | 98 | 2·2 |
| 6 | Before ,, | | | |
| | During ,, | — | — | — |
| | After ,, | | | |
| | | *Subject J.A.S.* | | |
| 1 | Before ,, | 190 | 245 | 1·1 |
| | During ,, | 192 | 185 | 1·5 |
| | After ,, | 170 | 78 | 2·5 |
| 2 | Before ,, | 204 | 216 | 1·7 |
| | During ,, | 171 | 148 | 2·1 |
| | After ,, | 165 | 56 | 1·8 |
| 3 | Before ,, | 150 | 293 | 1·3 |
| | During ,, | 185 | 184 | 2·9 |
| | After ,, | 150 | 61 | 1·1 |
| 4 | Before ,, | 137 | 127 | 1·0 |
| | During ,, | 190 | 208 | 3·7 |
| | After ,, | 148 | 53 | 1·9 |
| 5 | Before ,, | 160 | 250 | 1·4 |
| | During ,, | 200 | 250 | 6·0 |
| | After ,, | 195 | 134 | 2·7 |
| 6 | Before ,, | 165 | 175 | 1·4 |
| | During ,, | 195 | 97 | 6·3 |
| | After ,, | 180 | 48 | 1·5 |
| 7 | Before ,, | 180 | 173 | 1·7 |
| | During ,, | 200 | 151 | 12·1 |
| | After ,, | 210 | 120(?) | 3·2 |
| | | *Subject A.A.G.* | | |
| 1 | Before ,, | 135 | 155 | 0·78 |
| | During ,, | 188 | 310 | 1·24 |
| | After ,, | 187 | 30 | 1·12 |
| 2 | Before ,, | 167 | 155 | 1·24 |
| | During ,, | 190 | 308 | 3·23 |
| | After ,, | 245 | 82 | 1·64 |
| 3 | Before ,, | 160 | 465 | 0·93 |
| | During ,, | 193 | 430 | 3·90 |
| | After ,, | 172 | 79 | 0·92 |
| 4 | Before ,, | 140 | 74 | 1·04 |
| | During ,, | 192 | 138 | 6·4 |
| | After ,, | 180 | 80 | 1·87 |
| 5 | Before ,, | 152 | 238 | 0·95 |
| | During ,, | 191 | 360 | 15·10 |
| | After ,, | 192 | 54 | 0·40 |

amine in the plasma was less than 3 μg./l. Nevertheless, the effect of the infusion on the acid secretion was always pronounced and the corresponding increase in the urinary free histamine (total/basal) was 9, 5, and 19-fold respectively in the three experiments.

The histamine content of the gastric juice was estimated in only two of the subjects (Table I,

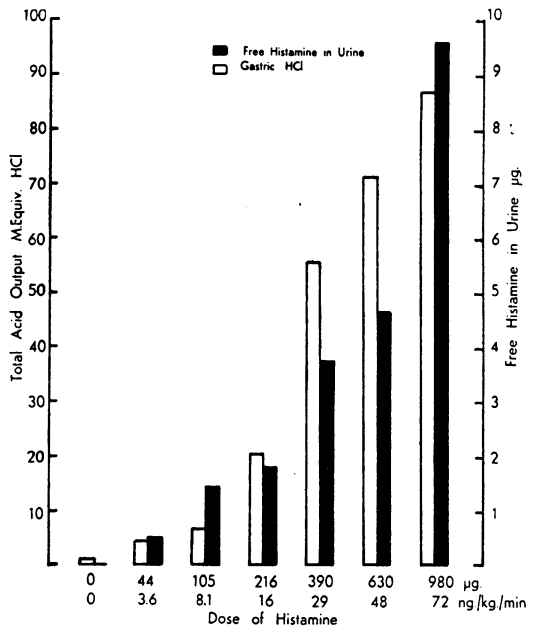


FIG. 1.—Subject J.A.S. Effect of intravenous infusions of histamine on acid gastric secretion and on urinary excretion of free histamine. The duration of each infusion was about 180 min. The dose is shown as the total amount infused (μg.) and as the rate of infusion (ng./kg./min.).

W.I.C., J.A.S.). Free histamine was present in the normal gastric juice under resting conditions, but the quantities varied widely. When the flow was stimulated by the infusion of histamine, the total amount of histamine in the juice increased in 7 out of 9 experiments, but the increase bore no close relationship to the dose infused. The concentration of histamine rose slightly in all but three experiments (Table I, J.A.S., Expts. 2, 6, and 7).

