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THE EXCRETION OF HISTAMINE IN URINE

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Attempts to demonstrate the presence of histamine in normal urine by biological methods have been, so far, unsuccessful. Even after injection of large amounts of histamine none could be detected in the urine [Dale & Laidlaw, 1910; Oehma, 1913; Guggenheim & Loeffler, 1916]. On the other hand, Best & McHenry [1930] report that neutralized HCl hydrolysates of dog's urine lower the blood pressure of the atropinized cat and suggest without, however, any further evidence, that the effect might be due to histamine. On this assumption they estimated the histamine equivalent of the two urine samples which they analysed as 0·2 μg./c.c. Macgregor & Peat [1933] detected histamine in the urine when large amounts of the substance were added to the blood in a perfused kidney. The concentration in the blood in these experiments must have been much higher than ever occurs in the intact animal. Ungar & Pocoulé [1937] found no histamine in human urine. On the available evidence Feldberg & Schilf [1930] and Gaddum [1936] conclude that histamine is probably not normally excreted by the kidneys.

The results of isolation of histamine by chemical methods from large amounts of urine were more satisfactory. According to Koch [1913] histamine can be isolated from the urine of parathyroidectomized dogs. Revoltella [1927] claims that histamine is excreted in eclampsia, and Kapeller-Adler [1941] isolated from the urine of a woman suffering from severe pre-eclamptic toxaemia about 1 mg. of histamine base per litre of urine. Ackermann & Fuchs [1939] obtained 0·9 mg. of histamine dipicrate per litre of normal urine; the amount was too small for unequivocal identification.

In the course of our experiments we found that extracts of normal urine prepared by a modified technique of Barsoum & Gaddum [1935] are inactive when tested for histamine unless they have been previously hydrolysed in acid. After hydrolysis in HCl the extracts show unmistakable evidence of containing histamine which greatly varies in amount in different specimens of urine. It will be shown that histamine is normally excreted in a conjugated and inactive form from which the active base can be released by hydrolysis.

Conjugated histamine

Conjugated histamine like the free base is readily adsorbed from the urine by charcoal (B.D.H. decolorizing charcoal) from which it can be released by repeated washing with acidulated alcohol. A large amount of conjugated histamine can be thus collected on a relatively small amount of the adsorbent. The eluate is almost free from inorganic salts and from a considerable amount of the organic substances of the urine. The following method is used by us for the preparation of conjugated histamine.

Method. 10 c.c. urine are shaken with about 0·25 g. charcoal and filtered under suction. The filtrate is preserved for further analysis and the charcoal on the filter is washed with cold water. After allowing most of the water to drain, 10 c.c. 0·3*N* HCl in 95% alcohol are passed through the charcoal. The first portion of acidulated alcohol removes the remaining water. Further portions of acidulated alcohol, 10 c.c. each, are then passed through the filter. Each portion is passed 3–4 times, about 40–60 c.c. being used in all. Beginning with the second portion the strength of the acidulated alcohol may be reduced to 75%. All the alcoholic eluates, including the first, are joined together and carefully neutralized. The charcoal filtrate and the alcoholic eluate are divided into two parts. One part of each is hydrolysed in HCl while the respective remainders are dried *in vacuo* on a boiling water-bath. For the hydrolysis 10 c.c. conc. HCl and about 50 c.c. water are added and the mixture is boiled for 1·5 hr., water being occasionally added to prevent desiccation. Towards the end of the hydrolysis the volume of the mixture is reduced to a few c.c. The two hydrolysates are evaporated to dryness on a water-bath under suction. Four portions of 10 c.c. 95% alcohol are added and distilled off in order to remove the HCl. The dried residues of the hydrolysed and non-hydrolysed fractions of the filtrate and of the eluate are then extracted by a technique similar to that of Barsoum & Gaddum [1935]. The extraction is made with four lots, 3 c.c. each, of absolute alcohol saturated with NaCl. The residue usually forms a crust at the bottom and on the sides of the flask, so that a certain amount of scraping with a glass rod is necessary during the extraction. The alcoholic extracts are filtered and evaporated to dryness. The dry residue is taken up in 4·4 c.c. water and neutralized to bromo-thymol blue with x c.c. *N* NaOH; $(0{\cdot}6-x)$ c.c. *N* NaCl (5·85%) are then added to make the total volume equal to 5 c.c. When more than 0·6 c.c. of the normal alkali is required the neutralization is completed with *N*/7 NaOH after which Tyrode solution is added to any desired volume.

As a result of this procedure we have now four extracts: a hydrolysed and a non-hydrolysed extract of the filtrate and a hydrolysed and a non-hydrolysed extract of the eluate. At this stage of preparation the four extracts are not

suitable for quantitative tests, since they all contain a variable amount of relaxing material which is especially noticeable when the assay is made on the rectal caecum of the fowl. The relaxing material can be removed by adsorption on aluminium oxide (B.D.H. 'for adsorption'). Each extract is treated with about 1 g. of the adsorbent and filtered. The *p*H of some extracts has to be readjusted after treatment with aluminium oxide by addition of a trace of HCl. Adsorption on Al_2O_3 should not be omitted unless the histamine equivalent of an extract is so high that it can be diluted 5–10 times. The four extracts are now ready for the assay. As a routine the tests were made on the guinea-pig's ileum and on the rectal caecum of the fowl. The test object was suspended in a slightly atropinized (1 in 10^7) Tyrode solution in a bath of 5 c.c. capacity at 36° C. In other details we followed the usual procedure as recommended by Barsoum & Gaddum [1935].

The charcoal filtrate. There is no significant difference between the hydrolysed and non-hydrolysed fractions of the filtrate. After adsorption on Al_2O_3 both evoke contractions of the ileum, which are, however, not typical of histamine. The contractions are not sustained; on repetition of the injection they diminish in strength, but large doses of the extract may cause a temporary diminution of the sensitivity of the ileum to histamine, and the contractions are not abolished by the selective paralysis of the ileum with large doses of histamine [Barsoum & Gaddum, 1935] or with traces of the drug F 933 [Ungar, Parrot & Bovet, 1937]. Expressed in terms of histamine acid phosphate the activity of these extracts varies for human urine between 0·03 and 0·12 μg./c.c. After five or six injections the ileum becomes insensitive to these extracts, still retaining its sensitivity to histamine. Although extracts of the filtrate contain no histamine their occasional assay is useful as providing a check on the completeness with which the histamine has been adsorbed on the charcoal.

