THE METABOLISM OF HISTAMINE.

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INTRODUCTION.

Since the discovery of histamine, the literature on its metabolism has expanded continually. It has been investigated and discussed with reference to the part it plays in physiological and pathological conditions. As the methods of investigation have become more sensitive, so a more detailed picture has been drawn of its functions in the body. As drugs have been discovered which influence its action in the living animal, so its functions seem to become more complicated and varied. However, in spite of all this investigation, the place and mode of origin of the body histamine is still ill-defined, the form in which histamine is carried in the blood stream is still uncertain, and the factors which control its release from the tissues in the normal animal are matters of controversy.

The present study is essentially an investigation of the metabolism of histamine in the normal animal. The rat was used throughout these experiments because its urinary excretion of histamine is known to be high. Several authors (2, 33, 42, 45, 47;) have made observations on the form in which histamine is excreted in the urine after its oral or parenteral administration in rats and mice. These workers used the measurement of the urinary excretion of histamine to determine the method by

which the body metabolises excess histamine. Anrep,
Ayadi, Barsoum, Smith & Talaat (3) measured the
urinary excretion of histamine in several different
species of animals, and observed the effects of
different diets, and of variations in the urinary
volume on the excretion of histamine. In so doing,
they made several deductions about the metabolism
of histamine in normal animals. In the present
experiments, it has, in fact, been shown that it is
justifiable to make such deductions by measuring
the excretion of histamine in the urine. Provided
that certain conditions are fulfilled regarding
constancy of diet and the excretion of an adequate
volume of urine, the excretion of histamine does
appear to reflect changes in the metabolism of
histamine in the body, and provides an easy method
of studying factors which may influence this
metabolism.

Interest in the possibility that the intestinal
flora might play an important part in the animal's
metabolism of histamine was first aroused by reading
the papers of Hanke & Koessler (19, 20;) on the
bacterial production of histamine. The powerful
action of the antibiotics and sulphonamides on the
bacterial flora of the intestine is well-known, and
it was a simple procedure to measure their effects

on the urinary excretion of histamine in the rat.
Since 1924 only Urbach (47) has suggested that
intestinal bacteria could influence the metabolism
of histamine under normal conditions.

A considerable amount of work has already been
done on the action of histamine liberators on the
metabolism of histamine in isolated tissues. The
efficiency of the histamine liberators has been
assessed in several different tissues, but no
observations have been made on the absolute
quantities of histamine released in the whole
animal by the different liberators. Measurement of
the urinary excretion of histamine following the
administration of various liberators enabled
comparisons of the efficiency of these substances to
be made, demonstrated differences in their actions
in the whole animal, and made it possible to use
the liberators as a tool for the investigation of
the metabolism of histamine in the normal animal.

The inter-action between adrenaline and histamine
which it has been suggested may occur physiologically
(44), can be confirmed only in the whole animal
under normal conditions. It is still under
discussion whether histamine is found in increased
quantity in the blood following the infusion of
adrenaline into man and operated animals.

Concentration in the urine of any histamine liberated from the tissues is easily detected in the rat. Thus the present method of investigation is satisfactory for determining whether adrenaline influences the metabolism of histamine in the normal animal.

The present work, therefore, consists of a description of the method used for measuring the urinary excretion of histamine, and observations on this in the normal animal. The effects of the oral administration of drugs affecting the bacterial flora of the intestine, and of the parenteral injection of adrenaline and the histamine liberators on the urinary excretion of histamine have also been investigated. The conclusions concerning the metabolism of histamine in the rat are discussed.

METHODS.

The methods which were employed for the investigation of the metabolism of histamine involved two procedures :-

(1) The collection of urine over suitable periods, and the extraction of the histamine from the different portions of the alimentary canal.

(2) The assay of the histamine in the urine and in the fluid extracts.

Experiments were performed to determine to what extent the experimental procedures to which the animals were subjected influenced the results. The validity of the results of the histamine assay on the urine and tissue extracts was also investigated in several different ways.

Throughout these investigations the albino rat was used as the experimental animal. The rats were obtained from four different sources; but before any experiment was performed they were kept for at least four days in the animal house in the department. Female rats aged 12 - 18 months and weighing 200 - 300 g. were used. They had each had about five pregnancies, and the last weaning had been completed 1-2 months before the experiments were started.

COLLECTION OF URINE.

Twenty-four hour and four hour collections.

The rats were kept in metabolism cages throughout the experiments, which lasted for three weeks or longer. Before any experimental values for normal 24-hour or 4-hour excretion were accepted, the rats were allowed three days to settle down in their cages. During this period the values for the urine volumes and for the histamine excretion were often rather inconstant. This inconstancy, at any rate during the first 24 hours, appeared to be due to adjustment to the water and food content of their diet. However, the rats became adapted to living in their cages during the first three days, and thereafter the urinary volumes remained fairly constant over 24 hour periods. Their histamine excretion remained correspondingly constant.

Diet. Throughout the experiment the rats received a standard diet and water **ad libitum**. They were weighed at the beginning and at the end of the experiments. Generally the rats maintained their weight during an experiment, but occasionally they lost 10 - 20 g. It was not found possible to give the rats rat-cake for their diet, as they scattered fragments of the cake over the floors of their cages, and it interfered with the urine collection.

This difficulty was overcome by using crushed rat-cake made up in bowls with water and edible gelatine. The recipe for the preparation of the diet was as follows:-

Rat-cake	17g.
Edible gelatine	5g.
Hot water	25ml.

Rat-cake manufactured by the North-Eastern Agricultural Co. Ltd. of Aberdeen was used in all the experiments. It has been estimated (25) that this cake contains:-

Protein	24.8%
Fat	1.7%
Carbohydrate (starch)	9.3%
Fibre	4.9%
Ash	9.5%

For the preparation of the experimental diet, crushed rat-cake from the bottom of the bin was used. The gelatine was manufactured by T. & G. Cox Ltd. Gorgie Mills, Edinburgh. The manufacturers state that its composition is about 98% gelatine, "the remainder consisting of a small amount of inorganic constituents". They also state that it should contain no fats, carbohydrates or histamine.

After preparation, the food was placed in the refrigerator for 8 - 12 hours, and then given to the rats. The cake was spread uniformly through the gelatine. The food was coherent and had a soft rubbery consistency. Metal plates, with holes

through which the rats could pass their heads only,
were placed over the bowls, to prevent them from
using their front paws for holding the food. By
these means scattering of the food over the floors
of the cages was reduced to a minimum, and little,
if any food reached the urine separators.

The water content of the food after it had been
in the refrigerator for eight hours was estimated

by weighing it before and after completely drying
it in the oven. The mean weight of three different
samples of food ready to feed to the rats fell from
44 g. to 28g. after drying. The rats therefore
received a water load of about 16 ml. in their
food. The histamine content of two different
samples of the food was estimated. A weighed
quantity of food was extracted with normal hydro-
chloric acid, boiled for one minute, and centrifuged.
The fluid was poured off, boiled in 20% HCl on a
sand-bath, then evaporated and reconstituted ready
for assay. This method is similar to that used for
tissue extraction of histamine. The mean histamine
content was 7.8μg./g. of food. A variable quantity
was left in the corners of the bowl, but this never
exceeded 8g. Most of the food was eaten during the
night, by which time it is likey that some of the
fluid in it had been lost by evaporation. Thus the

rats received probably not more than 12 - 16 ml.
water and 340 μg. histamine daily in their food.

Urine collection. The rats were kept in metabolism
cages made of wire mesh. The cages had a perforated
zinc floor and rested on 10" glass funnels.
Originally the urine was separated from the faeces
by pear-shaped glass balls resting in the collecting
vessels. This method of separation was fairly
satisfactory. The collecting vessels used were 50 ml
measuring cylinders. The tips of the stems of the
funnels rested just above the balls. Any faeces or
food which passed down the stems remained on the top
of the balls, while the urine passed round the sides
of the balls into the collecting vessels. If,
however, the food was not of uniform consistency, or
if the rats suffered from diarrhoea, the food or
faeces tended to lodge in the stems of the funnels.
Another disadvantage of this method was that the
stems had to be arranged very carefully to rest over
the centres of the balls; any large or sudden
movement of the rat tended to move the funnel, with
consequent faulty separation or loss of urine.
Another method of separation was tried, in which
the separator consisted of a small collecting funnel
2" in diameter, with a perforated zinc sieve. This
was found to be much more successful. It had the

advantage that the whole system was not affected by shaking or the movements of the rat, and it held up any food or faeces passing into the collecting funnel. University College Hospital separators were also tried. They consist of wide glass tubes, down the inside of which the urine passes from the collecting funnels, so that it is collected and passed into a small tube attached to the side of the main tube. The faeces dropped unobstructed down the centre of the main tube. These suffered from the disadvantages of the glass balls, and loss of urine tended to occur down the centres of the separators.

The second method of separation was found to be the simplest and the most practical. Provided the food was properly prepared and the cage floors were of suitable sized mesh, minimal food and faeces passed on to the separating mesh, and none into the urine. This method was used in the experiments in which 24 hour and 4 hour collections of urine were made. Initially, drinking water was provided in bottles, and the rats drank by sucking water through an angled glass tube. This method was abandoned, however, as the water tended to drip out of the bottles. Water was subsequently supplied in containers attached to the sides of

the cages, but outside the collecting funnels, so
that no mixture of water with the urine occurred.
Each morning at 0930 hours the food and faeces
were removed from the metabolism cages , the
collecting funnels were washed with soap and warm
water, and allowed to dry. Fresh food was put in
the cages and the urine collecting vessels changed.
When the urine was being collected at four hour
intervals the urine collecting vessels were
changed six times in the twenty four hours;
otherwise the experimental conditions were similar
to those described above for the twenty four hour
collection periods. Period 1 started at 0930
hours each day. Normal urine had a pH of about
6.5, though it varied from 5 to 7. The pH of the
urine was adjusted to 7.8 with normal NaOH, and
its volume measured. No preservative was added to
the vessels in which the urine was collected,
because it was found that if the urine was kept in
the refrigerator, no change occurred in the
concentration of the histamine in the urine. It
is also considered doubtful (2) whether any
preservative is necessary to prevent bacterial
growth in the urine.

Half-hour collections.

Measurements of the urinary histamine excretion

over 24 hour periods are suitable for the investigation of factors which cause gradual changes in the metabolism of histamine. In order to investigate more rapid changes, a method has been developed in which the histamine excretion is measured during 30 minute periods for several hours at a time.

The rats were placed on wire mesh platforms on eight inch glass funnels. In order to initiate and maintain an adequate urinary output, the rats were given a water load of 5% of their body weight by stomach tube. This was repeated half an hour later, and half an hour after this again a volume of water was given equivalent to the volume of urine passed in the first hour. Thereafter a volume of water was administered at hourly intervals corresponding to that excreted in the previous hour. The urine was collected at half-hourly intervals in graduated tubes and its volume measured to 0.1 ml. Luke warm distilled water was administered to the rats through a rubber catheter passed into the stomach through a glass gag held in the rat's mouth. Before any experiment was performed, a water load was given to the rats on the previous day, and water was administered at half-hourly intervals for three

or four hours in order to train the rats to swallow the tube and to sit quietly on their platforms. Administration of the water was always performed with the rat sitting on the platform, so that no urine was lost should micturition occur during the operation. Initially, emptying of the bladder at the end of the collection period was produced by holding the animal firmly round the chest until panting began, when the bladder was completely emptied. Compression of the chest was always preceded by gently stroking the back of the animal's neck. Subsequently this milder form of stress was sufficient to cause reflex emptying of the bladder.

The urine output remained fairly constant at about 6 - 10 ml. during each half-hour period after the first hour. After the first hour the urine was kept, its volume measured and the assay of histamine performed. The urine collections have been continued for as long as eight hours; and throughout this period the urine output remained remarkably constant, and the rats showed no ill-effects as the result of water administration. This method was used for the investigation of histamine liberators, but no rat was ever used more than once for this purpose, as it has been

reported (12) that the restoration of the skin histamine in rats which had been given Compound 48/80 was still incomplete forty days after its injection.

EXTRACTION OF THE TISSUES FOR HISTAMINE.

Extracts were made of the stomach, small and large intestines. A modification of the method of Douglas, Feldberg, Paton & Schachter (7) was used in order to make it applicable to the alimentary canal of the rat, and the method was further modified in order to estimate the histamine concentration in the contents of the intestinal lumen.

Prior to death the rats had been fed on Aberdeen rat cubes and water, _ad libitum_. The animal was killed with ether, Immediately after death the alimentary canal was dissected out as rapidly as possible, and its three parts were weighed with their contents. The stomach and intestines were split longitudinally, the contents lifted out with forceps, and the lumen washed with saline. The stomach and intestines were hung up and allowed to drain for about four minutes, and then weighed again. The contents and intestinal washings were kept and shaken together vigourously for several minutes. The extraction procedure was then carried

out as described for the gut, (7), and a colourless
opalescent fluid was finally obtained. The
intestinal contents and washings were brought to
pH 2 by the addition of normal HCl, and were then
treated by the same method.

After the gut wall and contents had been
separated by centrifuging, and the extracts had been
brought to pH7 by 2N. NaOH, the final volumes were
measured. It was found that if the fluid was then
assayed, it occasionally contained a substance
which depressed the contraction of the guinea pig
ileum to the standard solution of histamine. In
order to eliminate this substance, and also to
make certain that all the conjugated histamine was
freed, the extracts were boiled with strong HCl
for an hour. This procedure is described later.

PREPARATION AND ASSAY OF HISTAMINE-CONTAINING FLUIDS.

Histamine assay.

All the assays were performed by comparing the
contraction of the isolated guinea pig's ileum
caused by a standard solution of histamine with
those produced by the active substance in the
fluids tested. In all the assays the ileum was
suspended in Tyrode's solution containing atropine
in the concentration of 10^{-7}. A 2 ml. bath was

used, and the bath temperature was maintained at
35 - 36°C. The standard histamine solution
contained 0.2μg. histamine base per ml. and 0.10 ml.
of this solution was added to the bath. The
solution remained in the bath for 15 seconds, and
was then washed out. The gut was allowe 1.25
minutes to recover before the next active solution
was added.

Preparation of urine.

The concentration of free histamine in rats'
urine is usually so high that it can be measured
by direct addition of diluted urine to the organ
bath. The urine from 24 hour collections was
diluted 1:25 in order that the contraction of the
guinea pig ileum which it caused would be comparable
to that produced by the standard histamine solution.
The urine from the rats which had received a
water load was assayed without dilution, as the
concentration of histamine in it was so low. The
urine was always filtered before dilution and after
measurement of the volume. The evidence, on which
is based the assumptiom that the active substance
assayed in this way was free histamine, is given
later.

The urinary excretion of conjugated histamine
was estimated by obtaining the difference between

the total histamine, after the hydrolysis of the
acetyl histamine, and the free histamine. Hydrolysis
was performed on 1 ml. of urine by the method of
Roberts & Adam (38); the hydrolysate was evaporated
to dryness at 60 - 70°C. in a water bath under
suction, and the residue was dried off in 10 ml.
absolute alcohol. Finally it was taken up in
normal saline, neutralised with NaOH, and the final
volume made up to 25 ml. In the assay, the
concentrations of histamine in the unhydrolysed
and then in the hydrolysed solutions were determined
by comparison with the standard solution. Finally
it was confirmed that these volumes of the unknown
solutions produced equal contractions of the gut.

Preparation of tissue extracts.

In order to hydrolyse all the conjugated histamine
in extracts of the gut wall or contents, and also
to destroy the substances interfering with the
assay, the extracts were boiled with HCl, as
described above for urine. The final solutions of
the wall were yellowish brown and clear; those of
the contents were dark brown.

All the wall extracts were assayed first, and
then the contents. In spite of this it was found
that some contaminating substances were present in
the solutions - though certainly not in such high

concentrations as when hydrolysis was omitted. The histamine could not be assayed in solutions which contained the gut-depressing substance, but this occurred on only two occasions.

VALIDITY OF RESULTS.

A number of tests were performed in order to confirm that the active substance assayed in the urine was, in fact, histamine. Other experiments, designed to determine the influence of the different procedures employed on the excretion of histamine by the rats and on the validity of the results of the assays, were also carried out.

The values for the urinary excretion of histamine might have been influenced in two ways by the experimental techniques employed. The experimental procedures to which the rats were subjected could have influenced the volume of urine passed during the collection periods, and thus influenced the urinary excretion of histamine, as a result of physiological and psychological factors other than those caused by the drugs. The assay of the histamine might have been affected by the excretion of the drugs in the urine in concentrations sufficient to affect the sensitivity of the assay.

Identification of active substance in urine.

Addition of the urine diluted 1:25 to the bath containing the guinea pig ileum produced a contraction exactly similar to that produced by the standard histamine solution. Addition of atropine sulphate to the Tyrode's solution in the bath, to give a concentration of 10^{-7}, did not affect the size or quality of the contractions produced by the addition of diluted or undiluted urine. Contraction of the ileum was abolished by mepyramine; and the responses to the urine , diluted or undiluted, and to an equipotent dose of the histamine standard solution returned at the same rate. The record from a typical experiment is shown in Fig. 1.