## DISCUSSION

Little is known about the fate of histamine injected into the blood stream. The results obtained in the present experiments, and those previously reported (Adam, 1950) indicate that about 1% of the dose is excreted in the urine in the free form and that none is conjugated. The remaining 99% disappears in other ways, possibly by combination with an enzyme system similar to histaminase (Best and McHenry, 1930) or with tissues upon which it has no action (Gaddum, 1948).

Within the limits of the doses used in these experiments, the proportion that appeared in the urine was independent of the rate of infusion; it may also be independent of the concentration of histamine in the plasma (Teorell, 1933; Öbrink, 1948). Roberts and Adam (1950) have shown that free histamine appears continuously in the urine

and suggested that the plasma was the immediate source of this histamine. The results support this hypothesis, but, owing to the failure to detect histamine in the plasma, the evidence is indirect. The recovery of 1% of the dose in the urine suggests that the normal excretion (approximately 20 μg./24 hr.) represents a similar proportion of histamine that may be continuously entering the blood stream —either as a result of metabolic processes in the tissues, or of absorption from the alimentary tract, or of both (Gaddum, 1951). Calculation shows that this quantity would be of the order of 2,000 μg./24 hr., or roughly 20 ng./kg./min. for a man of 70 kg., which is within the range of infusion rates used.

When histamine is infused intravenously at a constant rate of about 10 ng./kg./min., the acid secretion is slightly stimulated and the excretion of histamine is measurably increased. On infusing still larger doses, the effect on the acid output and on the urinary excretion increases correspondingly. Headache and other effects on the circulation occurred only when the rate of infusion exceeded 30 ng./kg./min. It therefore seems possible that even slight changes in the plasma histamine concentration can be detected indirectly by determining the urinary free histamine. This suggests that the liberation of histamine in the tissues or its absorption from the gut may be studied by following its excretion in the urine. Such a method, however, would be more likely to detect the slow continuous passage of histamine into the blood stream than it would the sudden release of a large quantity which might disappear in other ways before an appreciable volume of urine could be formed.

When histamine is given by slow intravenous infusion it disappears rapidly from the blood (Emmelin, 1951) and this contributes to the difficulty of estimating it in the plasma, particularly when the dose is small. Our failure to detect histamine activity in the plasma may be explained in several ways. Histamine may have disappeared from the plasma before this was separated from the cells. However, it is also possible that some of the plasma histamine is loosely bound to proteins (Emmelin, 1945 ; Öbrink, 1948) or conjugated (Anrep *et al.*, 1944). By the Decalso method, it may be expected that these pharmacologically inactive forms of histamine would be lost in the percolate. If the plasma histamine consisted mainly of these forms, the quantity of histamine retained on the column as the cation and subsequently eluted as the free base would probably be less than could be detected by the method of assay. But the evidence for this view is inconclusive, since the results obtained by Code's method, which would be expected to measure the total plasma histamine, were also negative. Again, blood sampled at the antecubital vein may not have contained free histamine. This would be so if the dose infused per minute disappeared during a single passage of the blood through the capillaries. Some of these problems are at present being investigated, and until more data are obtained further discussion is unprofitable.

Free histamine is present in measurable amounts in the resting gastric juice, where its concentration is similar to that in the urine but more variable. The slight increase in concentration observed in six out of nine histamine infusions agrees with findings in the cat (Emmelin and Kahlson, 1944) and in the dog (Code, Hallenbeck, and Gregory, 1947). Emmelin and Kahlson concluded that the parietal cells are permeable to histamine given in this way. It is, however, difficult to explain this effect in man by simple diffusion, since the concentration of free histamine in the plasma was lower than in the gastric juice. Whether this histamine derives from the plasma or from the gastric mucosa (Trach, Code, and Wangensteen, 1944) cannot be decided on the present evidence.

## Summary

1. The effect on the urinary free histamine, and on the acid gastric secretion, of histamine given by slow intravenous infusion has been studied in three healthy men.

2. When histamine was infused at a rate that stimulated acid secretion there was a corresponding rise in the excretion of free histamine in the urine. The threshold rate for both of these effects varied from 7.4 to 13.0 ng./kg./min.

3. The proportion of free histamine that appeared in the urine during the infusion was about 1% of the dose and was independent of the rate of infusion.

4. There was no detectable increase in the histamine content of plasma obtained from the antecubital vein during the course of infusions which stimulated the acid gastric secretion.

5. Free histamine was present in the resting gastric juice, where its concentration was similar to that in the urine but more variable. The amount appearing in the juice bore no close relation to the rate of infusion.

6. The evidence obtained supports the view that the free histamine normally excreted in the urine derives from the plasma.

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