The charcoal eluate. The unhydrolysed extracts of most charcoal eluates of dog's and human urine are inactive when tested on the ileum and cause a conspicuous relaxation of the rectal caecum. The relaxation is abolished by treatment of the extracts with Al_2O_3. The histamine equivalent of those few extracts which evoke a contraction of the ileum is below 0·1 μg./c.c. Injection of non-hydrolysed extracts into an atropinized dog, in amounts equivalent to 40 c.c. urine, has no effect on the blood pressure. However, under certain experimental conditions and in some animals in which histamine is normally excreted in a free form or when some histamine has been added to the urine, the assay of the non-hydrolysed extracts gives positive results. As will be shown below, the non-hydrolysed extracts of the eluate can be used for quantitative estimation of free histamine in the urine. After acid hydrolysis the extract of the eluate shows a striking increase of activity. The extent of this effect can be seen from Table 1 and Fig. 1.

TABLE 1. Showing the activity of the charcoal eluate before and after hydrolysis in HCl. The examples given here are taken at random from experiments made under different conditions. The assays are made on the guinea-pig's ileum and the histamine equivalents are given in μg./c.c. histamine acid phosphate. Before testing, the extracts were treated with Al_2O_3.

Human urine		Dog's urine	
Before hydrolysis	After hydrolysis	Before hydrolysis	After hydrolysis
Trace	0·60	Trace	7·20
Trace	0·55	Trace	0·25
Trace	2·20	Trace	0·15
0·06	0·09	0·03	4·10
<0·08	0·30	Trace	10·50
<0·08	5·20	<0·05	54·00
Trace	1·90	0·08	70·00

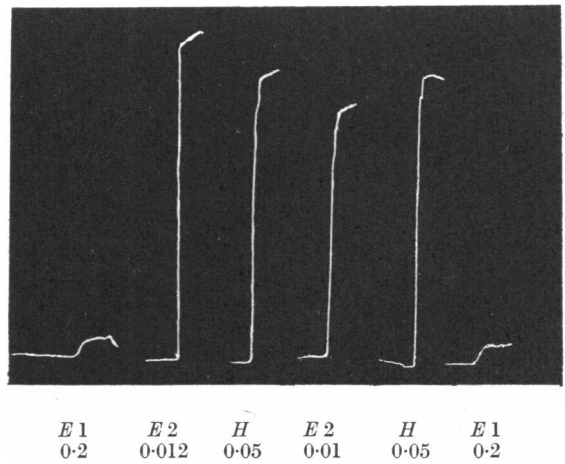

Fig. 1. Guinea-pig's ileum; dog's urine. Effect of acid hydrolysis of urine extracts prepared by the charcoal method. *E* 1, non-hydrolysed and *E* 2, hydrolysed extracts; doses in c.c. of urine. *H*, standard solution of histamine acid phosphate; doses in μg. All the extracts were adsorbed on Al_2O_3. The histamine equivalent of the urine was estimated as 5·5μg./c.c.

The increase in the histamine-like activity of urine extracts after hydrolysis can be due either to the destruction of some antagonizing substances interfering with the contraction of the ileum or to a release of the active principle from a conjugated and inactive form. The first possibility is excluded by the fact that histamine when added, even in traces, to the non-hydrolysed extracts can be quantitatively estimated on any of the usual test objects. No interfering substances can therefore be present. As a result of these observations we conclude that the histamine-like substance of urine is normally excreted in a conjugated inactive form. Table 1 shows that the concentration of this substance in the urine may vary to a remarkable extent. The study of its excretion in different species of animals and under different experimental conditions is of obvious importance.

Precautions necessary during the preparation and hydrolysis of conjugated histamine. The amount of charcoal used for the adsorption of conjugated histamine should not be reduced below 0·2 g. per 10 c.c. urine if quantitative results are desired. When half this amount is used some of the substance escapes adsorption. When large amounts of urine are employed at least 150–200 g. charcoal per l. urine should be used. The necessary amount will vary with the brand of charcoal. Variations in the *p*H of urine between 5·5 and 9·0 do not affect the completeness of the adsorption.

The recovery of conjugated histamine from the charcoal with non-acidulated absolute alcohol is incomplete. The acidity of the alcohol should not be below 0·15*N* HCl and the alcohol not below 70%. After many trials we found the recommendations given earlier to answer the purpose, namely 0·3*N* HCl in 95% alcohol for the first and in 75% alcohol for the subsequent washings. When large amounts of charcoal are worked on, it is advisable to remove it from the filter, mix with about 300–400 c.c. acidulated alcohol and refilter. The process must be repeated several times.

The duration of the hydrolysis should not be less than 1 hr. When shortened to 30 or 45 min. the yield of free histamine is about 75 and 90% respectively. We always continue the hydrolysis for 1·5 hr., but further prolongation is without harm. Conjugated histamine, like the free base, is soluble in alcohol and water and insoluble in ether.

Histamine can be released from the conjugated form also by hydrolysis in alkali. However, this method presents the disadvantage that the released histamine itself undergoes a gradual destruction. For example, hydrolysis of a 5 c.c. sample of urine in acid yielded 2·25 μg. histamine. Hydrolysis of a second sample in 0·2*N* NaOH released 0·85 μg., and a further hydrolysis of the same sample in acid yielded 0·8 μg., giving a total of 1·65 μg. The difference of 0·6 μg. (2·25–1·65) was presumably due to the destruction of histamine by the alkali. A similar alkali hydrolysis of 2·25 μg. of released histamine reduced it to 0·7 μg.

Non-hydrolysed eluates of urine can be kept for a long time; one sample remained in the laboratory for over 3 years without much deterioration. The dried residue of the eluate has a waxy appearance and obviously contains a large amount of impurities. In view of the limited facilities at our disposal we have not attempted to purify the extract. At present any speculation as regards the composition of conjugated histamine must be left over until this derivative is obtained in a pure form.

Evidence that the active substance is histamine. The typical contractions of the ileum and of the rectal caecum evoked by the hydrolysed extracts are abolished by selective paralysis with large doses of histamine or traces of piperidinomethylbenzodioxan—F 933 (Fig. 2). The extracts vigorously contract the guinea-pig's bronchi and lower the blood pressure of the atropinized

cat (Fig. 3). Assays performed on the ileum, rectal caecum and the blood pressure show good agreement. On intradermal administration in man the extracts evoke a conspicuous triple response with formation of blisters. Non-hydrolysed extracts are in all these respects completely inactive.