In a few experiments the histamine equivalent of the urine was estimated after adsorption on Amberlite I.R.C. 50. A column of the OH form of the resin, 5 cm. in length and 1 cm. in diameter, was prepared and washed with distilled water until its pH was reduced to 7.4; 40 ml. of 0.2 molar NaH_2PO_4 (pH 7.1) were then passed through, 10 ml. at a time, at the rate of 1 ml. per minute. The pH of the eluate initially rose to 8.3, but during the third wash fell again to 7.4. The rate of flow was then reduced to 0.5 ml. per minute, and the solution containing the histamine was poured on to the column
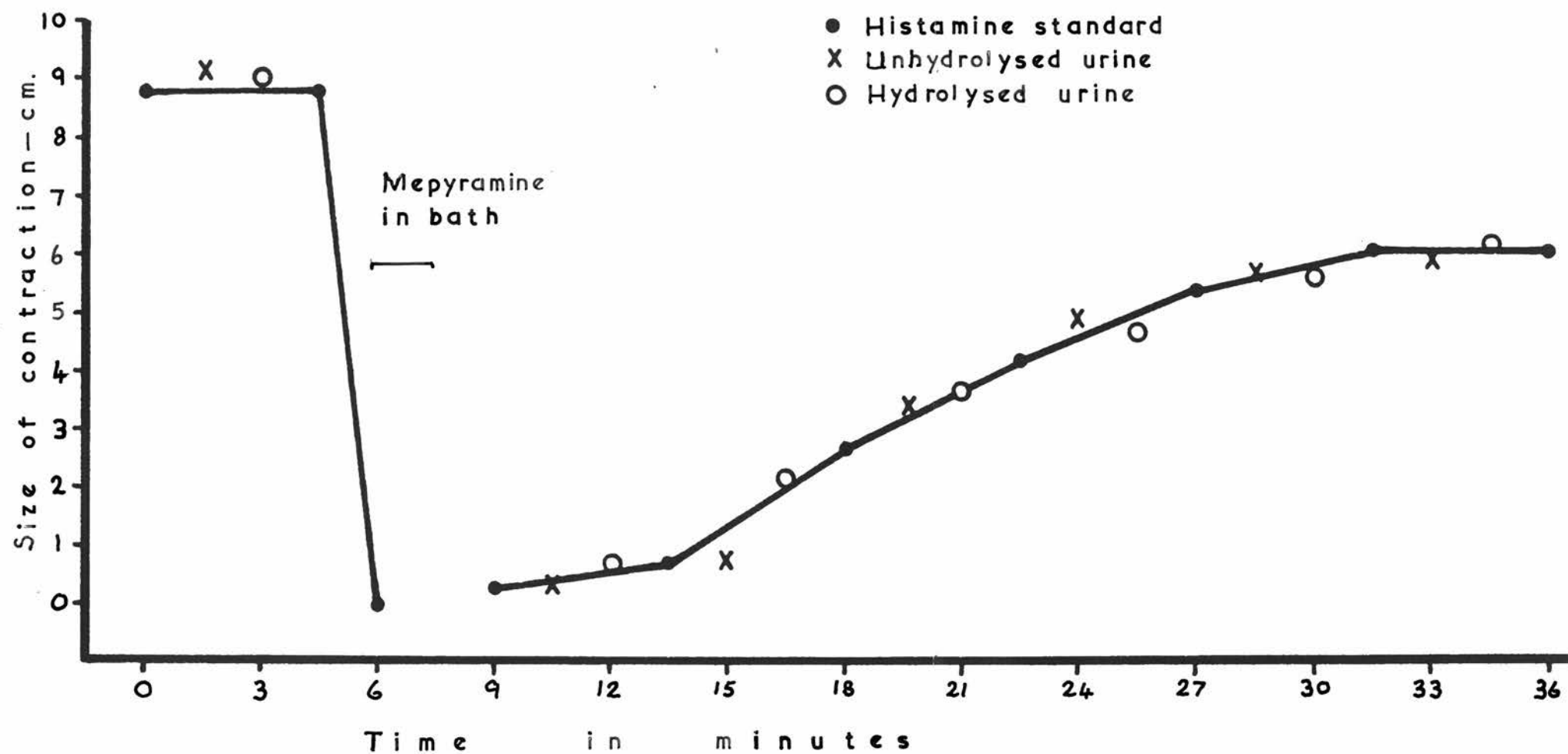
Fig. 1.

Effect of mepyramine on the size of contraction
of guinea pig ileum to doses of histamine standard
(0.2 µg./ml.) and to hydrolysed and unhydrolysed
urine.

and washed through with five washes of 2 ml. of distilled water. Elution was performed by passing 4 ml. of 2N. HCl, followed by 10 ml. 1/100 N.HCl through the column, which reduced the pH to 2. The pH of the eluate was adjusted to 7 with 4N. NaOH and its volume was measured. The final volume was about 16 ml.

When urine was passed through the columns it was diluted 1 in 3 in order to reduce the salt concentration to a level at which it would be unlikely to interfere with the adsorption of histamine. Six mils of urine were used, made up to 18 ml. with distilled water. The eluate was finally diluted with Tyrode's solution before the assays were carried out. Four control experiments were carried out in which 25 µg. of histamine were passed through the columns in 30 ml. of 0.02 N. NaH_2PO_4. The recoveries of histamine were 25.6, 23.3, 24.4, and 25.6 µg, giving a mean of 24.7µg. This is equivalent to a recovery of almost 100%.

A comparison between direct estimates of the histamine equivalent of rats' urine and estimates obtained after adsorption on Amberlite is shown in Table 1.

There was no significant difference between the results obtained by these two methods. It was

Table 1.

Comparisom of the values for histamine excretion by direct assay of the urine and assay following adsorption of histamine on columns of I.R.C. 50.

Rat.	Histamine excretion (µg./24 hr.)	
	Assay after adsorption.	Direct assay.
1.	64	65
2.	157	156
3.	182	195
Mean	134	139

considered that these results justified the use of
the method of direct assay by the introduction of
the urine after suitable dilution into the organ
bath. The results in themselves do not prove that
the active substance in the urine was histamine,
but they do show that it resembled histamine in the
fact that it was adsorbed at pH 7.4 and eluted at
pH 2. This at least suggests that it was an organic
base.

Boiling the urine for one hour in 20% HCl, (38),
increased its histamine equivalent. This is stated
to cause complete hydrolysis of acetyl histamine.

It is probable that this treatment would also
destroy any other substances in the urine which
would cause contraction of the gut. (5). Addition
of mepyramine to the bath abolished the response of
the gut to the hydrolysed solution, and the response
returned at the same rate as the response to histamine.

It is considered (7) that histamine itself is
responsible for the histamine-like effects on the
guinea pig's ileum of extracts of the alimentary
canal. The added precaution of boiling the extracts
with HCl made the elimination of any impurities
which might have interfered with the assay more
certain. The addition of mepyramine to the bath
containing the guinea pig ileum abolished the effect

produced by the extract, and the sensitivity of the
preparation to histamine returned together with that
to the principle present in the extract.

Thus it is concluded that the active substance in
the urine and extracts which caused contraction of
the guinea pig ileum was histamine, and that the in
increase in the concentration of histamine produced
by boiling the urine in strong acid was due to the
hydrolysis of the acetyl histamine present in the
urine.

Effects of various experimental procedures on urinary histamine excretion.

During the course of different experiments in
which the 24 hour and 4 hour urinary histamine
excretion was measured, various drugs were given to
the rats by stomach tube or by subcutaneous injection.
The stomach tube was either a soft rubber catheter,
introduced through a glass gag, or a No. 3 Emesay
gum-elastic catheter. No volume greater than 2 ml.
was ever introduced. Daily administration of
distilled water by stomach tube did not affect the d
daily excretion of histamine, although for the first
day or two the urinary volume might diminish
slightly. Two mils of water were always administered
from the beginning of the experiments, so that by
the time the drug was given, the animals had become

used to the procedure. The values for the daily histamine excretion and urine volumes from one rat for a period of eight days, during which the rat received 2 ml. water daily by stomach tube, are given in Table 2.

Intraperitoneal injection of normal saline did not affect significantly either the urinary volume or the urinary excretion of histamine in rats which had received a water load and in which the histamine excretion was being measured at 30 minute intervals. The mean values for the histamine excretion and histamine concentration, and the urine volumes in two rats which had received a water load are shown in Table 3. In this experiment the histamine excretion began to diminish three and a half hours after the injection of the saline, that is, five and a half hours after the beginning of the experiment, though the urine volumes did not alter significantly. The histamine concentration also diminished slightly towards the end of the experiment. There was no significant difference between the control values and the values for the excretion up to the end of the third hour after the injection of the saline. The histamine excretion and urine volumes remained fairly constant for five hours, and generally for considerably longer, in each experiment.

Table 2.

Daily histamine excretion and urine volume from one rat which was receiving 2 ml. of water daily by stomach tube.

Day.	Histamine excretion. µg./24 hr.	Urine volume. ml.
1.	117	21.2
2.	88	14.0
3.	102	18.4
4.	97	23.2
5.	136	24.5
6.	115	32.3
7.	115	27.5
8.	109	30.5
Mean	110	23.9

Table 3.

Half-hour excretion of histamine in the urine;
0.5 ml. saline injected intraperitoneally at zero
time. M.C. is mean of the control values during the
first two hours.

Time hours.	Histamine Excretion µg.	Urine vol. ml.	Histamine Concentration. µg./ml.
1.5	1.4	5.9	0.2
1.0	1.7	6.6	0.3
0.5	2.0	6.6	0.3
0	1.6	6.6	0.3
0.5	1.6	5.2	0.3
1.0	1.7	7.8	0.2
1.5	1.5	4.8	0.3
2.0	1.4	2.9	0.3
2.5	1.7	7.3	0.2
3.0	1.4	5.1	0.3
3.5	1.0	4.5	0.2
4.0	1.0	6.6	0.2
4.5	1.0	4.8	0.2
5.0	1.0	5.8	0.2
M.C.	1.7	6.4	0.3

Influence of the drugs on the assay of histamine.

The drugs themselves which were administered to the rats, or their metabolic break-down products, were generally excreted in the urine. Experiments were performed to determine the concentration at which the drugs would interfere with the assay of histamine.

The effects of the drugs were tested in two ways. The drug in 0.10 ml. of Tyrode's solution was introduced into the 2 ml. bath and left there for 90 seconds. It was then washed out and the histamine standard added in the usual way. When the response to the histamine standard had returned to normal, 0.10 ml. of the drug solution was added to the bath at the same time as the histamine standard. The drugs were left in the bath for 15 seconds in the usual way, and then washed out. This was repeated three or four times in succession.

1) Chloramphenicol. It is stated that chemical analysis of the urine over a 24 hour period in the rat never disclosed the presence of nitro-compounds accounting for more than 24.3% of a given daily dose of chloramphenicol (15). This would give a concentration of 0.016 mg./ml. of chloramphenicol in the diluted urine as it was used for assay. At a concentration 350 times greater than this , a

slight but definite effect was observed in the bath

tests on the sensitivity of the gut to histamine

(Fig. 2.) .

2) Aureomycin. It has been shown that rats excrete

2% of an oral dose of 20 mg./kg. of aureomycin

during the first six hours following administration

(21). Its excretion continues actively for six to

twelve hours, though no further figures for higher

doses have been given. On the assumption that 5%

of the administered dose is excreted in the urine,

a concentration of 0.003 mg./ml. would be obtained

in the diluted urine as it was used for assay. At

a concentration 1000 times as great as this, no

inhibiting effects were observed in the bath tests.

3) Phthalyl sulphathiazole. It is stated that 3.5%

of the administered drug is excreted in the urine

(27). This would give a concentration of

approximately 0.025 mg./ml. in the urine as it was

used for assay, the rats having received a dose of

250 mg. daily. No effect was observed on the

response of the ileum at 100 times this concentration.

4) Adrenaline. It was found that the intra -

peritoneal injection of adrenaline resulted in the

excretion of sufficient adrenaline in the urine to

interfere with the assay of histamine on the

guinea pig's ileum. The excretion of adrenaline
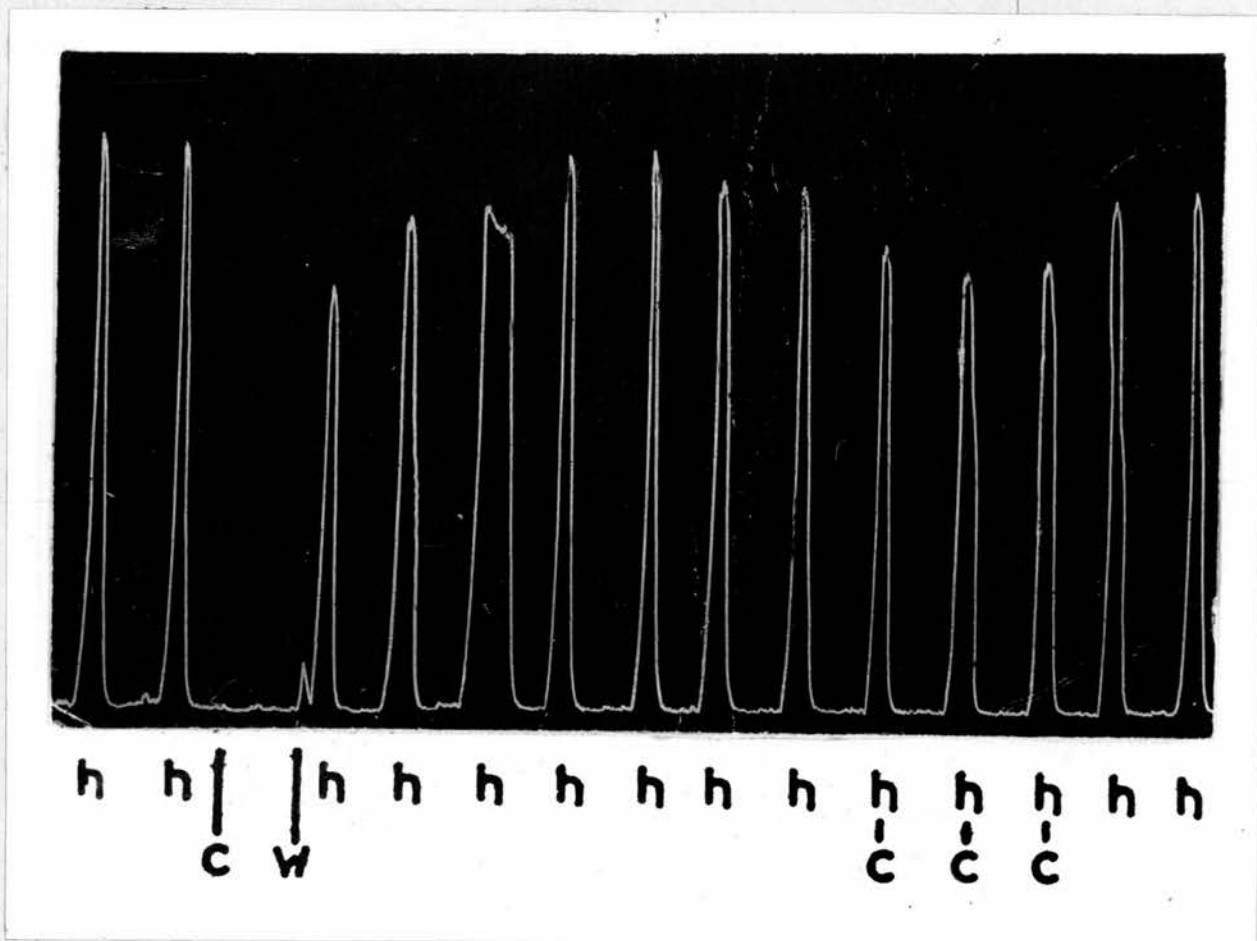
Fig. 2.

Inhibition of the response of the guinea pig
ileum to histamine, caused by chloramphenicol.

h : addition of histamine standard to the 2 ml.
bath; 0.1 ml. of a solution containing 0.2 µg./ml.

c : addition of chloramphenicol; 0.1 ml. of a
suspension containing 5.6 mg./ml.

After the second contraction chloramphenicol
was added and remained in the bath until it was
washed out at w.

began during the first half hour following its

injection, and depending on the size of the dose,

continued for three hours or longer. The excretion

of adrenaline was detected by its effect in

inhibiting the response of the guinea pig's ileum

to histamine during the assays, and a method of

destroying the adrenaline in the urine while

leaving the histamine intact was therefore employed

in all the experiments in which the effect of

adrenaline on the urinary excretion of histamine

was observed.

The following method was used :- After its

volume had been measured, solid Na_2CO_3 was added

to the urine until its pH was brought to 8. The

urine was placed in a boiling water bath for half

a minute, and after it had cooled down it was

ready for assay. In order to test the value of the

method, Adrenaline (10^{-5}) was added to a solution

of histamine (5×10^{-6}) in Tyrode's solution. After

treatment with Na_2CO_3 the solution had the same

action on the ileum as the same ammount of histamine

without the addition of adrenaline. Thus in the

concentrations to be expected in the urine,

adrenaline caused no interference with the assay of

histamine. The effect of the destruction of the

adrenaline in the urine on the excretion of histamine

is shown in Fig. 3. The effect of the adrenaline
is obvious with large and small doses, but its
interference during the histamine assay is
considerably more pronounced with the larger doses.
5) Propamidine isethionate also affected the
sensitivity of the ileum to histamine , at low
concentrations. This effect is discussed below.