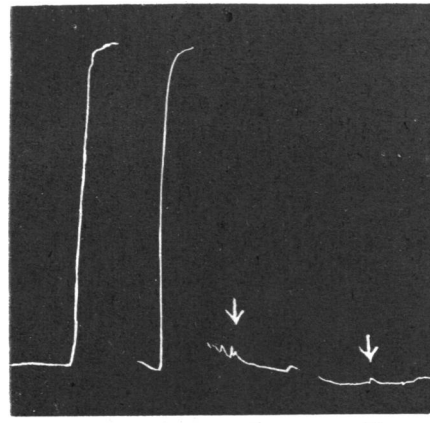

Fig. 2. Effect of selective paralysis of the guinea-pig's ileum with F 933 on its response to histamine (*H*) and to the hydrolysed extract of dog's urine (*E*). Doses in c.c. urine and μg. histamine acid phosphate. F 933 was administered after the second contraction of the ileum. The ileum continued to respond normally to KCl and alkali.

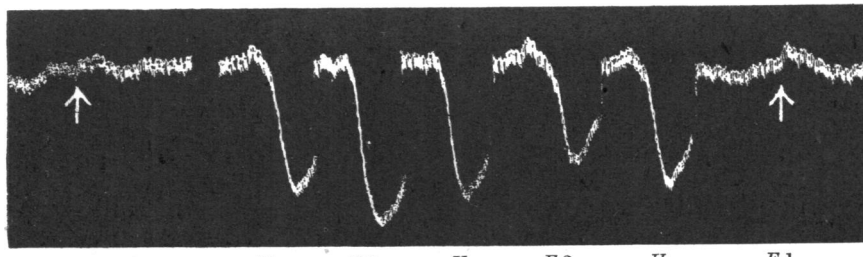

Fig. 3. Effect of urine extracts on the blood pressure of the atropinized cat. *E* 1, non-hydrolysed extracts of dog's urine; *E* 2, hydrolysed extracts of the same urine; *H*, histamine. Doses in c.c. urine and μg. histamine acid phosphate.

Attempts to demonstrate the action of histaminase on urine extracts were at first unsuccessful. It was found that the enzyme is entirely inactive in the presence of urine, and that this inhibitory influence is retained by the extracts of the urine even after acid hydrolysis. On diluting the urine or the extracts with water the enzyme becomes more active. With a tenfold dilution the inhibition of the enzyme is negligible. Taking this into account we tested the action of histaminase on the hydrolysed and non-hydrolysed extracts after different degrees of dilution with water (Table 2).

TABLE 2. Action of histaminase prepared by the method of Anrep, Barsoum & Talaat [1936]. (A), on histamine acid phosphate added to human urine (sp. gr. 1·025); (B), on histamine acid phosphate added to Tyrode solution; (C), on the non-hydrolysed urine extract (conjugated histamine); (D), on the hydrolysed extract of the same urine (released histamine). The samples were incubated at 37°C. for 30 min. The same amount of enzyme solution was added to each sample. The amount of histamine added to the total volume of each sample A and B and the histamine equivalent of each sample C and D before incubation was 30 μg. The histamine equivalent of samples C was determined after acid hydrolysis. The dilution of the samples is shown in the first column. The amount of histamine in the total volume of each sample after incubation is given in the table in μg. of histamine acid phosphate.

Dilution of sample	A Histamine in urine	B Histamine in Tyrode solution	C Conjugated histamine	D Released histamine
0	30·00	30·00	30·00	30·00
4	18·00	0·50	30·00	16·00
8	6·00	0·70	30·00	4·50
16	0·75	0·30	30·00	0·50
32	0·50	0·50	30·00	0·60

It is evident from Table 2 that histaminase does not act on conjugated histamine whatever the dilution (C), while the product of its hydrolysis is destroyed at the same rate (D) as histamine which has been added to urine (A). All these tests support the conclusion that the active principle of hydrolysed urine extracts is histamine.

QUANTITATIVE DETERMINATION OF TOTAL CONJUGATED AND FREE HISTAMINE

Total histamine. Adsorption on charcoal is not essential for quantitative estimation of the total histamine in urine. Satisfactory results can be obtained by directly hydrolysing 0·1–5·0 c.c. urine in HCl and then extracting the active principle by the modified technique of Barsoum & Gaddum. For this purpose the urine is treated in the same way as described for the charcoal eluate. Before the assay the final extracts must be adsorbed on Al_2O_3. The importance of this step is made clear by Table 3 and Figs. 4 and 5, which

TABLE 3. Showing the effect of adsorption of extracts of human urine with aluminium oxide. The assay of each extract was made on the guinea-pig's ileum and on the rectal caecum of the fowl. The histamine equivalents are given in μg./c.c.

	Guinea-pig's ileum		Fowl's rectal caecum	
	Before adsorption	After adsorption	Before adsorption	After adsorption
1	2·2	2·2	Relaxation	2·0
2	0·6	0·9	Relaxation	0·8
3	0·7	0·8	Relaxation	0·8
4	0·9	1·3	Relaxation	1·1
5	0·2	0·5	Relaxation	0·6
6	<0·05	0·12	Relaxation	No relaxation
7	0·25	0·3	Relaxation	0·25
8	Relaxation	0·07	Relaxation	No relaxation

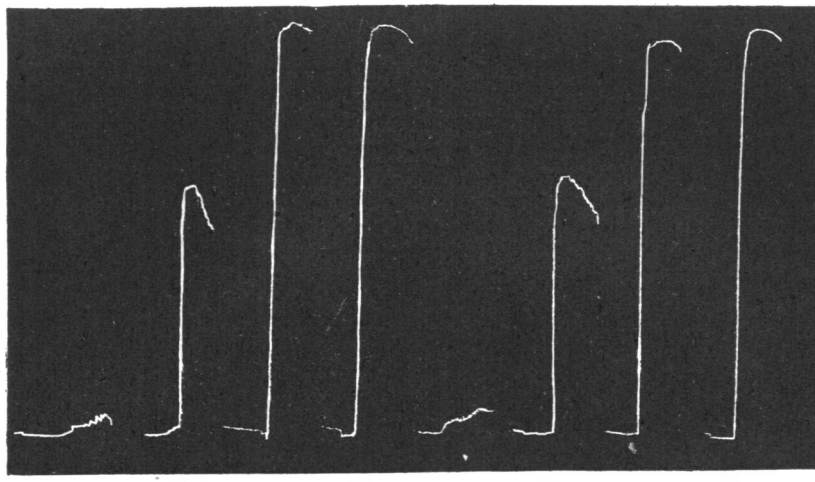

Fig. 4. Guinea-pig's ileum. Effect of adsorption of an extract of human urine with Al_2O_3. The urine extract was prepared by the method of direct hydrolysis. *E* 1, non-hydrolysed extract of urine. *E* 2, non-hydrolysed extract of urine adsorbed on Al_2O_3. *E* 3, hydrolysed extract of urine. *E* 4, hydrolysed extract of urine adsorbed on Al_2O_3. *H*, histamine. Doses in c.c. urine and μg. histamine acid phosphate.