MODE OF EXPRESSING RESULTS.

In all the experiments in which the excretion
of histamine was measured at intervals of 24 hours
and 4 hours, groups of three rats were used. In
the experiments in which the excretion was measured
half-hourly, two rats were used in each investigation
unless otherwise stated. In order to compare the
results from different groups of rats, the mean
excretion of histamine during the control periods
preceding the administration of the drug has been
taken as 100%, and the excretion for each period
throughout the experiment has been calculated as
a percentage of this. This procedure has been
followed in the majority of the experiments. In a
few, the results have been expressed as micrograms
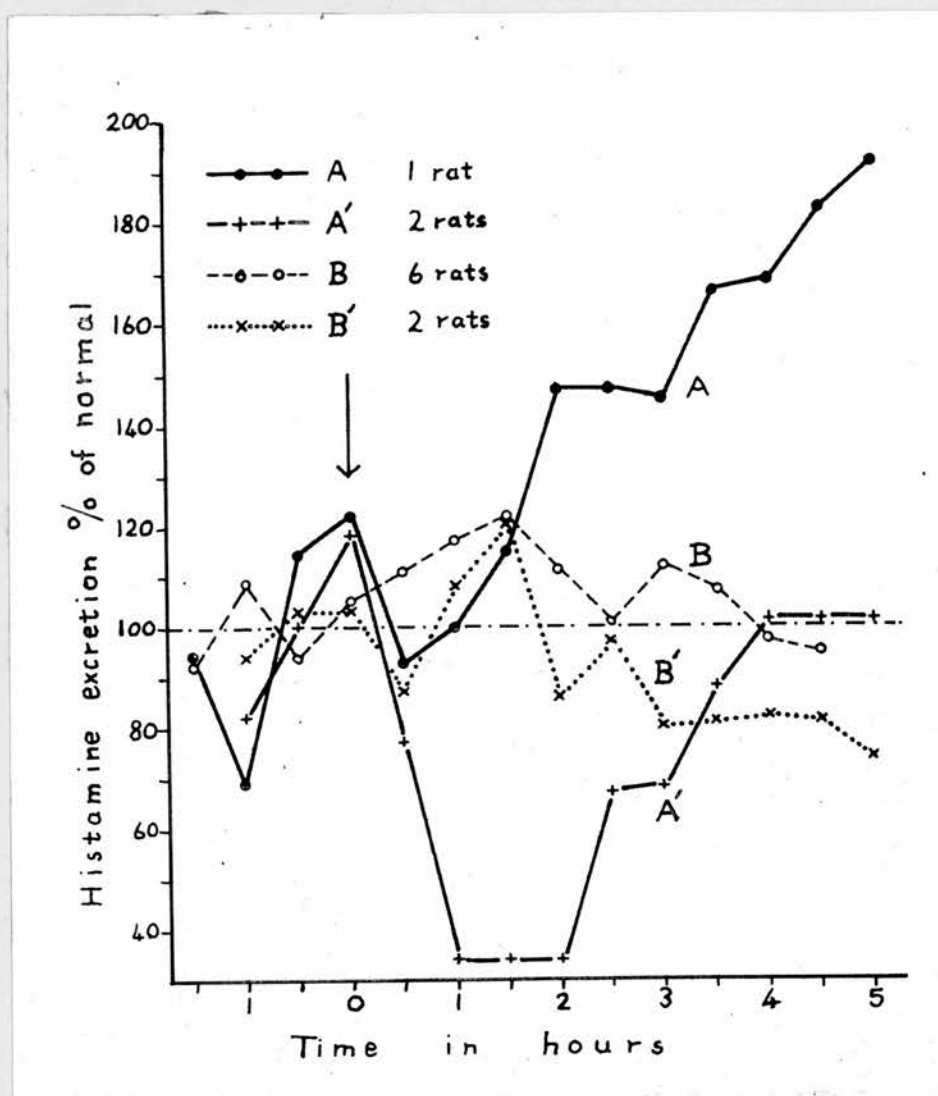of histamine excreted in each collection period.

Fig. 3.

The effect of the destruction of adrenaline on the apparent urinary histamine excretion: adrenaline given by intraperitoneal injection at the time shown by the arrow.

A : adrenaline destroyed) adrenaline 370 µg./kg.
A': adrenaline present) given I.P.I.

B : adrenaline destroyed) adrenaline 37 µg./kg.
B': adrenaline present) given I.P.I.

RESULTS.

The experimental results will be presented in four sections. The first section consists of the results obtained from observations on the urinary excretion of histamine under normal conditions, and on the concentration of histamine in the contents and walls of the alimentary canal. Certain conclusions regarding the constancy of the urinary excretion of histamine in the rat, and the normal variations which it undergoes, can be drawn from these observations. In the second section the experimental results obtained after the administration of various drugs with potent bacteriostatic effects on the organisms in the lumen of the alimentary canal, are presented. These results enable certain conclusions to be drawn concerning the origin of histamine in the normal animal. Adrenaline affects the urinary excretion of histamine; the results obtained from the administration of adrenaline on the urinary excretion of histamine are presented in section three. Finally, the results obtained from the administration of different histamine liberators on the urinary excretion of histamine are presented in section four.

SECTION 1.   THE METABOLISM OF HISTAMINE.

Twenty-four hour and four hour excretion of histamine.

The twenty four hour excretion of histamine remained fairly constant from day to day in individual rats, and also varied little between different rats. This is illustrated in Tables 2 and 4.

It was not possible to determine over the limited weight range of the rats used in these experiments whether there was any correlation between the excretion of histamine and the weight of the rats. However, it was quite clear that rats of this weight always excreted approximately the same quantity of histamine every day under the same conditions. In later experiments, in which the effect of factors influencing the excretion was measured, the changes in the histamine excretion were of a magnitude sufficient to exclude any possibility that they could have been due to day-to-day variation in the normal excretion.

The percentage recovery of free and conjugated histamine in the urine following its administration by oral and parenteral routes was estimated. Two different doses of histamine, dissolved in 1 ml. distilled water, were given by stomach tube to two

Table 4.

Twenty- four hour excretion of histamine in the
urine from three rats, expressed in μg./24 hr. period.

Day.	Rat 1.	Rat 2.	Rat 3.	Mean.
1.	95	114	175	128
2.	96	107	148	117
3.	90	116	142	116
4.	85	125	121	111
5.	92	128	142	121
6.	90	114	151	118
7.	94	110	153	119
Mean	92	116	147	

different groups of rats in the morning for several days. About two and a half hours later the rats began to pant and twitch occasionally. Their ears, tails, and paws became flushed. This reaction continued for two or three hours, after which it gradually disappeared. The reaction occurred with 10 mg. and 5 mg. of histamine, and appeared to be equally severe with both doses. The effects of the administration of the different doses of histamine are shown in Table 5.

Assuming that excretion continued for 24 hours after the last dose had been given, approximately the same proportion of the administered histamine was recovered as free histamine in the urine of both groups of rats; 5.6% and 6.0% were recovered from the rats receiving 10 mg. and 5 mg. respectively. Of the administered histamine, 2.4% was recovered as conjugated histamine, making a total recovery from the rats receiving 10 mg. of 8%.

The effect of a subcutaneous injection of histamine on the urinary excretion was also observed. Histamine acid phosphate was injected in normal saline, after neutralisation with NaOH, into three rats for four days. The excretion of free and conjugated histamine was measured. The results are given in Table 6. It was assumed that

Table 5.

The effect of the oral administration of histamine
on the urinary excretion of free and conjugated
histamine. Mean histamine excretion expressed as
μg./24 hr. collection period. Values are mean values
for the excretion from the rats used in each
experiment.

Day.	Dose of histamine mg.	Urinary excretion Free	Dose of histamine mg.	Urinary excretion. Free	Conjugated
1.	-	132	-	63	13
2.	-	132	-	73	12
3.	-	151	-	88	15
4.	5	434	10	755	187
5.	5	483	10	665	361
6.	5	363	10	632	206
7.	-	184	10	610	245
8.	-	122	-	144	15
9.			-	102	11
10.			-	84	25
No. of rats	2.		3.		

Table 6.

The effect of the subcutaneous administration of histamine on the urinary excretion of free and conjugated histamine. The mean histamine excretion is expressed as µg./24 hr. collection period from three rats.

Day.	Dose of histamine by s.c.i. mg	Urinary excretion. Free	Conjugated
1.	-	101	5
2.	-	83	17
3.	10	2477	0
4.	10	2682	0
5.	10	2183	0
6.	10	2231	0
7.	-	137	2
8.	-	134	13
9.	-	141	0
10.	-	112	0

histamine excretion had returned to normal on the fourth day after the last dose had been given. The recoveries from the three rats were 24.1%, 20.7%, and 24.8%, giving a mean recovery of 23.2%.

Comparison of these figures shows that the recovery after injection is more than four times as great as after oral administration of the drug. The histamine loss in the faeces after oral administration has not been measured, but this may well be large, and account for some of the difference. However, it is certain that at least three-quarters of the drug must be metabolised by the animal before final excretion in the urine, or else be excreted by some other route. During the period o f injection, no histamine was excreted in the conjugated form, in contrast with the excretion of 2.4% of the dose after oral administration. This suggests that conjugation must occur in the alimentary canal.

Estimation of the histamine excretion four-hourly from two rats was performed for two days. The values for the histamine excretion are shown in Fig. 4, together with the values for the urine volumes and the concentration of histamine in the urine. The histamine excretion is at its maximum just before midnight, and during the early hours of
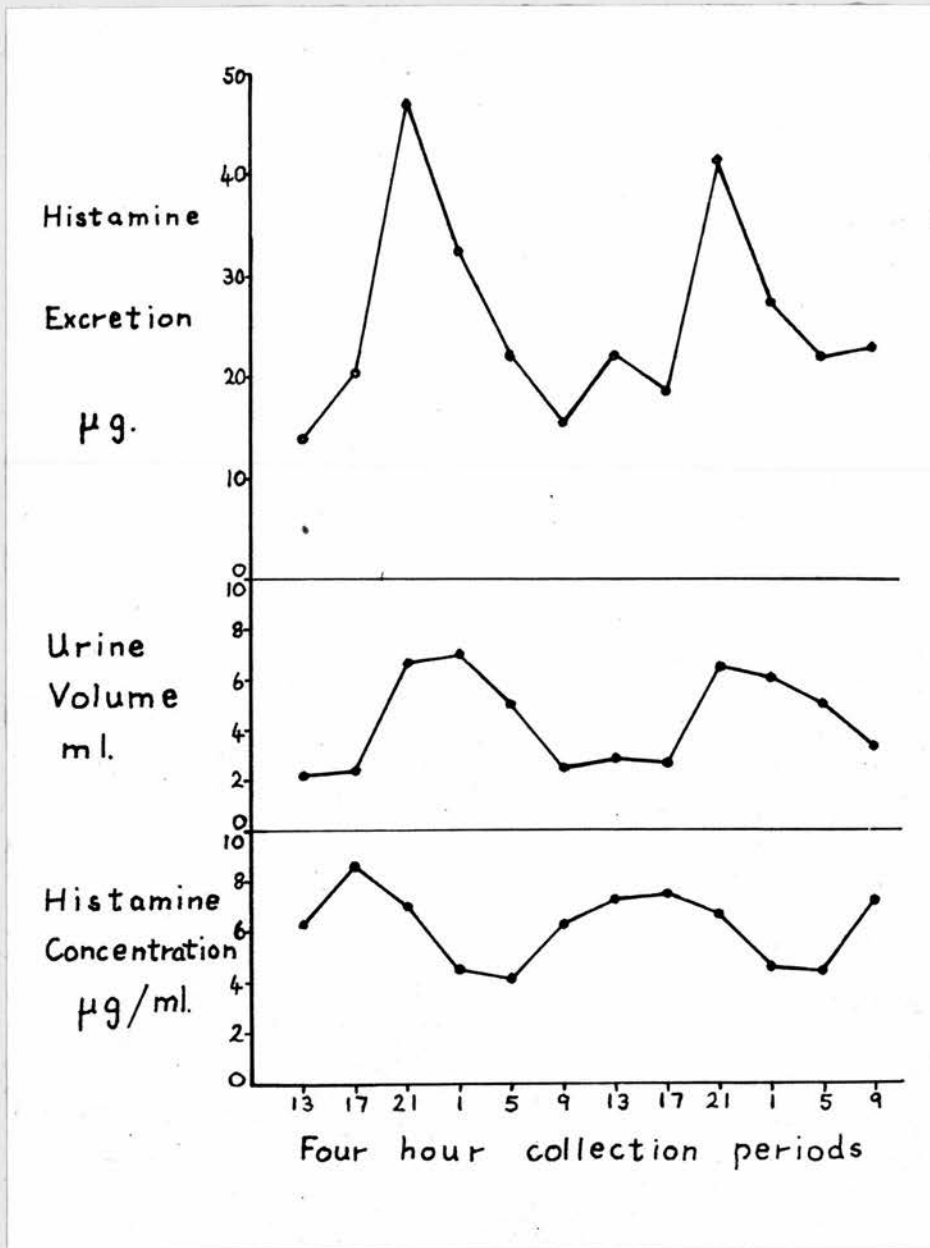
Fig. 4.

Histamine excretion, urinary concentration of
histamine and the volume of urine passed four -
hourly during a period of two days.

the morning; it reaches its minimum about 12 hours
later. The excretion of histamine fluctuated
throughout the 24 hours in the same way as the
excretion of urine. The concentrations of histamine
varied relatively little throughout each 24 hour
period.

Half-hour excretion of histamine.

The urinary excretion of histamine per half-
hour period varied considerably between individual
rats. The highest values obtained were in the
region of 4 - 5 µg., and the lowest were about
0.4 - 0.5 µg. The histamine excretion varied little
in any individual rat during the first five or
six hours. Thereafter in some animals there was
a tendency for the excretion to diminish. This
decrease was more pronounced in the animals which
initially had an excretion of more than 1.5 ug.
per half hour period, and generally it did not
occur at all in animals which excreted less. A
spontaneous increase in the histamine excretion
was never observed. Some fluctuation in the daily
excretion of histamine tended to occur. The
values for the histamine excretion in a series of
successive experiments are shown in Tables 7 and 8.

The intraperitoneal injection of histamine
caused an immediate increase in the excretion of

Table 7.

The histamine excretion in one rat during a
water load on four successive days. Histamine
excretion is expressed in µg./half-hour period.
Water given at -1, -0.5, 0, 1, and 2 hours as
described in the text.

Day.	Time in hours.						Mean
	0.5	1.0	1.5	2.0	2.5	3.0	
1.	1.4	1.2	1.3	0.9	1.2	1.9	1.3
2.	1.8	1.1	1.7	2.4	1.7	1.9	1.8
3.	1.7	2.0	1.8	1.7	1.8	1.7	1.8
4.	1.7	0.8	0.7	0.8	-	-	1.0

Table 8.

Mean half hour excretion of histamine from five
rats on four successive days during a water load.
Histamine excretion is expressed in μg./half hour
period and each value is the mean of six determinations
on each day.

Day	Rats.					Mean.
	1.	2.	3.	4.	5.	
1.	1.4	5.7	1.5	1.5	1.3	2.3
2.	3.4	2.1	1.4	1.2	1.8	2.0
3.	3.3	3.1	1.4	1.4	1.8	2.4
4.	3.3	1.7	3.1	1.4	-	2.1

histamine, which had returned to normal again an hour and a half after injection. The experiment was performed on two rats, and the individual values for the histamine excretion from both rats are shown in Fig. 5. In all the other experiments the degree of variation in the excretion between individual animals was similar to this. Twenty micrograms of histamine were injected, and 1.47 μg. of histamine in excess of normal, calculated from the mean values of both animals, had been excreted in an hour and a half. This corresponded to 7.4% of the dose administered. This experiment demonstrated how rapidly histamine is absorbed after intraperitoneal injection, and how quickly it appears in the urine. All the excess histamine which was not metabolised in the body had been excreted in one and a half hours. This dose of histamine appeared to cause no discomfort or any other pharmacological effect.

Comparison of the histamine excretion in the same animals in experiments in which the excretion was estimated during periods of 24 hours and at half-hourly intervals is of interest. It enables the influence of the urine volume on the histamine excretion to be assessed.

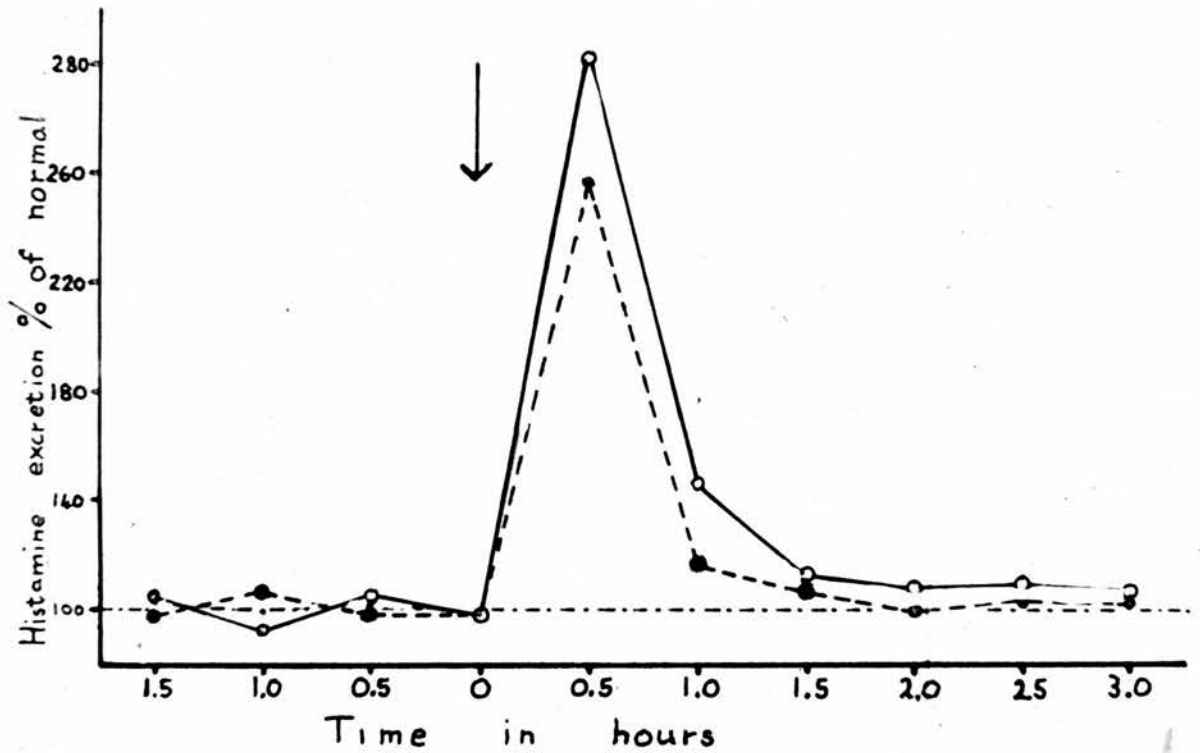The twenty four hour histamine excretion was

Fig. 5.

The increased urinary excretion of histamine from two rats produced by the intraperitoneal injection of 130 µg./kg. of histamine at the time shown by the arrow.

determined in two rats for three days. The mean
excretion from the two animals was 101.6 µg./24
hour (± 24.5 s.D.) . The mean volume of urine
passed during the same period was 26.1 ml. The
histamine excretion was determined from the same
rats at half-hourly intervals following a water
load. The experiment commenced at 0830 hours,
and was maintained for three hours. The mean exc
excretion from the two animals was 1.66 µg./half
hour period, and the mean urine volume was 6.4 ml./
half hour period. If this quantity of histamine
had been excreted throughout the 24 hours, the
daily excretion would have been 79.5 µg./ 24 hr.
with a standard deviation of 13.9, and the urine
volume would have been 307.2 ml. There is no
significant difference between the daily histamine
excretion determined in these two ways. This
confirms the view that the urinary volume does
not influence the excretion of histamine (3).

Histamine concentration in the alimentary canal.

The histamine concentrations in the walls and
contents of the stomach, small and large intestines
were measured. (Table 9). There was no significant
difference between the histamine concentrations
in the walls of the small and large intestines,
but the concentrations in the gastric walls were

Table 9.

The histamine content of the wall and contents
of the alimentary canal. Values expressed as
μg./g. tissue.

	Stomach.		Small intestine.		Large intestine.	
	wall	contents	wall	contents	wall	contents
	48.2	1.7	22.6	6.2	21.7	0.8
	38.6	1.6	9.7	1.9	18.3	0.6
	41.4	2.6	9.7	-	13.2	0.5
	50.5	2.3	12.8	3.5	23.2	0.4
Mean	44.7	2.1	13.7	3.9	19.1	0.6

significantly higher. The values for each part of
the canal were higher than those of other workers
(26, 40, 41.). The reason for this is unknown,
though the animals used in the experiments were
heavier than those used by the other investigators,
and in some cases of a different sex (40, 41). The
values for the histamine content of the gastric an d
small intestinal walls bear approximately the same
ratios to one another as was found by the other
workers. The ratio of the mean values of the
histamine content of the large intestine to the
small was found to be approximately 4:6 (26). It
is not stated whether there was a significant
difference between the values for the two parts of
the intestine.

The concentration in the contents of the small
intestine was higher than that in the stomach; the
concentration in the contents of the large intestine
was least. These results, in spite of the small
number of experiments on which they are based,
suggest that there is some factor present in the
small intestine which increases the concentration
of histamine within its lumen, and that considerable
absorption of histamine occurs from the lumen of
the large intestine. Although the gastric wall
contains a high concentration of histamine, the

gastric contents contain less histamine than do the contents of the small intestine. Thus it appears unlikely that the higher concentration of histamine in the lumen of the small intestine arises as a result of the excretion of histamine from the gastric wall into the lumen of the alimentary canal.

SECTION 11. THE EFFECTS OF ANTIBIOTICS AND PHTHALYL SULPHATHIAZOLE ON THE METABOLISM OF HISTAMINE.

Various workers have shown that some bacterial formation of histamine and its conjugate occurs in the alimentary canal.(19, 20, 47). However, it is not clear how important the histamine produced in the animal's alimentary canal is in the animal's general metabolism of histamine. To investigate this point, and to test the conclusion that histamine is produced in the alimentary canal by intestinal bacteria, (19, 20.) drugs with potent effects on the bacteria of the alimentary canal were administered to the rats, and their urinary histamine excretion was measured before, during and after the administration of the drugs. The effect of chloramphenicol was investigated most fully, but the effects of aureomycin, penicillin and phthalyl sulphathiazole were also observed.

Extracts of the alimentary canal were also made in
order to determine what effect the chloramphenicol
had on the concentration of histamine in its walls.
The dose of chloramphenicol used in all the

experiments but one, and also the doses of the other
antibiotics, corresponded on a weight basis with
the maximum doses recommended for human beings. The
doses used were all considerably below those
causing toxic effects in rats or mice. (17, 21, 27,
39, 48.).

The effect of chloramphenicol on the excretion of

free histamine.

Chloramphenicol was administered twice daily by
stomach tube in a dose of 16 mg., suspended in 1 ml.
water. The mean reduction in the urinary excretion
of histamine, which it caused in three rats, is
shown in Fig. 6. The individual values for the
histamine excretion from the rats are also shown.
The degree of variation between the animals was
similar in all the other experiments; the changes
in the excretion always occurred at the same time
in the different animals, and changed in the same
direction. The histamine excretion began to
diminish on the second day, and reached its minimum
on the third day after administration had started.
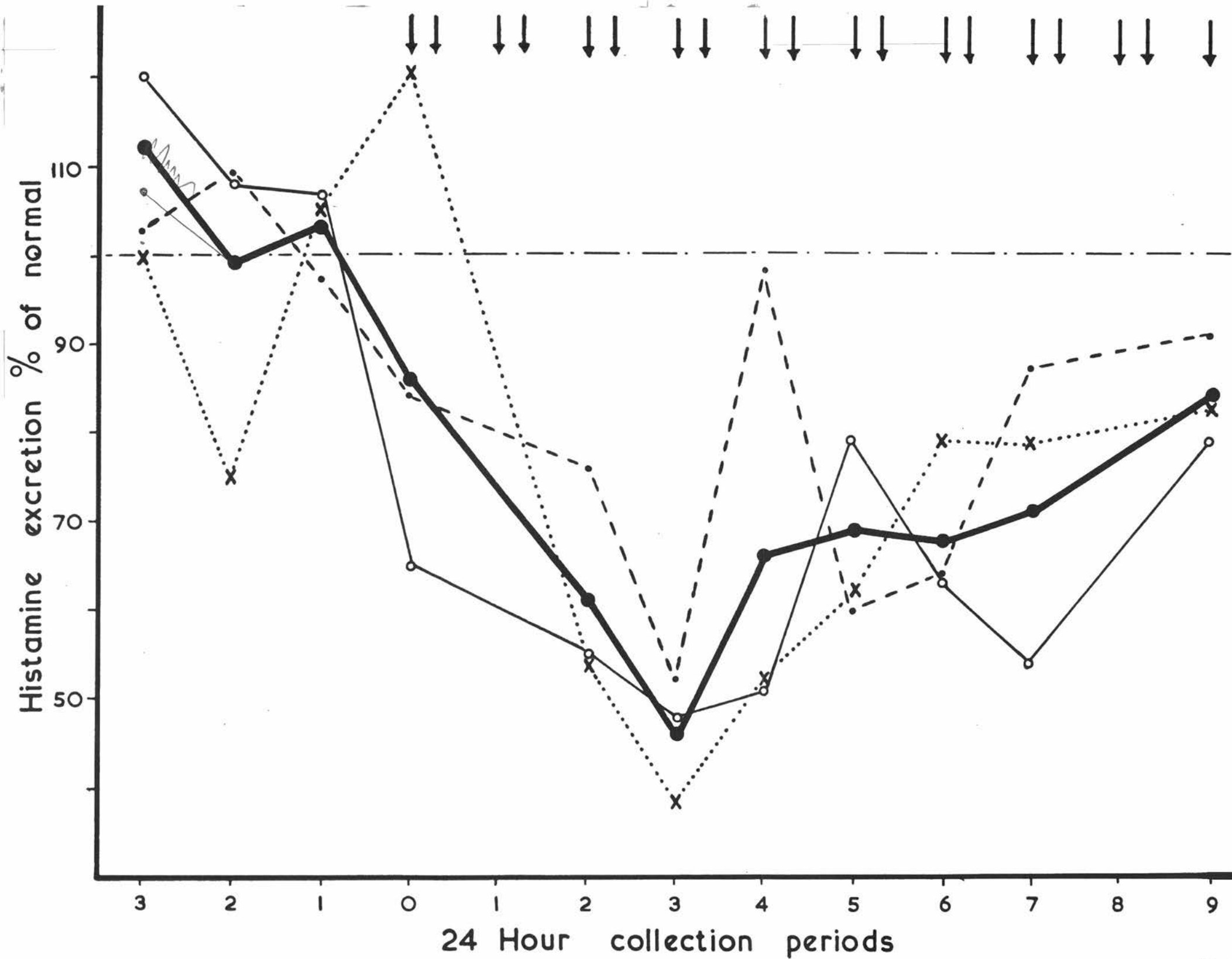After this, in spite of continued administration of

Fig. 6.

Effect of oral administration of chloramphenicol on the urinary
excretion of histamine. Chloramphenicol was given at the times
shown by the arrows in a dose of 16 mg. The mean excretion from 3 rats
is shown by the thick line. Values from individual rats are shown
in the other tracings.

chloramphenicol, the histamine excretion gradually
returned to normal, and had almost reached its
original level on the ninth day of administration.
In all the other experiments in which chloramphenicol
was used, administration was continued for four days
or less. It was always confirmed that the excretion
of histamine remained low during the first three
days, and began to increase on the fourth day.

The period required for the histamine excretion to
return to normal, after the administration of
chloramphenicol had been stopped, was determined fo r
two different doses of the drug. Doses of 48 and 16
mg. suspended in 1 ml. water were given twice daily
by stomach tube. Their effects are shown in Fig. 7.
There is no significant difference in the effects of
these two doses during the three days of minimum
histamine excretion. In both experiments the
values for histamine excretion following the
administration of the drug gradually increased, and
finally considerably exceed the control values. This
effect was more pronounced in the rats receiving the
larger dose.

Determination of the histamine excretion at
intervals of four hours showed that the effects of
chloramphenicol on the excretion appeared twelve
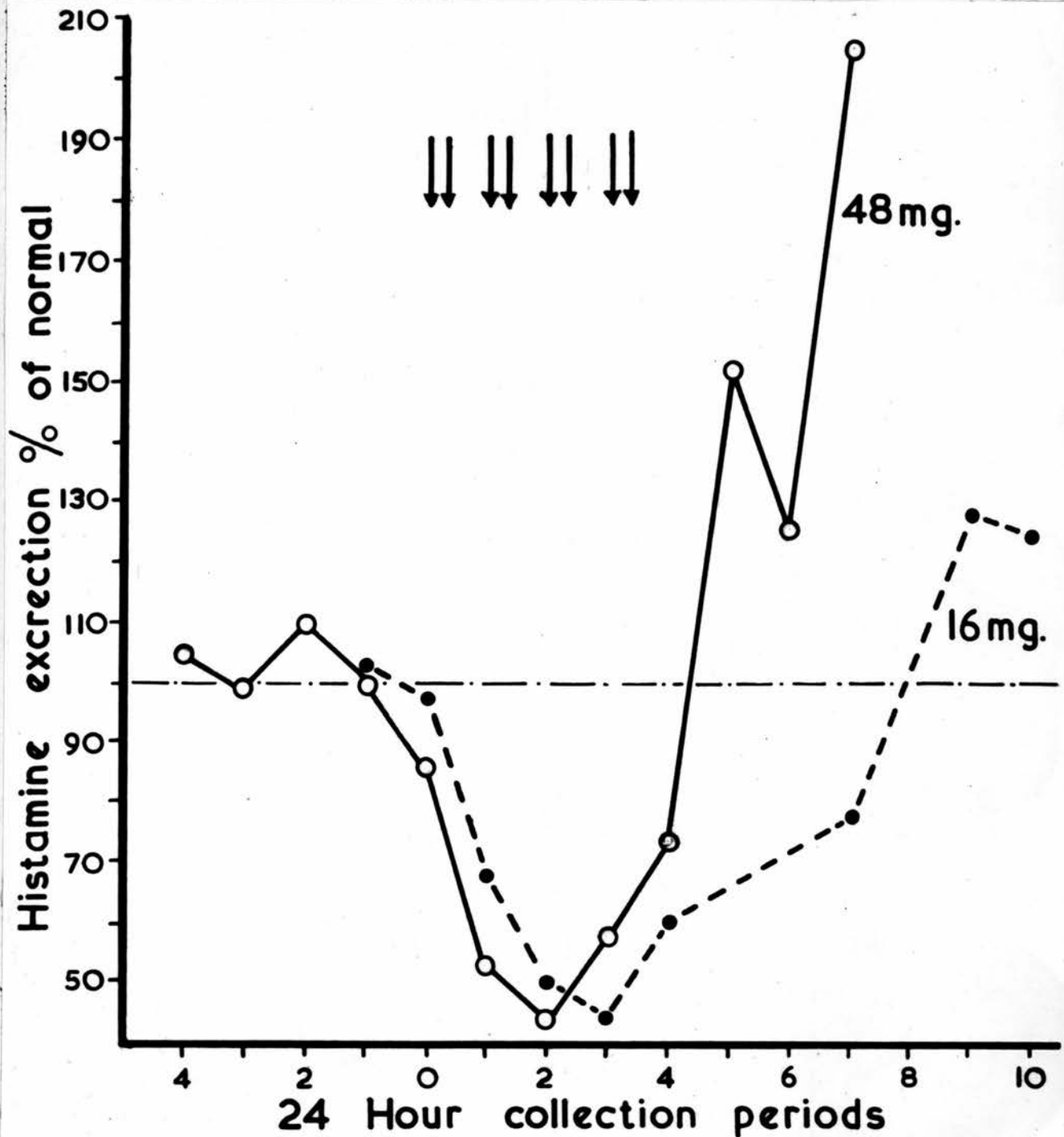hours after the first dose had been given. The

Fig. 7.

Effects on the urinary excretion of histamine following the administration of different doses of chloramphenicol. Chloramphenicol was given at the times shown by the arrows.

effect became more pronounced, until administration was stopped after the fourth dose. The results of a typical experiment in which two rats were used are shown in Fig. 8, in which the histamine concentrations in the urine are also shown.

The decrease in histamine excretion appeared about twelve hours after the first administration of chloramphenicol; the night maximum was diminished and the minimum values for the second day were both diminished. The histamine excretion reached its previous normal value 48 hours after the last administration of chloramphenicol. The changes in histamine excretion were largely due to alterations in the concentration of histamine in the urine. During and following the administration of chloramphenicol, the daily cycle of histamine excretion retained its normal form, though at a lower level than normal.

Rate of passage of contents through the gastro-intestinal tract.