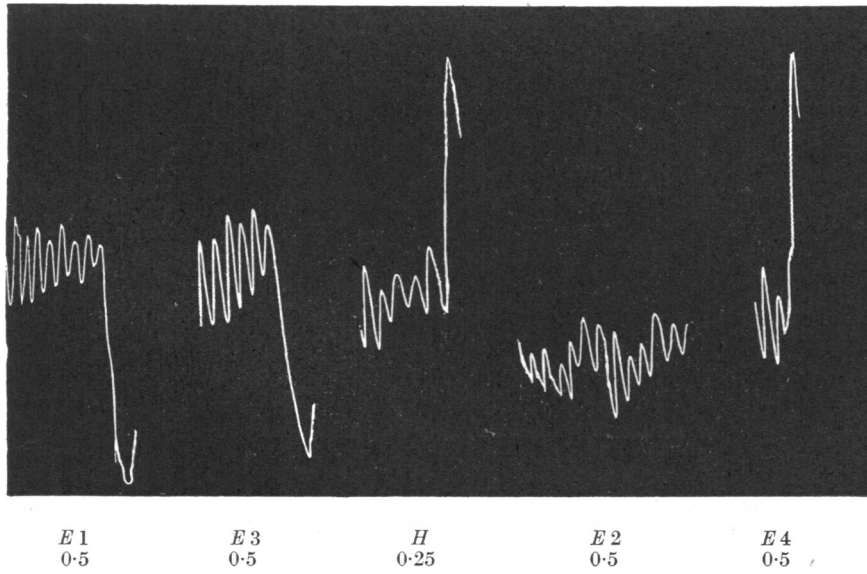

Fig. 5. Action of the same extract of human urine as in Fig. 4 on the rectal caecum of the fowl. The histamine equivalent of the urine as determined on the ileum (Fig. 4) and on the caecum is 0·5μg./c.c.

show the response of the ileum and of the rectal caecum to the administration of the extracts at different stages of their preparation.

The effect of adsorption on Al_2O_3 differs from one sample to another. When the histamine equivalent is high as in sample 1 adsorption is not necessary for tests made on the guinea-pig's ileum. The assays made on the two test objects show good agreement.

The method of direct hydrolysis and of charcoal adsorption can both be used for quantitative estimation of total histamine in urine. The final extracts prepared by the second method are less contaminated with impurities. On the other hand, a certain loss may occur during the release of the conjugated histamine by acidulated alcohol. The recovery, therefore, depends on the thoroughness with which the charcoal has been extracted.

Urine extracts prepared by the method of direct hydrolysis contain the unknown stimulating material which was described as present in the charcoal filtrate. This does not materially interfere with the assay, since the sensitivity of the ileum to this material rapidly declines. The charcoal method is to be preferred when the histamine equivalent of the urine is low. A larger amount of urine is then used so as to make the final extract more concentrated. The accuracy of the two methods was tested by estimations of urine histamine and of histamine added to water, Tyrode solution or urine. Some of the results are given in Table 4.

TABLE 4. (A), determination of the histamine equivalent of urine and recovery of added histamine by the method of direct hydrolysis and (B), by the method of charcoal adsorption. All the results are given in μg./c.c. of histamine acid phosphate.

Medium	Histamine added	A Recovery by direct hydrolysis	B Recovery by charcoal adsorption	A Percentage recovery by direct hydrolysis	B Percentage recovery by charcoal adsorption
Water	0·20	0·19	0·18	95	90
	0·50	0·50	0·45	100	90
Tyrode	0·50	0·46	0·46	92	92
	2·00	1·80	1·70	90	85
Human urine 1	—	0·25	0·20	—	80
	0·50	0·80	0·70	110	100
Human urine 2	—	1·55	1·40	—	90
	0·50	2·10	1·80	110	80
Dog's urine 3	—	6·20	5·80	—	94
	5·00	11·00	10·40	96	92
Human urine 4	—	0·30	0·25	—	83
Human urine 5	—	1·50	1·40	—	93
Dog's urine 6	—	8·00	8·00	—	100
Dog's urine 7	—	4·30	3·90	—	90

Table 4 shows that the recovery of histamine added to water, Tyrode solution or urine and the determination of the total histamine present in urine extracts is satisfactory with either of the two methods. The method of direct hydrolysis gives somewhat higher results, which are probably due to a small loss of the active substance on the adsorbent.

Conjugated and free histamine. When free histamine is present in the urine it can be quantitatively determined in extracts prepared by the charcoal method before they are hydrolysed. The total histamine is then determined by a second assay after hydrolysis of the extract. The difference between the two results represents the conjugated histamine of the sample. As a check the total histamine can be determined once more by the method of direct hydrolysis. It will be seen later that free histamine is normally present in the urine of some species or may appear in the urine under some experimental conditions.

THE HISTAMINE EQUIVALENT OF THE URINE OF DIFFERENT SPECIES OF ANIMALS

We should like to express our thanks to Dr Kadry, Director of the Cairo Zoological Garden, for the facilities which he placed at our disposal for the collection of urine samples from various animals. When possible, 24 hr. samples were collected, but most urines were taken as single samples either by catheter or during micturition. A few urines were collected from anaesthetized animals directly from the bladder. The samples were collected without regard to the previous history of the animal in respect to the time of feeding, etc. The results are grouped according to whether the animals are (*a*) typical Herbivora, (*b*) animals on varied diet, or (*c*) typical Carnivora. The number of animals used for each species is given in brackets. No number is given for man, dog and rat, since these served for our routine experiments.

In the group of Herbivora the urine of the following species was analysed: rabbit (12), horse (5), donkey (4), water buffalo (5), elephant (3), camel (5), llama (2), anthropoid apes (2). The extreme variations of the urine histamine of these species are between 0·02 and 0·2 μg./c.c. histamine acid phosphate; in one horse we found 0·4 μg./c.c. There is no appreciable difference between the urines of the different species belonging to this group. The histamine equivalent is uniformly low. Amongst the animals on varied diet the variations in the urine histamine is very considerable, the extreme values for the white rat are 0·1–10·2, the cat (8) 0·3–15·0, the dog 0·2–45·0 and for man 0·1–6·2 μg./c.c. In the group of typical Carnivora the variations are again less but the histamine equivalent of all the urines is uniformly high, for the lion (6) 10–15, tiger and leopard (6) 25–40 and the cheetah (1) 15 μg./c.c. Urines with a histamine equivalent below 0·2 μg./c.c. were analysed by the charcoal method, those of a higher equivalent by the method of direct hydrolysis.