To determine the rate of transit of the contents of the alimentary canal, powdered charcoal suspended in 1 ml. water was given to two rats by stomach tube at the time when the morning dose of chloramphenicol was normally given. Eight hours later charcoal was found irregularly scattered through the whole length
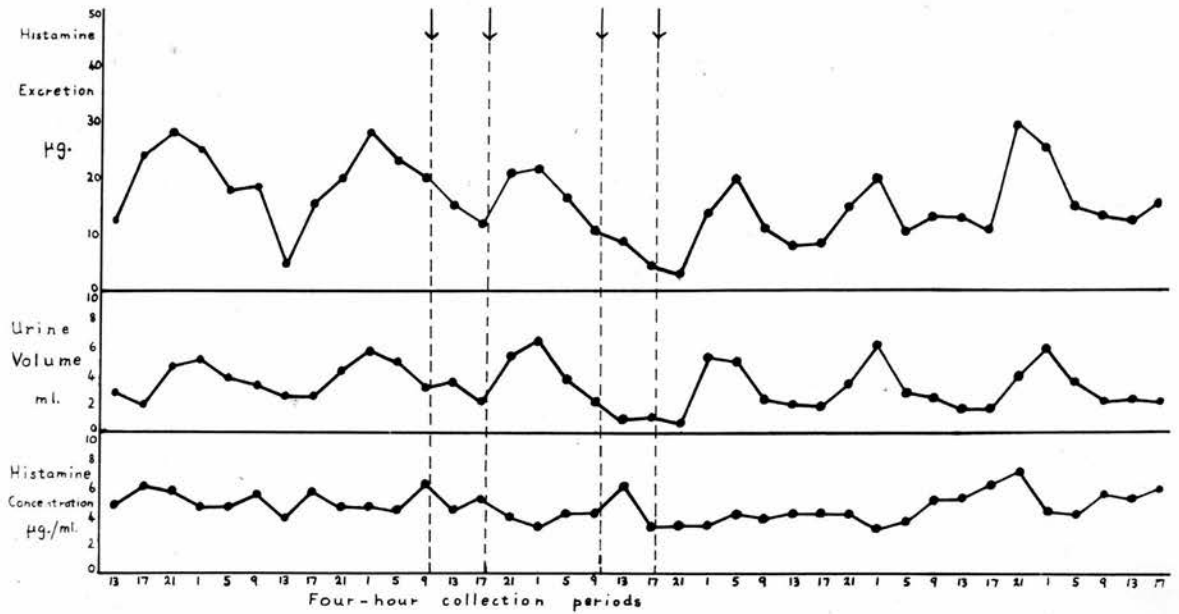
Fig. 8.

Changes in the four hour excretion and urinary
concentration of histamine , and record of the
urinary volume, during the administration of 16 mg.
of chloramphenicol twice daily. Administration of
chloramphenicol shown by the arrows.

of the jejunum, and thickly concentrated in the
ileum and caecum. Charcoal was found in formed
stools in the lower part of the descending colon,
but was absent from stools in the rectum.
Chloramphenicol, therefore, could have proceeded
along the alimentary tract at least as far as the
descending colon by the time that an effect on the
excretion of histamine in the urine was manifest.
Any chloramphenicol which had not been absorbed
would thus have exerted its effect on the organisms
of the small intestine, and would have started to
affect the bacteria of the large intestine by this
time.

THe effect of chloramphenicol on the excretion of
conjugated histamine.

The low control values for the excretion of
conjugated histamine made it difficult to measure
any changes imposed by the administration of
chloramphenicol. In order to increase the excretion
of conjugated histamine and so more easily to
observe any changes in its excretion, histamine
was given orally to the rats for eleven days, and
from the fifth tb the eighth days chloramphenicol
in a dose of 48 mg. twice daily was also
administered by stomach tube. During the four day
control period before the administration of

chloramphenicol, the daily histamine excretion increased continuously. The excretion diminished rapidly after the chloramphenicol administration was started, and the lowest value which it reached was 40% of the mean control value. The increase in the excretion of histamine was averted, and it reappeared only after the chloramphenicol was stopped. (Fig.9.). The time relations of the reduction in the excretion of conjugated histamine were similar to those produced on the excretion of free histamine when the same dose of chloramphenicol was given alone. The reduction in the excretion of both forms of histamine was approximately the same with the same dose of chloramphenicol.

The effects of other drugs on the excretion of free histamine.

Penicillin, aureomycin and phthalyl sulphathiazole all diminished the urinary excretion of free histamine in the same way as chloramphenicol. All except penicillin were given twice daily; penicillin was given once daily in the morning. The drugs were suspended in 1 ml. fluid, and were given by stomach tube. They were given in the following doses:-

chloramphenicol .... 16 mg.

aureomycin .... 16mg.

phthalyl sulphathiazole ..... 125 mg.

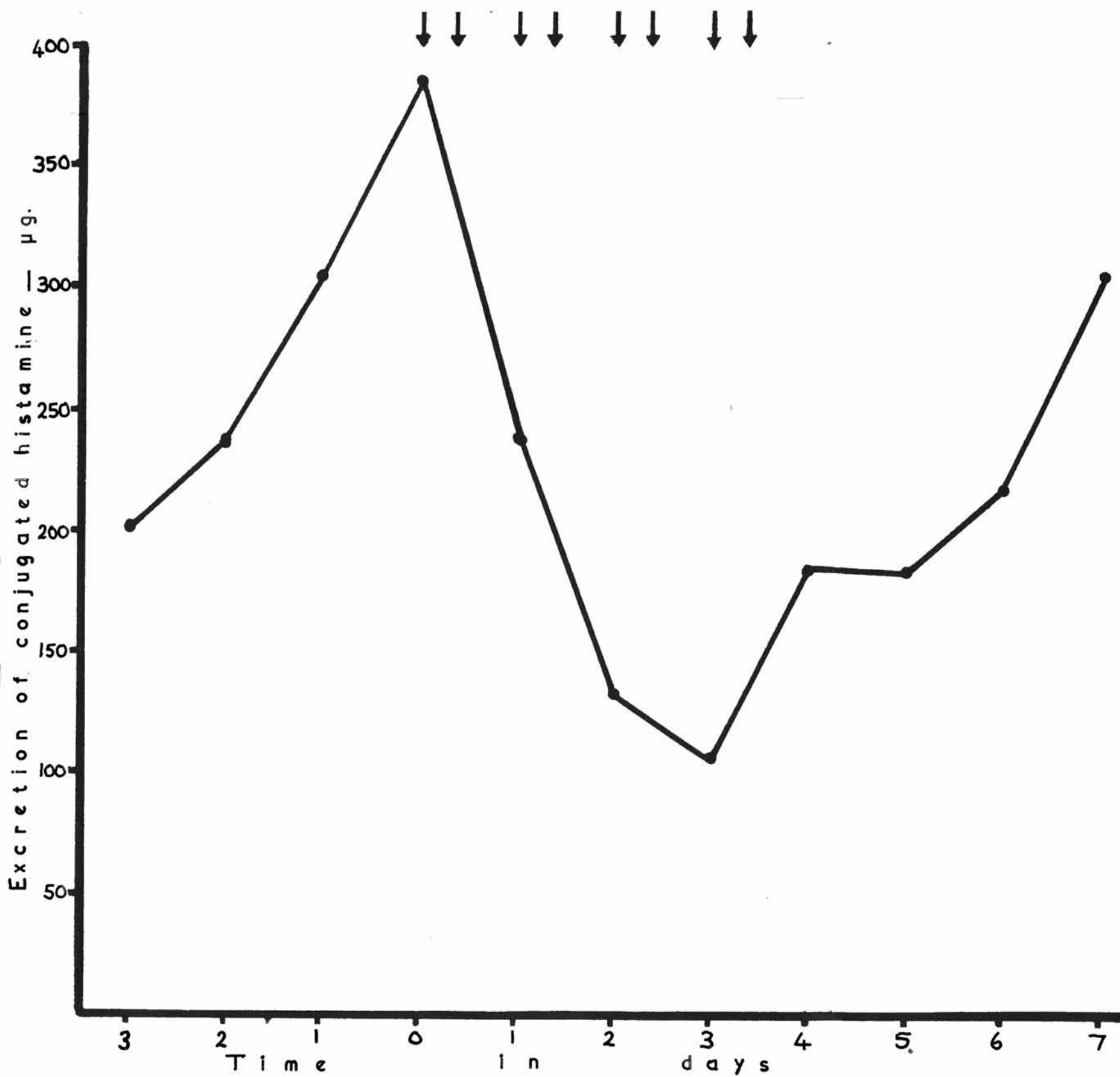penicillin ....30,000 units daily.

Fig. 9.

Effect of chloramphenicol on excretion of conjugated histamine in urine.
During the experiment 10 mg. histamine was given by stomach tube at the
beginning of each collection period. Chloramphenicol was given in doses
of 48 mg. at times shown by the arrows.

During the four days of the administration of
the drugs, there were no significant differences
between the histamine excreted b y the different
groups of rats. The minimum value which has ever
been obtained was with chloramphenicol on day 3,
when the excretion was 45% of normal. Following the
return of the excretion to normal, it rose
considerably above the normal level in each group
of rats, it then returned towards the control
value, which it began to approach eight days after
the administration had ceased. (Fig. 10.). The
increase in the excretion for all the drugs together
during the two days at which it was at its maximum
was significantly different from the control values.

The effect of chloramphenicol on the tissue content of histamine.

The histamine concentration in the wall of the
alimentary canal was estimated in four normal rats,
and in four rats after the administration of
chloramphenicol for three days. Each rats received
16 mg. of a suspension of chloramphenicol twice
daily, and the animal was killed on the morning of
the fourth day. Chloramphenicol had no significant
effect on the concentration of histamine in the
walls of the stomach or large intestine, but it
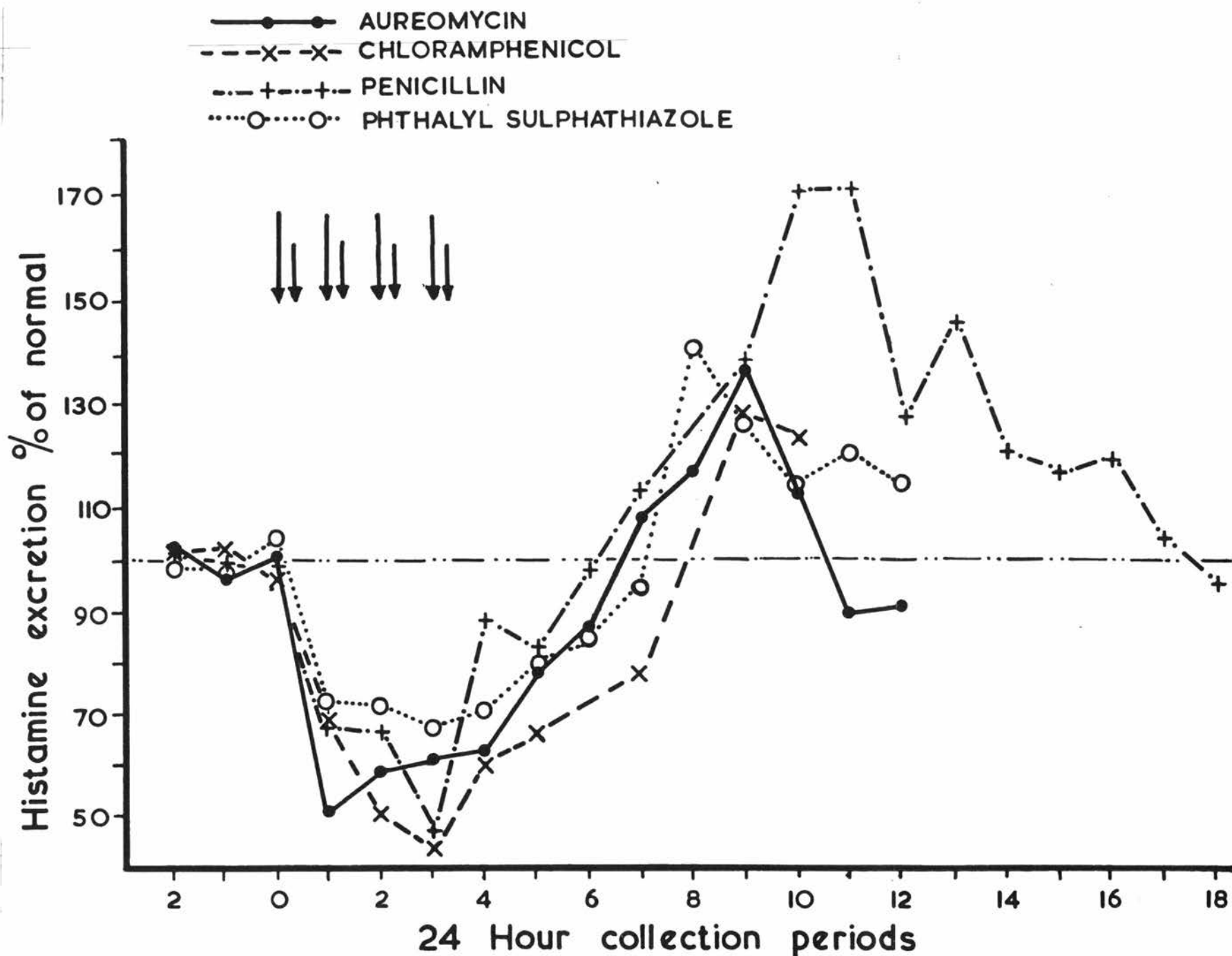reduced the mean concentration in the wall of the

Fig. 10.

Effects of aureomycin, chloramphenicol, penicillin and phthalyl sulphathiazole on the excretion of histamine in the urine. For doses see text. The drugs were given twice daily by stomach tube at the times shown by the arrows. Penicillin was given only at the times shown by the longer arrows. Values showing effect of phthalyl sulphathiazole were obtained from two rats; all other values were obtained from three rats.

small intestine by more than 50%. (Table 10).

SECTION 111. THE EFFECT OF ADRENALINE ON THE METABOLISM OF HISTAMINE.

Adrenaline had a much more transient effect on the urinary excretion of histamine than had any of the other drugs previously described. Its effect was more similar to that of the histamine liberators

The effects of adrenaline and the histamine liberators were observed by measuring the excretion of histamine half-hourly.

The intraperitoneal injection of adrenaline caused an increase in the excretion of histamine which began a half to one hour after the injection, gradually increased, reached a plateau, and then diminished again. A small response was produced with 37 μg./kg., and a response which lasted for about seven hours occurred with a dose of 370 μg./kg. (Fig. 11).

The rats which received doses of 37 μg./kg. often developed haematuria, and the urine volume was always reduced for an hour following the injection. They became restless, began to twitch, the skin felt cold, and their respiration increased. These signs had generally disappeared 4 - 5 hours

Table 10.

The histamine content of the walls of the
alimentary canal ofcontrol rats and chloramphenicol
treated rats. The treated rats received 16 mg.
chloramphenicol twice daily by stomach tube for three days
days preceding death. Values expressed in µg./g.
fresh tissue.

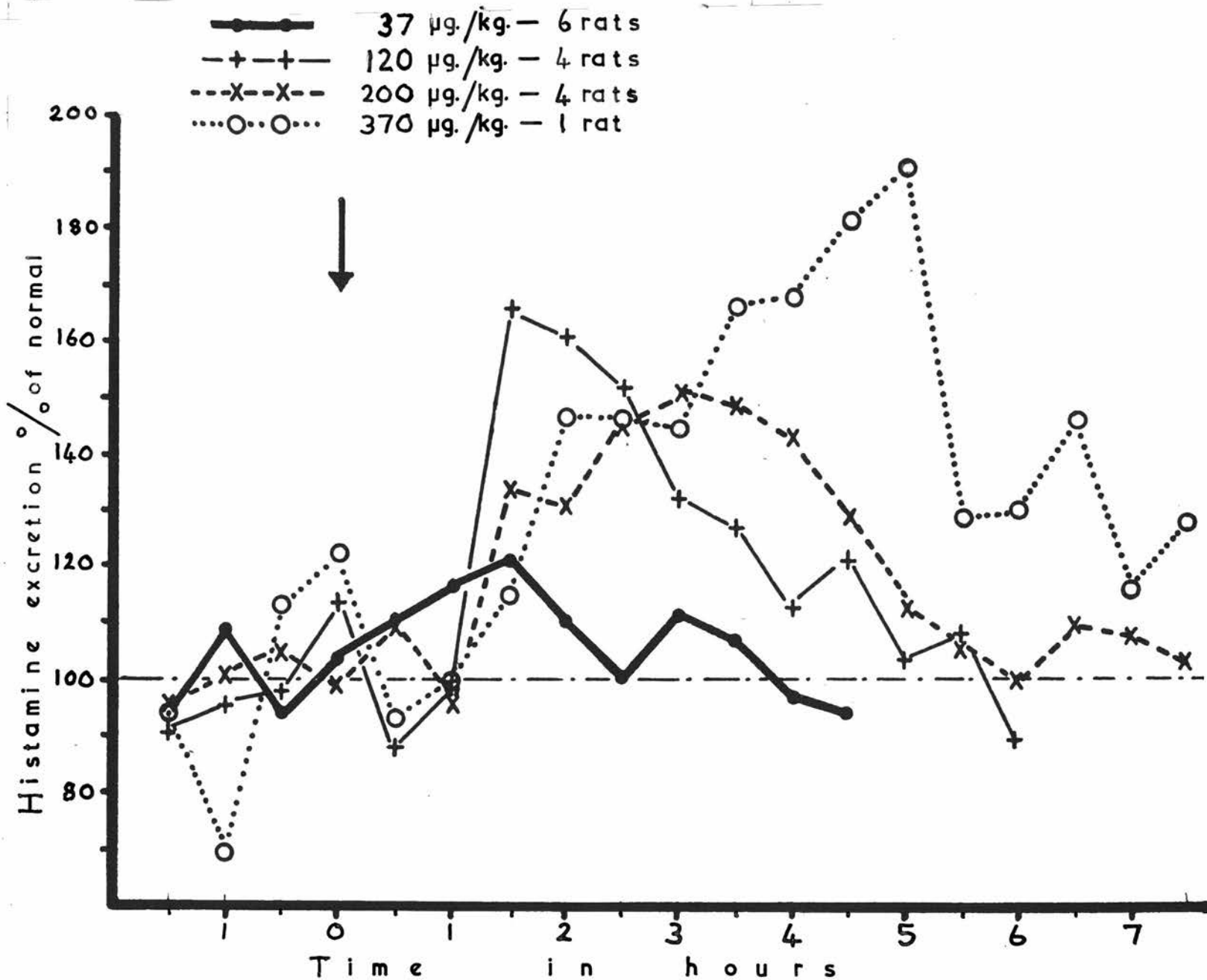|	Control rats			Chloramphenicol treated rats		
	stomach	intestine		stomach	intestine	
		small	large		small	large
	48.2	22.6	21.7	43.5	6.3	14.2
	38.6	9.7	18.3	63.4	6.0	16.5
	41.4	9.7	13.2	43.7	4.0	15.7
	50.5	12.8	23.2	44.1	4.0	13.4
Mean	44.7	13.6	19.1	48.7	5.1	14.9

<u>Fig. 11.</u>

Effects of different doses of adrenaline on the

excretion of histamine in the urine. Adrenaline

given by intraperitoneal injection at the time shown

by the arrow. Numbers of rats used in each

experiment shown in the figure.

after the injection. With larger doses these
effects became more severe. Increased excretion of
histamine occurred in four of the six rats which
received 37 μg./kg. adrenaline. With the larger
doses, increased excretion of histamine always
occurred, though the size of the increase did not
appear to depend entirely on the dose of adrenaline,
but seemed to fluctuate considerably between
individual rats. The largest dose of adrenaline
which was investigated, 370 μg./kg., only caused
an increase in the excretion of histamine up to
190% of normal five hours after injection with the
adrenaline. The responses resembled those caused
by the alkylamines, described in section 1V, but
the response to the largest dose of adrenaline
was less than that produced by 100 μg./kg. of
octylamine, which reached 257% of normal five and
a half hours after injection.

During the first hour after the injection, an
increase in the urinary excretion of histamine
occurred, although the volume of urine was
diminished. This is shown in Fig. 12. Thereafter
the urine output remained fairly constant, and the
urinary concentration of histamine remained high.
Thus the increase in the excretion of histamine
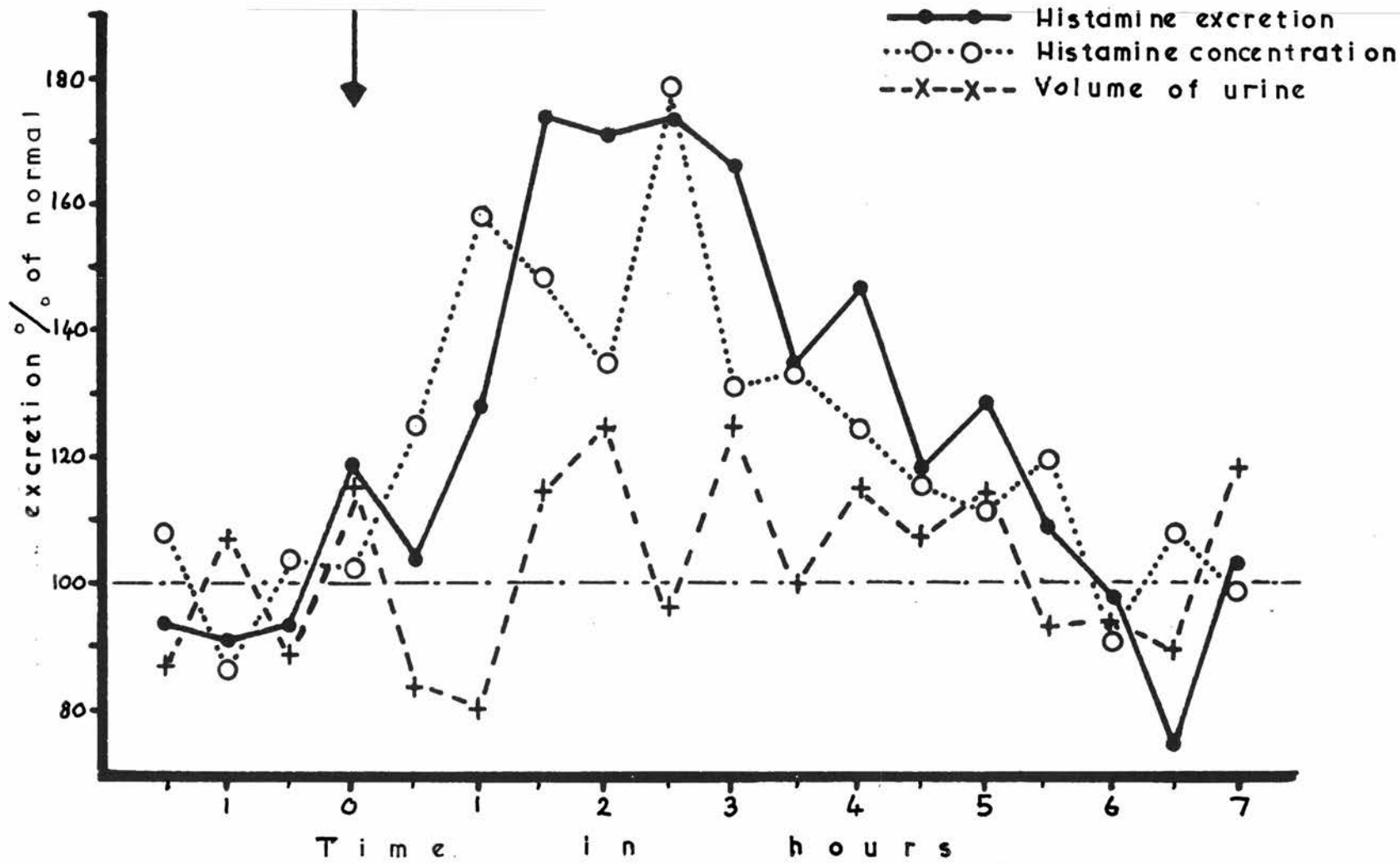was due to an increase in the concentration of

Fig. 12.

Changes in the urinary excretion and concentration
of histamine and the volume of urine passed
following the intraperitoneal injection of 120 µg/ kg.
adrenaline at the time shown by the arrow. All values
are expressed as percentages of the mean control
values.

histamine in the urine, which lasted for about six hours.

In these experiments, high doses of adremaline were used, which caused toxic effects in the rats. It is thus probable that the results of the injection of adrenaline on the excretion of histamine which were observed in the present experiments are not entirely comparable with the action of adrenaline in the normal animal under physiological conditions. However, it was obvious that adrenaline did influence the metabolism of histamine and that its effects could be compared with those of the histamine liberators described in section 1V.

The doses of adrenaline which were used were comparable with those used in the investigation of the histamine liberators, although with the liberators no toxic effects were observed which could be attributed to the action of these drugs, It is possible that the histamine liberating actions of adrenaline were due to its toxic actions, and so comparisons between adrenaline and the histamine liberators must be made with caution. It is obvious that, used in these doses, adrenaline was a histamine liberator which produced its effects after a short delay and caused an increased

excretion of histamine which continued over a period of several hours. The increased excretion produced by the largest dose was not, however, great, and began to diminish again six hours after the injection of the adrenaline. Thus adrenaline, although it may resemble octylamine in the course and time relations of the histamine release which it caused, did not appear to be so efficient a liberator of histamine.

SECTION 1V. THE EFFECTS OF HISTAMINE LIBERATORS ON THE METABOLISM OF HISTAMINE.

The injection of a histamine liberator into the cat causes the release of histamine within a few seconds of injection.(28). It was thought that the collection of rats' urine for 24 hours would demonstrate whether a drug did actually liberate histamine, but it would give very little idea of the time when it was most effective. For this reason the method of giving the rats a water load and making urine collections every half hour was adopted. In practise the method has worked very well, but it has the disadvantage that the liberator itself may also be excreted in the urine and interfere with the assay of histamine.

The effect of propamidine isethionate.

The minimum dose of propamidine isethionate
which causes a detectable release of histamine
is reported to be 0.3 mg./kg. (28). This dose
was therefore injected, and subsequently the
effects of other doses, both greater and smaller,
were observed. Following the subcutaneous
injection of propamidine isethionate into guinea

pigs, about 60% of the drug is excreted in six
hours (13). On the assumption that a similar
excretion of the drug occurs in rats following an
intaperitoneal injection, concentrations of the
drug were made up in Tyrode's solution,
corresponding to the estimated concentration in
the urine in six hours. The doses were made up as
shown in Table 11. The drug was tested in the

2 ml. bath as described above, at these concentrations

and a slightly antagonistic effect was observed
at a concentration of 9.5×10^{-7}. As the
concentration of the drug was increased, so the
antagonistic effect also increased, until at a
concentration of 1.5×10^{-5}, the drug reduced
the effect of the histamine standard by more than
a quarter (Figs. 13 and 14).

Propamidine has been shown to act as a histamine
antagonist on the guinea pig's ileum in dilutions

Table 11.

The effect of the calculated urinary excretion of
propamidine on the sensitivity of the guinea pig
ileum to histamine.

Dose of propamidine mg./kg.	Calculated conc. in urine mg./ml.	Conc in Tyrode solution in bath.	Size of contraction to histamine standard Normal = 100.
10.0	0.30	1.50×10^{-5}	71
5.0	0.15	7.50×10^{-6}	81
1.2	0.08	4.0×10^{-6}	91
0.6	0.019	9.5×110^{-7}	96
0.15	0.0095	4.8×10^{-7}	100
0.06	0.0047	2.4×10^{-7}	100

Fig. 13.

Inhibition of the response of guinea pig ileum to histamine produced by propamidine isethionate.

h : addition of 0.10 ml. of a solution containing 0.2 μg./ml. histamine standard to the 2 ml. bath.

p : addition of 0.10 ml. of a solution of propamidine isethionate to give a concentration of 4×10^{-6} in the bath.

After the second contraction propamidine was added and remained in the bath until it was washed out at w.
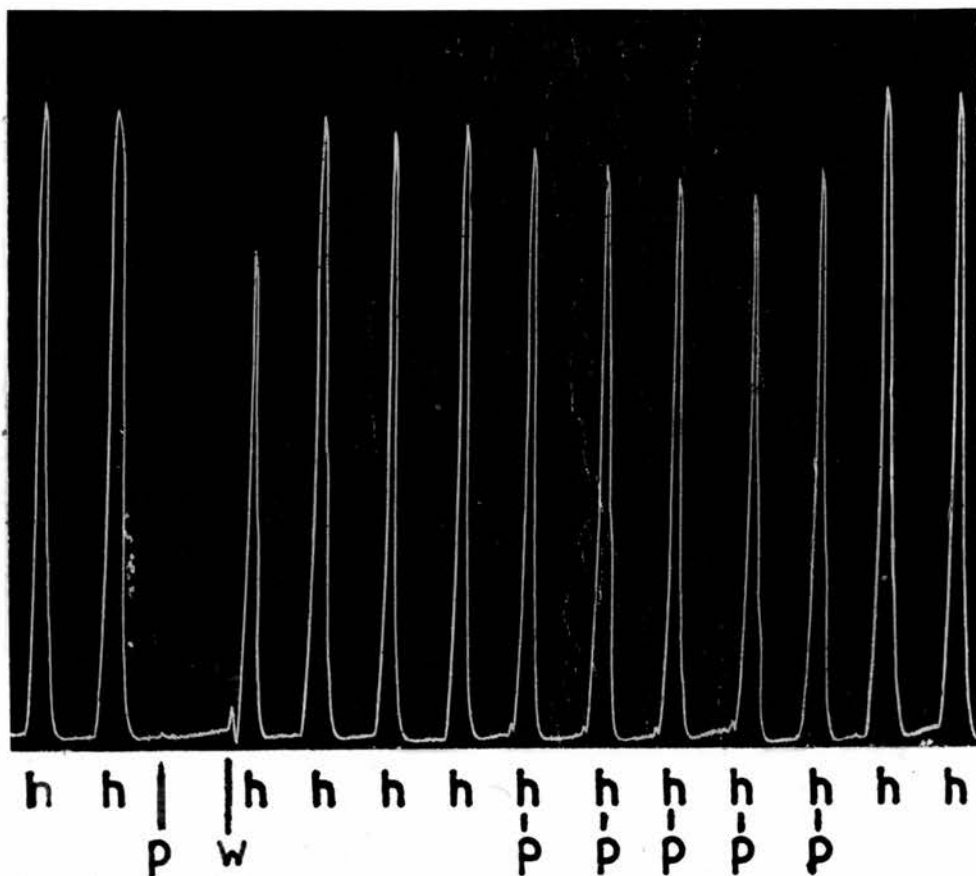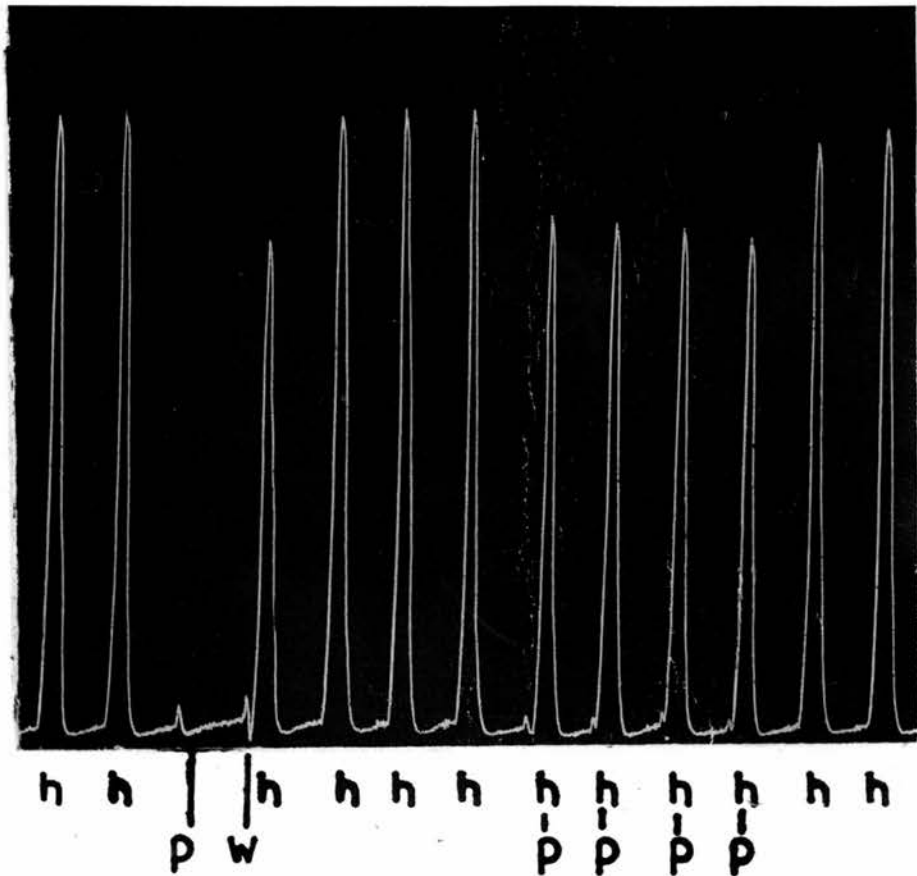
Fig. 14.

Inhibition of the response of guinea pig ileum to histamine produced by propamidine isethionate.

h ; addition of 0.10 ml. of a solution containing 0.2 µg./ml. of histamine standard to the 2 ml. bath.

p : addition of 0.10 ml. of a solution of propamidine to give a concentration of 7.5×10^{-6} in the bath.

After the second contraction propamidine was added to the bath and remmainedthere until it was washed out at w.

of 10^{-5} or less (28). No comments were made about any difficulties in the assays due to the effects of propamidine. However, in these experiments the plasma itself was assayed for histamine, and it is probable that the concentration of propamidine in the plasma after its injection intravenously into the cat would not be suuficient to interfere with the assay on the guinea pig's ileum.

Doses of 0.06, 0.15, 0.30, 0.60, 1.20, 5.0, and 10.0 mg./kg. of propamidine isethionate were injected into rats which had received a water load. No signs attributable to the release of histamine were observed in the rats, but in all the animals except those receiving 0.6 mg./kg. or less, there was some reduction in urine volume during the hour following the injection. Haematuria developed in those receiving 10 mg./kg. The experimental results are shown in Figs, 15 and 16. A considerable release of histamine was caused by 0.6 mg./kg. and doses less than this. With doses greater than 0.6 mg./kg. the apparent excretion of histamine diminished as the dose was increased, until at 10 mg./kg. it was not increased at all. It was, in fact, slightly less than normal. This effect was attributed to the histamine antagonism of the excreted propamidine in the assay. This conclusion

Fig. 15.