The obvious conclusion from these observations is that the excretion of histamine in the urine depends on the character of the diet. No relation seems to exist between the histamine equivalents of the blood and urine. In the rabbit in which the blood histamine is high there is very little histamine

in the urine; in the cat in which the blood histamine is extremely low the histamine of the urine may be as high as 15 μg./c.c.

Carnivora and Herbivora differ not only in respect of the concentration of histamine excreted in the urine but also the form in which it is excreted. In Carnivora almost the entire histamine (98–100%) is excreted in the conjugated form, while in Herbivora most if not the whole of it is free. In view of the very low histamine content and the frequent presence of relaxing substances which are not completely removed by Al_2O_3 we did not analyse the urine of Herbivora any further.

The urine of the rat occupies an intermediate position; free and conjugated histamine are present together but in different proportions. Frequently 40–50% of the histamine is excreted in a free form. In many samples the entire histamine is free. Small amounts of free histamine are also found in most samples of human urine.

Effect of diet on the excretion of histamine

The difference in the histamine content of the urine of Herbivora and Carnivora led to the investigation of the effect of administration of meat. The observations were made on rats, dogs and man.

Experiments on rats. Rats weighing 250–300 g. were kept in Hopkins's metabolic cages with the usual precautions and care.

The observations were divided into three periods. During the first the animals were placed on a carbohydrate-fat diet consisting of boiled starch, sugar, olive oil and occasionally some boiled or fried starchy potatoes. During the second period the diet consisted exclusively of slightly roasted or boiled buffalo meat, and during the third period the rats were fed again on a mixture of carbohydrates and fat. The meat was given outside the cages so as not to contaminate the urine. The diets were supplemented by the usual salt mixture, cod-liver oil and clover. The urine was collected in 24 hr. samples, some toluol or thymol being added to prevent putrefaction. Since rats' urine contains conjugated, as well as free histamine, we usually analysed the urine extracts for both, i.e. before and after acid hydrolysis. Urea was analysed by the hypobromite method.

An example of this type of experiment is given in Fig. 6. During the first period of carbohydrate diet the histamine excretion fell to below 10 μg./day, and almost the whole of it was eliminated in a free form. During the next period the total histamine gradually increased to 90 μg., the increase being mainly accounted for by the conjugated histamine. The excretion of free histamine also increased but to a much smaller extent. On return to the carbohydrate diet the excretion of free histamine abruptly diminished to the previous low figures, while the conjugated histamine declined more gradually and almost completely disappeared in the course of 3 days.

Experiments on other rats gave similar results differing only in degree. The maximal elimination of total histamine was in some rats as high as 230 μg., almost the whole of it conjugated. In other animals it did not exceed 50 μg./day. On carbohydrate diet not less and usually more than 50% of the total histamine was eliminated in a free form.

When rats are kept on a meat diet for a long time their histamine excretion shows fairly wide fluctuations which can be usually traced to the different amounts of meat consumed. As a very rough approximation the elimination of 1 g. of urea corresponds in the rat to the excretion of 100 μg. of total histamine. This relationship is not apparent in 24 hr. samples, but it becomes more obvious with longer periods of observation: for example, a pair of rats on a mixed diet excreted in 18 days 11·6 g. urea and 1070 μg. total histamine. Another pair which received a larger proportion of meat excreted during the same time 23·2 g. urea and 2234 μg. histamine.

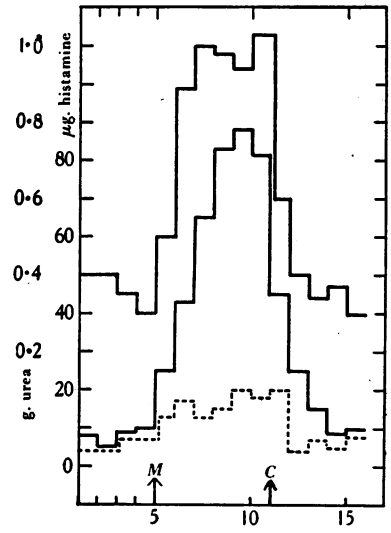

Fig. 6. Effect of administration of meat on the daily excretion of conjugated histamine, free histamine and urea by the rat. From below upwards—free histamine, total histamine in μg. histamine acid phosphate and urea in g./24 hr. The excretion of conjugated histamine is represented by the difference between the total and the free histamine. Meat was given for 6 days between *M* and *C*. Abscissae, days.

The time relations of the excretion of histamine and urea are not the same. The changes in the excretion of histamine lag behind those of urea. In some animals this is so considerable that it can be noticed in 24 hr. samples of urine (Table 5).

TABLE 5. Showing the effect of a single administration of meat on the daily excretion of urea and of total histamine

Days	Diet	Vol. urine c.c.	Urea g. %	Total histamine μg./c.c.	Excretion of urea g.	Excretion of histamine μg.
1	Potatoes	18	0·6	0·5	0·11	9·0
2	Potatoes	16	0·5	0·7	0·08	11·2
3	Potatoes	20	0·5	0·6	0·10	12·0
4	Potatoes with 29·5 g. meat	20	7·8	2·5	1·56	50·0
5	Potatoes	14	3·0	5·0	0·42	70·0
6	Potatoes	18	0·8	2·5	0·14	45·0
7	Potatoes	10	0·8	1·1	0·08	11·0
				Total	2·49	208·2

The maximal excretion of urea occurred in the above experiment on the day of administration of the meat, and that of histamine on the following

day. On the third day the excretion of urea returned to the original level while that of histamine was still increased. Since a more exact determination of these time relations could not be easily made on the rat the experiments were continued on dogs.

Experiments on dogs. The observations were made on female dogs weighing 14–16 kg. As a preliminary an incision was made in the perineal region to facilitate catheterization. Urine samples were collected at intervals of 0·5–4 hr. and analysed for total histamine and urea. Determinations of free histamine were made only occasionally, since it was shown that dogs in common with other Carnivora do not excrete free histamine even when fed on meat.