Effects of different doses of propamidine on the urinary excretion of histamine. Intraperitoneal injection of propamidine at the time shown by the arrow.

Erratum : The maximum value produced by a dose of 600 µg./kg. should read 226% of normal and not 196% of normal.
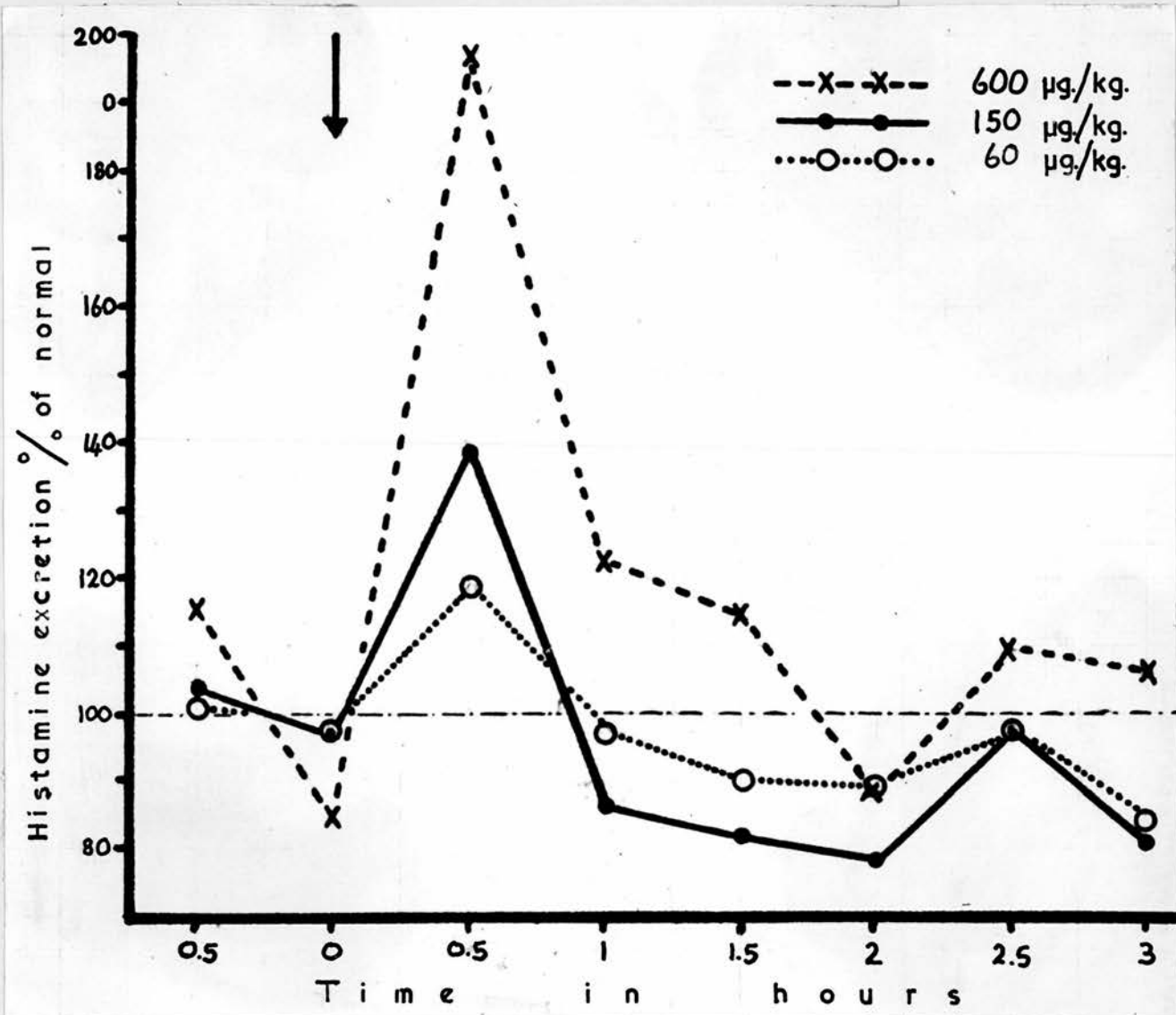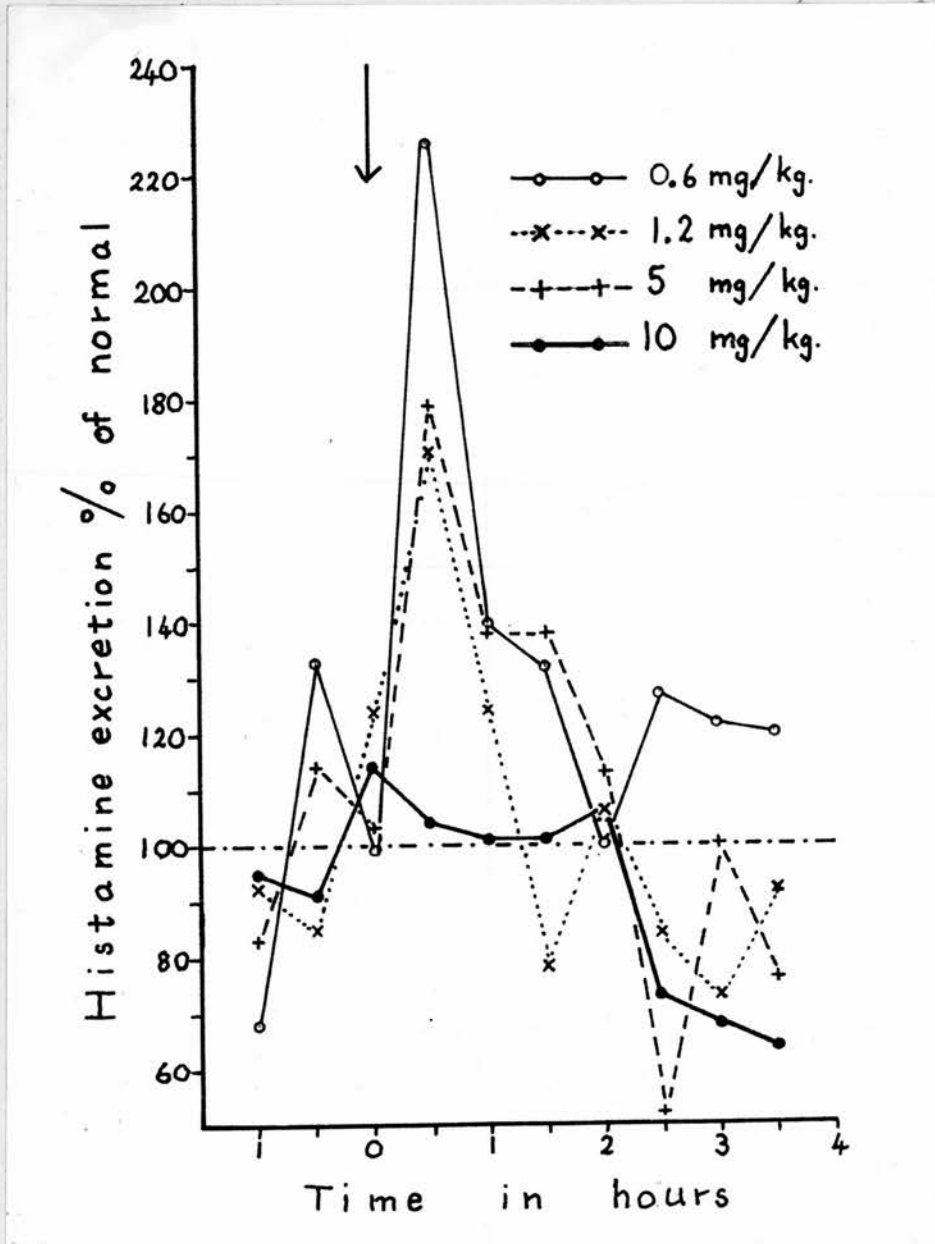
Fig. 16.

Effects of different doses of propamidine on
the urinary excretion of histamine. Intraperitoneal
injection of propamidine at the time shown by the
arrow.

was supported by the fact that the antagonistic
effect of the propamidine became greater as the
dose was increased, and that at doses below 0.6 mg.
/kg., the histamine excretion did not fall
significantly below normal. At these doses the
propamidine concentration in the urine probably
caused little histamine antagonism during the
assay.

The increased excretion of histamine produced
by the injection of propamidine closely resembled
that produced by the injection of histamine itself.
The immediate and transient rise of the histamine
excretion in both cases is very similar, and
suggests that the histamine produced by propamidine
is as rapidly available for excretion in the urine
as when histamine itself is injected intraperiton-
eally.

The effect of compound 48/80.

Compound 48/80 produced an immediate release of
histamine in the urine. (Fig. 17). The excretion
rapidly returned to its normal value after the
injection of the liberator, and the type of
response closely resembled that produced by the
intraperitoneal injection of histamine. Doses of
compound 48/80 of 15 and 5 µg./kg. did not
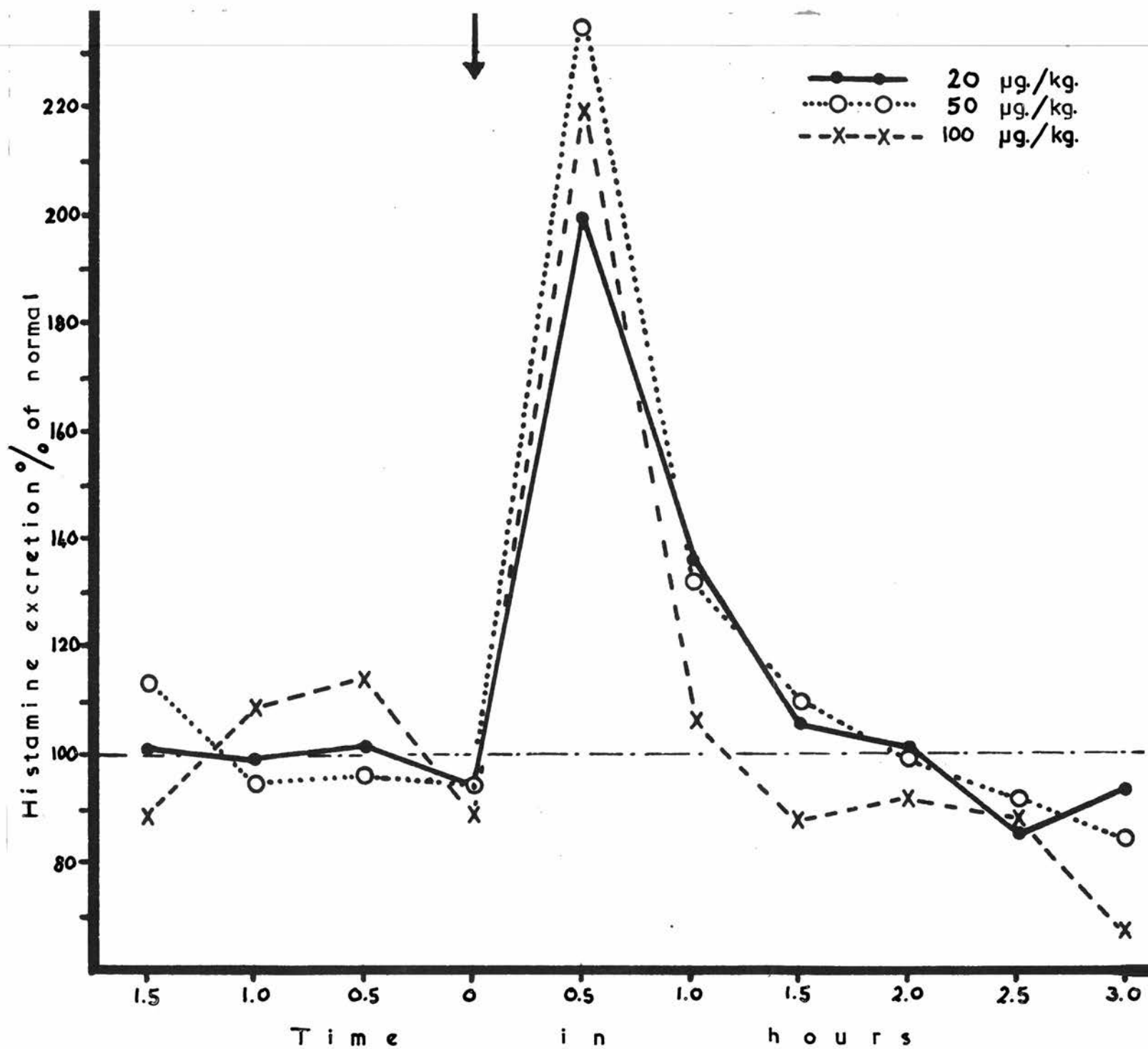produce any effect on the histamine excretion.

Fig. 17.

Effects of intraperitoneal injection of different
doses of compound 48/80 on the excretion of histamine
in the urine. Injection made at the time shown by
the arrow.

Larger doses produced increased excretion of histamine, but increase of the dose caused no change in the duration of the response as shown by this method. The appearance of a slow-contracting substance in cat's plasma one or two minutes after the injection of 48/80 has been reported. (36). No effects attributable to the presence of such a substance in rats' urine were ever observed. It has been reported that oedema and other effects in rats were produced by intraperitoneal injection of 5 mg./kg. of 48/80, (6), but no signs attributable to the release of histamine were seen in the present experiments.

The effect of the n-alkylamines.

Octylamine caused the liberation of histamine into the urine with doses similar to those used with compound 48/80, namely, 20, 50 and 100 ug./kg. The time relations and course of the response of octylamine however, differed considerably from those produced by 48/80, propamidine and histamine itself. No response at all was obtained with a dose of 10 μg./kg. With doses of 20 μg./kg. and 50 μg./kg. there was a delay of half an hour before the response began to appear. The excretion then increased and remained high for three hours before it began to diminish again. A dose of

100 µg./kg. caused a gradually increasing excretion of histamine and, five and a half hours after the injection, the excretion was more than twice its normal value and still increasing. (Fig. 18). As in the case of compound 48/80, no signs were observed in the rats attributable to the release of histamine, even with the largest dose at the end of the experiment.

Decylamine was found to be less active in this test than octylamine. Doses of 20 and 100 µg./kg. caused no increase in the excretion of histamine. (Fig. 19). A dose of 500 µg./kg. elicited a small but definite response; the excretion reached 130% of normal before returning to its normal value two and a half hours after the injection of the drug. The response to decylamine resembled that to octylamine in that the excretion was maintained at an increased level for one and a half hours before it began to fall. This high level of excretion was maintained or gradually increased with both decylamine and octylamine. As a result of this, the effect produced by these drugs on the excretion of histamine can be differentiated from the effects produced by propamidine, compound 48/80, and the injection of histamine base itself, which all cause an immediate rise and fall in the excretion
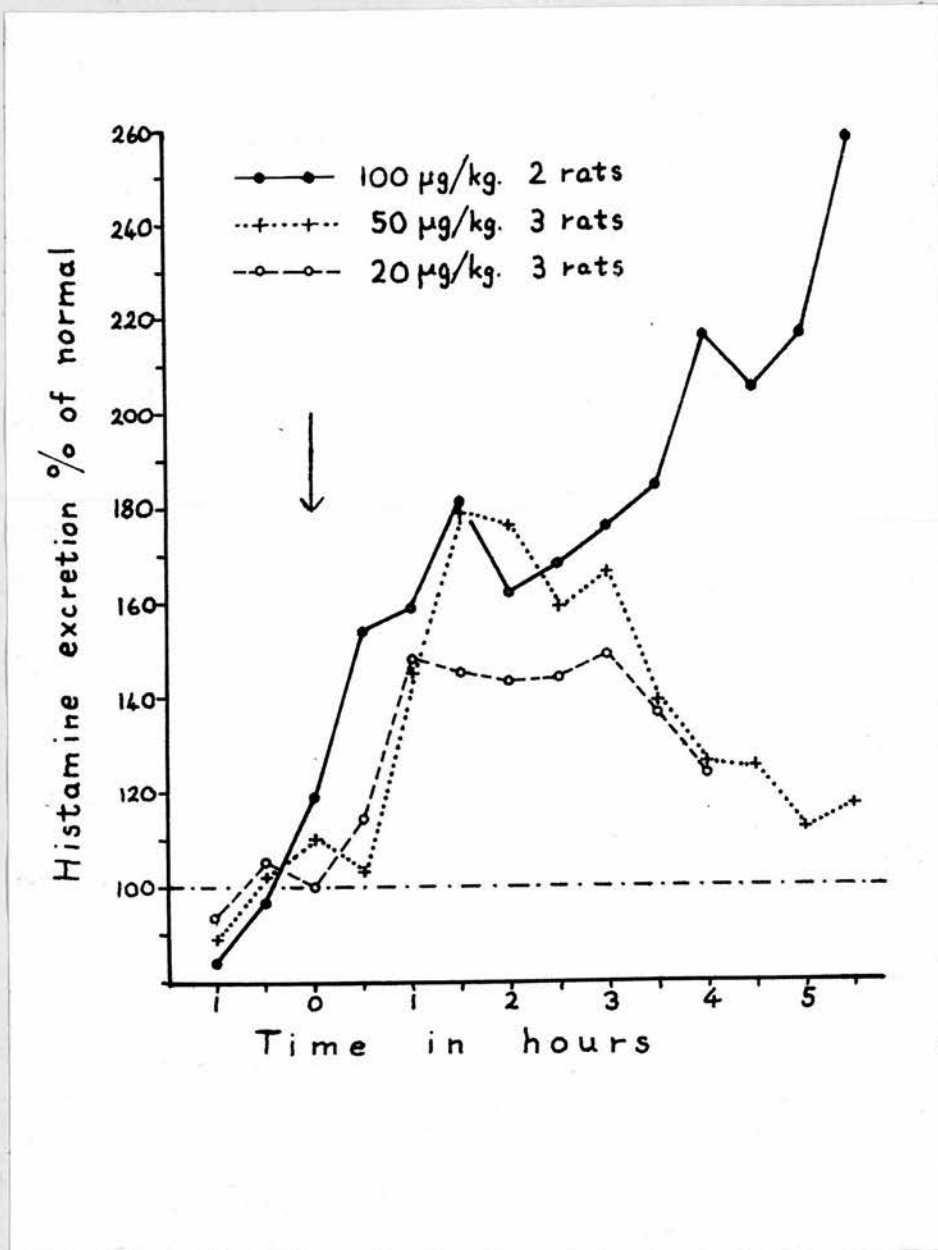
Fig. 18.