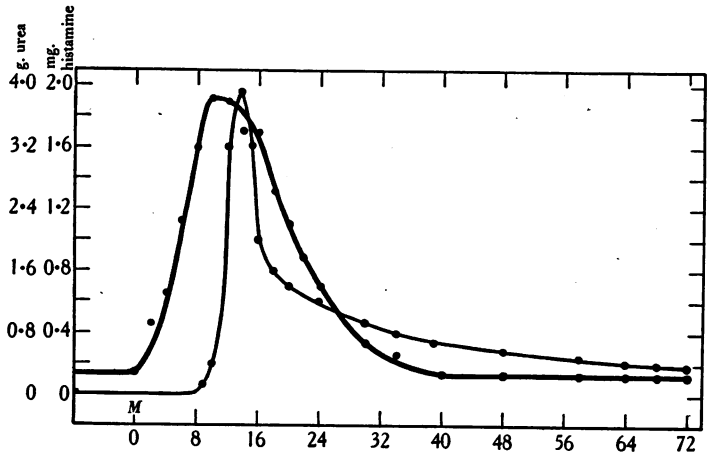

Fig. 7. Effect of administration of 1 kg. of meat on the excretion of conjugated histamine and urea by the dog. Thick line, urea, g.; thin line, conjugated histamine, mg. histamine acid phosphate. Abscissae, hr. The meat was administered at *M*.

In the experiment the results of which are given in Fig. 7, the animal was deprived of food for 36 hr. and then given a large meal of raw minced buffalo meat. The excretion of urea began to increase within 2 hr. after the meal; the maximum was reached on the 10th hour after which the amount of urea gradually dropped to the pre-feeding level in 34 hr. The excretion of histamine began to increase only 6 hr. after the meal and reached a maximum about 4 hr. later than urea. The decline of histamine excretion was at first more rapid and then much slower than that of urea, so that the pre-feeding level was not quite reached even 72 hr. after the meal. Altogether about 20 mg. of histamine were excreted, the entire histamine being present in the conjugated form.

Table 6 is computed from another similar experiment to show the changes in the concentration as well as in the total excretion of histamine and urea following administration of meat.

TABLE 6. Dog, 16 kg. Effect of administration of 1 kg. of minced meat on the excretion of histamine and urea and on their concentration in the urine. During the first 36 hr. after feeding the urine samples were collected at intervals of 4 hr. and then at 8 and 12 hr. Histamine in μg. of histamine acid phosphate.

Hours before and after feeding	Vol. urine c.c.	Urea g. %	Histamine μg./c.c.	Total urea excreted g.	Total histamine excreted μg.
8– 4	32	4·8	0·40	1·5	13
4– 0	26	6·0	0·35	1·6	9
		Administration of 1 kg. meat			
0– 4	53	10·7	0·26	5·7	14
4– 8	89	12·6	1·40	11·2	125
8–12	127	12·6	26·00	16·0	3302
12–16	115	11·8	53·00	13·6	6095
16–20	87	12·1	37·00	10·5	3219
20–24	57	11·3	40·00	6·4	2280
24–28	33	10·0	43·00	3·3	1419
28–32	27	9·0	50·00	2·4	1350
32–36	24	8·1	43·00	1·9	1032
36–44	66	4·8	32·00	3·2	2112
44–56	124	3·9	10·00	4·8	1240
56–68	69	6·1	3·50	4·2	242

A total amount of about 22 mg. histamine, all in the conjugated form, was excreted in this experiment. Experiments on other dogs gave similar results. The excretion varied with the amount of meat consumed. For example, on administration of 500 g. it was 10·2 mg. or about half the amount excreted after consumption of 1 kg. meat. The highest concentration of conjugated histamine found in dog's urine on meat diet was 87 μg./c.c. and the maximal amount excreted 2·1 mg./hr.

Experiments on man. These will not be described in detail since they present no points of interest beyond those established for the dog. One subject (G.V.A.) excreted on a mixed diet 23–25 g. urea and 1·2–1·4 mg. histamine per day. On the fourth day of a strictly carbohydrate-fat diet the subject excreted 7·5 g. urea and 190 μg. histamine. The excretion of urea declined more rapidly than that of histamine. Beginning from the fifth day the subject was given large amounts of roasted meat. The excretion of urea increased rapidly reaching a maximum of 38 g. on the first day of the meat diet. The excretion of histamine increased more gradually taking about 2 days to reach 2·4 mg. in 24 hr. Almost the whole histamine was excreted as conjugated histamine. Traces of free histamine were found in all the samples of human urine, but they were too small for accurate estimation. The relation between histamine and urea excretion in the dog is higher and in man lower than in the rat. Apparently the facility with which conjugated histamine is formed or excreted differs, being highest in the dog, smaller in the rat and least in man.

Effect of diuresis. Histamine excretion bears no relation to urine flow. This becomes evident after administration of large amounts of water or of urea.

In one experiment a dog on a mixed diet secreted during 4 consecutive hours 15–28 c.c. urine and 190–250 μg. histamine/hr. After administration of 20 g. urea in 100 c.c. milk the urine flow was 81 c.c. during the first and 118 c.c. during the second hour and the elimination of histamine 243 and 206 μg. respectively. Similar results are obtained with animals which are kept on a meat-free diet. For example, in another dog, the urine flow increased from 12 to 95 c.c./hr., while the histamine excretion remained almost the same, 2·2 and 3·8 μg./hr. respectively. Experiments on rats and man gave the same results.

The origin of conjugated histamine

Effect of administration of histamine-free food. The experiments with different diets established the fact that administration of raw or slightly cooked meat greatly increases the excretion of conjugated histamine in Carnivora and in

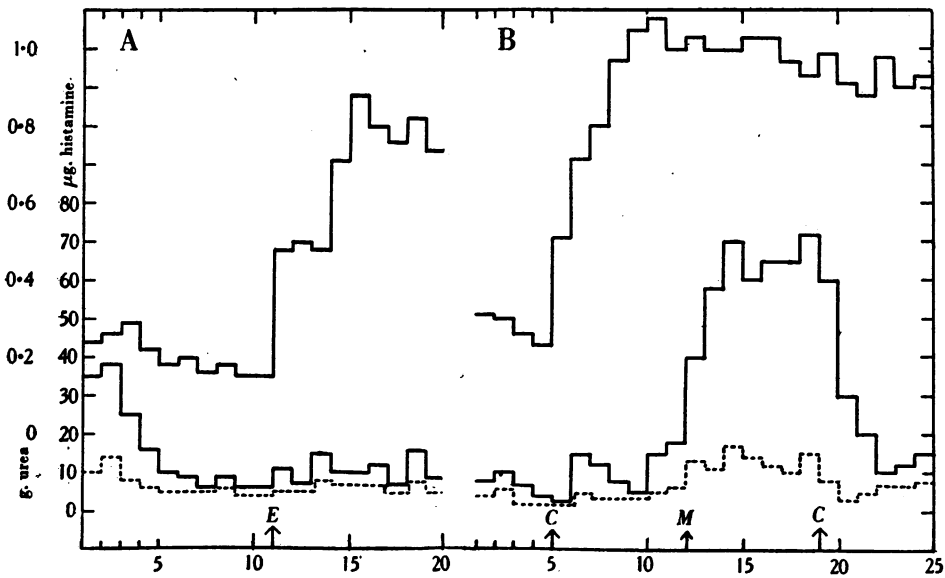

Fig. 8. A. Effect of administration of egg-white on the excretion of histamine and urea by the rat. At *E* the carbohydrate-fat diet was changed to egg-white. B. Effect of administration of casein and of meat to the same rat as in A. At the first *C* the carbohydrate-fat diet was changed to casein, at *M* to meat and at the second *C* again to casein. From below upwards; free histamine, total histamine in μg. histamine acid phosphate and urea in g. Abscissae, days.

man and also, but to a much smaller extent, the excretion of free histamine in the rat. The experiments do not throw any light on whether conjugated histamine derives from the protein component of meat or from its extractive substances.

This question assumes special importance, since all samples of buffalo meat which we analysed contained histamine, varying from 6 to 20 μg./g. The effect of administration of meat was, therefore, compared with the administration

of histamine-free proteins and with the administration of histamine acid phosphate.

The estimation of histamine in the food was made after extraction with trichloracetic acid following the method of Barsoum & Gaddum [1935]. The food extracts were assayed for free and conjugated histamine by testing the extracts before and after hydrolysis in HCl. The histamine equivalent of the hydrolysed and non-hydrolysed extracts of meat was found to be always the same, which suggests that the entire histamine of meat is probably present in a free form. On the other hand, casein, fresh white cheese and egg albumen were found to contain neither free nor conjugated histamine.

Fig. 8 A and B shows the effects of feeding rats on egg albumen and on casein. In Fig. 8 B the rats were fed for 7 days on casein; during the following 7 days they were given meat and then again casein. While on the protein diet the urea excretion remained on a high level independently of whether the food was meat or casein. The histamine excretion increased only during the meat period, and the increase was mainly due to the conjugated histamine. Similar results were obtained on dogs. Administration of a large meal consisting of casein, egg white, fresh cheese and milk had no appreciable effect on the elimination of histamine. The effect of more prolonged feeding with histamine free food was not investigated.

Subcutaneous administration of histamine. The dogs on which the effects of administration of histamine were studied were kept on a bread and milk diet during and for some days before the experiment. A few minutes after an injection of 6–50 mg. histamine acid phosphate the dog becomes restless, begins to pant, defaecates and sometimes vomits. The secretion of urine is diminished or stopped, depending on the dose, for 0·5–2 hr. The period of anuria is followed by diuresis which continues for 2–3 hr. The excretion of conjugated histamine is not changed. On the other hand, free histamine, which is normally not found in the urine of dogs and other Carnivora, appears in measurable amounts. Table 7 is an example of the effect of subcutaneous injection of 10 mg. histamine.

TABLE 7. Dog, 14 kg.; 10 mg. histamine acid phosphate in 5 c.c. saline were injected at the beginning of the third hour.

Time hr.	Urinary secretion c.c./hr.	Conjugated histamine μg./hr.	Free histamine μg./hr.	Total histamine μg./hr.
1	23	26·5	—	26·5
2	18	21·6	—	21·6
3	0	—	—	—
4	16	32·0	107·2	139·2
5	54	30·0	37·8	67·8
6	105	25·1	20·5	45·6
7	99	18·9	9·9	28·8
8	34	20·2	—	20·2
9	25	19·8	—	19·8
10	19	27·8	—	27·8

In this experiment injection of 10 mg. histamine led to an excretion of 175 μg. free histamine. We find no relation between the amount of histamine injected and excreted in the urine. With doses varying from 10 to 50 mg. histamine the amount excreted did not exceed 200 μg. In fact, with the larger doses it was usually less. The excretion of free histamine seems to be related to the duration of the period of anuria which follows the injection, more histamine being excreted in those experiments in which the suppression of urine flow is short. In some experiments with long periods of anuria hardly any free histamine appeared in the urine. For example, injection of 50 mg. histamine suppressed the urine flow in one dog for 60 min. and in another for 150 min. In the first the amount of free histamine excreted was 126 μg., while in the second only traces appeared in the urine. It is probable that most of the injected histamine is destroyed in the animal during the prolonged suppression of the urine. When the period of anuria is short some free histamine escapes through the kidney, but even under the most favourable conditions the amount so excreted is extremely small.

Oral administration of histamine. Histamine acid phosphate was given to dogs in amounts of 0·005–0·5 g. dissolved in 100 c.c. milk. Only traces of free histamine appeared in the urine. On the other hand, the excretion of the conjugated histamine was greatly increased. The excretion begins to increase about 1 hr. after the administration; it gradually reaches a maximum in 7–14 hr. and then slowly declines to its original level, which it reaches, depending on the dose, in 12–60 hr. The total amount of conjugated histamine excreted is between 3 and 5% of the free histamine administered to the animal. Fig. 9 shows the rate of the excretion after oral administration of different doses of histamine acid phosphate.

Administration of conjugated histamine. Subcutaneously injected conjugated histamine is almost quantitatively excreted by the kidneys in an unchanged form. In most experiments we could recover from the urine 85–100% of the amount injected. It will be shown in a later communication that in these experiments no conjugated histamine can be detected in the blood. This indicates that the excretion of conjugated histamine proceeds at the same rate as its absorption and that the kidney threshold to it is very low. Injection of conjugated histamine neither diminishes nor suppresses the urinary secretion probably because, unlike free histamine, it has no vasomotor action. The excess excretion of conjugated histamine begins almost immediately after the injection, reaching a maximum in 2–3 hr. and being completed in about 20 hr. (Fig. 9). The maximal concentration of conjugated histamine observed in the urine was 145 μg./c.c. and the maximal amount excreted 2·50 mg./hr.

Oral administration of conjugated histamine leads to a much greater and more rapid excretion of it in the urine than administration of histamine acid

phosphate. Between 50 and 60% of the amount administered is excreted in an unchanged form.

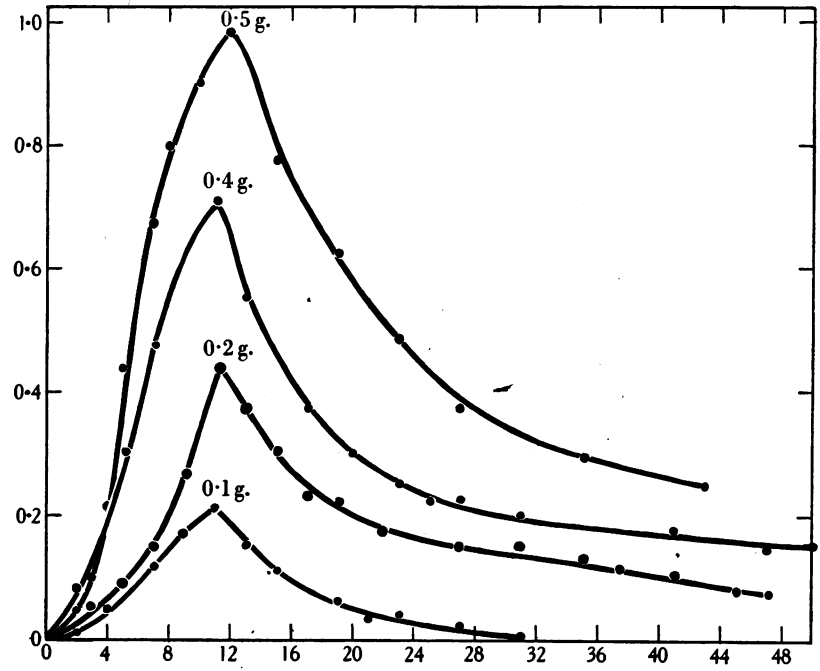

Fig. 9. Excretion of conjugated histamine by the dog after oral administration of histamine acid phosphate. Abscissae, hr.; ordinates, conjugated histamine in mg. histamine acid phosphate. The amounts of histamine administered are given on the figure. The total amounts excreted in the urine were 3·4, 6·4, 16·2 and 24·5 mg. from below upwards.

Comparison between the administration of conjugated and free histamine. In order to make a quantitative comparison between the administration of conjugated and free histamine and of meat, experiments were made in which these substances were administered to the same dog in amounts of approximately equal histamine content. In the series of experiments given in Table 8

TABLE 8. Showing the effect of administration of free and conjugated histamine and of meat on the histamine excretion

Substance and mode of administration	Excess histamine in urine μg.	Duration of excretion hr.	Remarks
Conjugated histamine by subcutaneous injection, 4·5 mg.	4365	9	All conjugated
Histamine acid phosphate by subcutaneous injection, 4·5 mg.	100	2	All free
Conjugated histamine orally, 4·5 mg.	2345	10	All conjugated
Histamine acid phosphate orally, 4·5 mg.	244	14	All conjugated
400 g. of ox meat containing 4·5 mg. histamine	3794	45	All conjugated

all substances were administered in amounts containing 4·5 mg. of histamine acid phosphate or its equivalent.

Table 8 shows the main points of interest concerning the excretion of conjugated and free histamine on their subcutaneous or oral administration. In addition, it shows that the amount of conjugated histamine excreted after administration of meat cannot be accounted for by the 4·5 mg. of histamine of the meat. Had the histamine of meat been the only source of histamine in the urine we would expect an excess excretion of not more than 5% of the amount administered or about 240 μg. instead of 3794 μg. The investigation of this point is being continued.

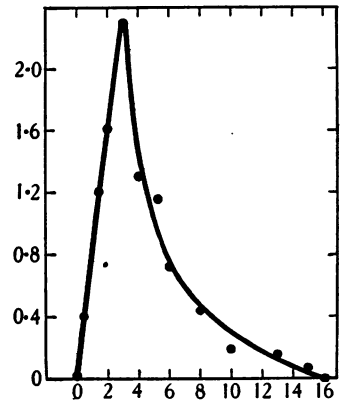

Fig. 10. Excretion of conjugated histamine by the dog after subcutaneous administration of an amount of conjugated histamine equivalent to 10 mg. histamine acid phosphate. Abscissae, hr.; ordinates, conjugated histamine in mg. histamine acid phosphate. The total amount excreted was 9·05 mg.

Discussion

The experiments described in this communication show that histamine is a normal constituent of urine of all the animals which have been so far investigated. In Herbivora it is excreted in a free form and in small amounts. In Carnivora it is excreted in a conjugated and inactive form from which free histamine can be released by hydrolysis.

The amount of conjugated histamine excreted in 24 hr. by the dog varies, depending on the diet, from less than 0·1 mg. to over 30 mg. of histamine acid phosphate, while the concentration of histamine acid phosphate may reach 100 μg./c.c. Subcutaneously injected histamine is excreted in the urine in negligible traces and in a free form; none is converted into the conjugated form. Orally administered histamine is excreted in the urine in a conjugated form to an extent not exceeding 5% of the amount administered. These results indicate that most of the injected histamine is destroyed or fixed in the body, the elimination by the urine playing an unimportant role. On the other hand, a part of the histamine which is absorbed from the alimentary tract is conjugated and excreted in the urine in an inactive form.

The possibility is not excluded that the conjugation of histamine takes place in the kidney although the experiments with injections of histamine point to the intestine or the liver as the more likely organs responsible for the formation of conjugated histamine. No conjugated histamine could be detected in the blood during histamine absorption, which places this substance amongst the low threshold bodies. This is confirmed by the fact that sub-

cutaneously injected conjugated histamine is quantitatively excreted in the urine in a few hours (Fig. 10). On oral administration about 60% of conjugated histamine is excreted in the urine.

Administration of meat greatly increases the excretion of conjugated histamine. The source of it is most likely the histamine present in the meat itself. Administration of histamine-free proteins do not lead to excretion of conjugated histamine. The question whether some other extractive substances of meat besides histamine participate in the formation of conjugated histamine has not yet been answered. Experiments now in progress may explain why on oral administration of histamine not more than 5% is excreted in the urine in the conjugated form, while a much larger proportion of the histamine content of meat can be recovered as conjugated histamine from the urine.

Inactivation of histamine by conjugation must be considered as the third method of detoxication of histamine. Together with the action of histaminase and the inactivation of histamine by the corpuscular elements of the blood, conjugation presents an effective method of dealing with this substance.

SUMMARY

1. A method is described for quantitative estimation of histamine in urine.

2. Histamine can be eliminated in a conjugated and a free form. Carnivora excrete mainly the conjugated and Herbivora the free form. In the rat both forms are present in varying proportions.

3. Administration of meat leads to a considerable increase in the excretion of conjugated histamine.

4. Orally administered histamine is eliminated in a conjugated form to the extent of about 5% of the amount administered. Injected histamine is eliminated in traces in a free form.

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