Effects of different doses of octylamine on the excretion of histamine in the urine. Octylamine given by intraperitoneal injection at the point marked by the arrow.
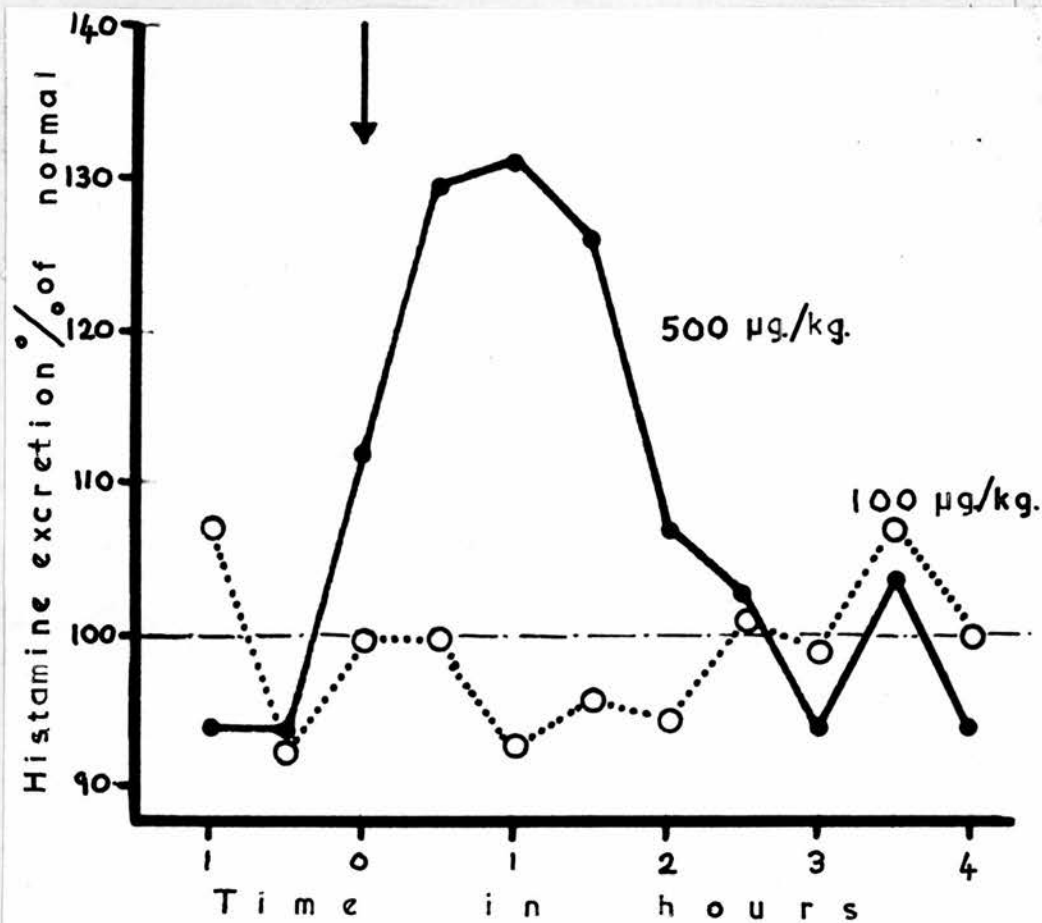
Fig. 19.

Effects of different doses of decylamine on the urinary excretion of histamine. Intraperitoneal injection of decylamine at the time shown by the arrow.

of histamine.

A comparison of these effects.

In spite of the variability observed in the half-hourly histamine excretion between different rats, a comparison can be made between the histamine releasing activity of the different liberators. It is assumed that all the histamine excreted following the injection of the liberator in excess of the mean control value is released by the liberator. This quantity was found to bear some relationship to the normal level of urinary histamine excretion. This observation was to be expected in view of the results reported by Emmelin (10). As a result, the quantity of histamine excreted in excess of the mean control value has been expressed as a percentage of this value. The quantities of histamine excreted and the periods over which the excretion occurred for each of the liberators are shown on Table 12.

Table 12.

TABLE 12

Urinary excretion of histamine caused by different histamine-liberators
compared with that caused by injection of histamine.

M.C. - mean control value.

Liberator	Dose µg./kg.	Period prior to maximum release - hours	Total period of release - hours	M.C. µg./30 min. period	Total Histamine excretion in excess of M.C. µg.	Histamine release in excess of M.C. as % of M.C.
Histamine	130	0.5	1	0.68	1.47	216
Propamidine	60	0.5	1	1.39	0.57	41
	150	0.5	0.5	1.53	0.88	51
	600	0.5	1.5	1.80	3.60	200
48/80	20	0.5	1.5	1.47	2.12	144
	50	0.5	1.5	1.99	3.50	176
	100	0.5	1	2.21	2.79	126
Octylamine	20	1.0	4.0+	0.65	1.33	204
	50	1.5	5.0+	1.24	5.55	448
	100	5.5	5.5+	0.37	3.62	978
Decylamine	500	1.0	2.5	2.70	2.62	97

DISCUSSION.

Throughout these investigations, estimations
of the urinary excretion of histamine, supplemented
by measurements of the concentration of histamine
in the alimentary canal, have been used as a
method of observing changes in the metabolism of
histamine. This method has the advantage that
it is simple to perform, appears to portray
accurately changes in the metabolism of histamine
in the normal animal under physiological conditions,
and can yield information about the way in which
different agents influence the metabolism of
histamine. From the observations made in this
way and the conclusions drawn from them, some
points regarding the origin, the metabolic path
and the factors which influence it in the normal
animal under physiological conditions, emerge.

The method used for the estimation of urinary
histamine is rapid and easy to perform. It has
been shown that the active substance in the urine
which was being assayed was histamine. The
problem of how to extract histamine from the
urine so that it can be accurately estimated has
been investigated. (3, 38.). The method suggested
by Roberts and Adam (38.) has been most successful,
but it suffers from the disadvantage that 100%

extraction of the histamine is not possible. Also
the process of elution with ammoniated chloroform
and the subsequent evaporation of the eluate are
time consuming. In the small number of experiments
which have been performed, it appears that the use
of columns of I.R.C. 50 for the histamine extraction
has several advantages over the method described
by Roberts and Adam, in which columns of Decalso
were used. The greatest advantage is that an
almost complete recovery of histamine is obtained.
The method is simple to apply, and can be performed
more than twice as quickly as the Decalso method.
Comparisons of the values for the active substance
after extraction on I.R.C. 50 and after direct
assay of the urine together with the other tests,
justified the method of assaying the urine
directly for histamine.

The method of collecting urine over 24 hour
periods is a standard biological procedure.
Administration of a water load and collection of
the urine half-hourly with subsequent measurement
of the urinary histamine excretion, provided a
useful method of measuring rapid and transient
changes in the excretion of histamine. The
histamine excretion could be measured sufficiently
accurately and was sufficiently constant under

normal conditions to enable small changes to be
measured in response to the administration of
drugs. With all the drugs used, except adrenaline,
the doses could be so adjusted that changes in the
excretion of histamine could be measured without
the drug appearing in the urine in sufficient
concentrations to interfere with the assay of
histamine. By making use of the stability of
histamine relative to that of adrenaline, the
interference with the assay caused by the excretion
of adrenaline in the urine could be eliminated.

By the use of these experimental procedures a
method of investigating the metabolism of histamine
in the normal animal has been evolved. The
administration of the antibiotics and phthalyl
sulphathiazole, the histamine liberators and the
alkylamines provided methods of influencing the
metabolism, which enabled conclusions to be drawn
about the metabolism of histamine. It appears
that the administration of these drugs, particularly
the antibiotics, may provide a new tool for the
investigation of the metabolism of histamine in
the rat.

There is a considerable body of evidence to
show that the urinary excretion of histamine reflects
changes in the level of histamine in the tissues of

the rat. Anrep et al.(3) could find no relation
between the amount of histamine injected in the
dog, and the quantity excreted in the urine.
However, they used doses of histamine which were
associated with anuria, and they concluded that
the excretion of free histamine was related to the
period of anuria which followed the injection. They
suggested that more histamine was excreted in
those animals in which the suppression of urine
flow was short. The albino rat was used in the
present experiments as the experimental animal
because it is relatively resistant to the effects
of histamine compared with many other animals. (33) .
These workers have also shown that a large
percentage of the histamine administered to rats
by parenteral injection is excreted in the urine
unchanged. They suggest that this indicates that
their tolerance for histamine is not based upon
its conjugation or destruction. Emmelin (10) has
shown that injected histamined disappears very
quickly from the plasma, and enters the tissues
in the cat. His experiments show that removal of
the kidneys and other abdominal viscera, excluding
the liver, considerably reduced the rate and
completeness of removal of histamine from the
plasma. The observation confirms the suggestion

(5) that the kidneys and the intestinal mucosa
are of importance in the inactivation of histamine.
Emmelin (10-) further showed that the kidney
removed large amounts of histamine from the blood
passing through it. Samples of urine collected
from the ureter whilst histamine was being infused
into the renal artery showed that doses of histamine
large enough to cause a fall of blood pressure in
the cat also caused the excretion of histamine
in the urine. Small doses of histamine did not
cause a detectable excretion of histamine, but
when a threshold dose had been reached, increasing
the dose of histamine which was being injected
into the artery caused excretion of histamine in
the urine.

Several of these observations have been
confirmed in the present experiments. It was
found, in support of other observations, (3), that,
provided the flow of urine was adequate, the
excretion of histamine was uninfluenced by the
volume of urine passed. The rapid elimination
of histamine following its intraperitoneal
injection shows that when histamine is present in
the tissues in a form which can be excreted in
the urine, it is rapidly excreted in this fashion.
It appears that the quantity of histamine in the

tissues influences the proportion that is excreted
in the urine. Recovery of 23% of a dose of
administered histamine was obtained in 24 hours
from rats which had received 40 mg./kg. of
histamine by subcutaneous injection, but a
recovery of only 7.4% was obtained in rats which
had received 0.02 mg./kg. by intraperitoneal
injection. These observations are consistent
with those of Emmelin (10) and show that in the rat
the urinary excretion of histamine is dependent
upon the concentration of histamine in the body,
provided that this histamine is in a form which
can be excreted, and is present in the blood in a
concentration great enough to be excreted by the
kidney. Since in these experiments there was
little variation in the output of urine, and no
anuria occurred, it was considered justifiable to
accept the quantity of histamine excreted in the
urine as a measure of the change in the metabolism
of histamine. The exception to this was in the
case of adrenaline, and in view of the abnormal
effects which this drug had on the whole condition
of the animals, as well as on their secretion of
urine, the results of these experiments must be
considered with caution.

The twenty-four hour excretion of histamine

remained fairly constant from day to day. The
half-hourly excretion of histamine also remained
fairly constant from day to day in the same rats.
Analysis of the excretion of histamine throughout
the day, however, showed that there was considerable
fluctuation. The excretion tended to vary in
relation to the activity of the rats, being
greatest at night, and least when they were asleep
during the day. However, the urinary concentratration
remained fairly steady. This provides further
evidence that, provided that the excretion of
histamine is measured over periods during which
it is not influenced by the diurnal rhythm, such as
24 hour or half-hour periods, the excretion of
histamine can be taken as a reflection of the
factors influencing its metabolism in the body.

The experiments with the antibiotics and
phthalyl sulphathiazole make it clear that a
number of drugs differing widely from each other
in chemical structure, but having in common the
fact that they reduce the intestinal flora, diminish
the urinary excretion of free histamine in the rat.
One of them, chloramphenicol, has also been shown
to affect the excretion of conjugated histamine
in a similar way. The observed effect of the
antibiotics might be produced by their interference

with the normal metabolism of histamine in the
tissues of the rats, or by their action on the
histamine-producing organisms in the lumen of the
alimentary canal.

The antibiotics have no known effect on tissue
metabolism in the dosages used in these experiments.
The doses were small and corresponded approximately
to the doses recommended for use in human beings
for treatment of infections of the alimentary canal.
The fact that the 24 hour cycle of the excretion of
histamine was maintained, even when the administration
of chloramphenicol produced a fall in the 24 hour
excretion of histamine, suggests that the
chloramphenicol did not interfere with the renal
mechanism for excreting histamine. In incubation
experiments on minced rabbit's kidney, aureomycin,
chloramphenicol, and phthalyl sulphathiazole did
not influence the metabolic activity of histidine
decarboxylase. (49). The doses at which toxic
effects are reported to occur when the antibiotics
are given by parenteral injection are much larger
than the doses used in these experiments. For
these reasons it is considered unlikely that the
observed effects on the urinary excretion of
histamine produced by the antibiotics were caused by
any effects on the metabolism of the tissues of the

rats.

The explanation of the observed effects in relation to changes in the bacterial flora remains. It is known that the intestinal flora include organisms which are capable of producing the free and conjugated forms of histamine. (23, 24, 47.) It seems possible that the effect of the drugs administered in the present experiments on such flora might account for the observed changes in the urinary histamine excretion. That antibiotics and sulphonamides do, in fact, influence the flora of the alimentary canal is well known. Thus penicillin, (18), aureomycin (37), and phthalyl sulphathiazole (14, 32), have been shown to reduce the counts and influence the types of bacteria present in the alimentary canal of rats. Similar observations have been made in man (31). There is little direct evidence that the organisms affected are, in fact, those responsible for histamine production, but it has been shown (31) that the administration of aureomycin does not totally eliminate the organisms from the stools, but considerably reduces the numbers of coliforms, yeasts and anaerobes, amongst which there are histamine-forming bacilli. (19, 20). The drugs exert pronounced effects in the distal part of

the small intestine, whence it has been shown that
absorption of histamine occurs principally in the
dog (30). It has also been shown that histamine is
continuously released under normal conditions into
the blood passing from the intestinal tract into
the portal vein. (4).

The time relationships of the effects of these
drugs on the intestinal flora are closely correlated
with the times at which the histamine excretion
showed fluctuations. During eight days of
administration of aureomycin, the bacterial count
diminished during the first four days, and on the
seventh and eighth days a marked increase in
coliforms occurred in conjunction with a decrease
in the sensitivity of the organisms to aureomycin.
(31). Furthermore, it was shown that if
administration was stopped for two or three days,
when the count had fallen considerably, the count
reverted to its original level, and by the third
post-administrative day it might rise to seven
time the pre-administrative level. A similar
relationship between the reduction in the number
of micro-organisms and the time when these
reductions take place, occurs after the
administration of chloramphenicol. Counts have
been made of E. Coli grown from smears of the stools

of children who were receiving chloramphenicol
orally (16). The figures have been transformed
into percentages of the control growth of E. Coli
before administration of the drug, and compared
with the reduction of the urinary excretion of
histamine in rats in Fig. 20. The time relations
between the two effects are remarkably similar,
but it can be seen that a dose of chloramphenicol
greater than that required to reduce the numbers
of organisms to zero, only reduced the excretion to
about 50% of normal. A reduction in the number of
E.Coli on the third day after the oral administration
of phthalyl sulphathiazole had been started has
been observed. (14). In spite of continued
administration of the drug, the number of E. Coli
gradually began to increase after eleven days, and
in four weeks the count had returned to normal.
Quantitative changes also occur in the bacterial
flora of the intestines and caeca of rats while
they are receiving penicillin in their diets. (18).
During the first fortnight the anaerobes diminish
while the coliforms increase.

The experiments with charcoal suggest the drug
could have reached the pelvic colon two hours
before the earliest reduction in the excretion of
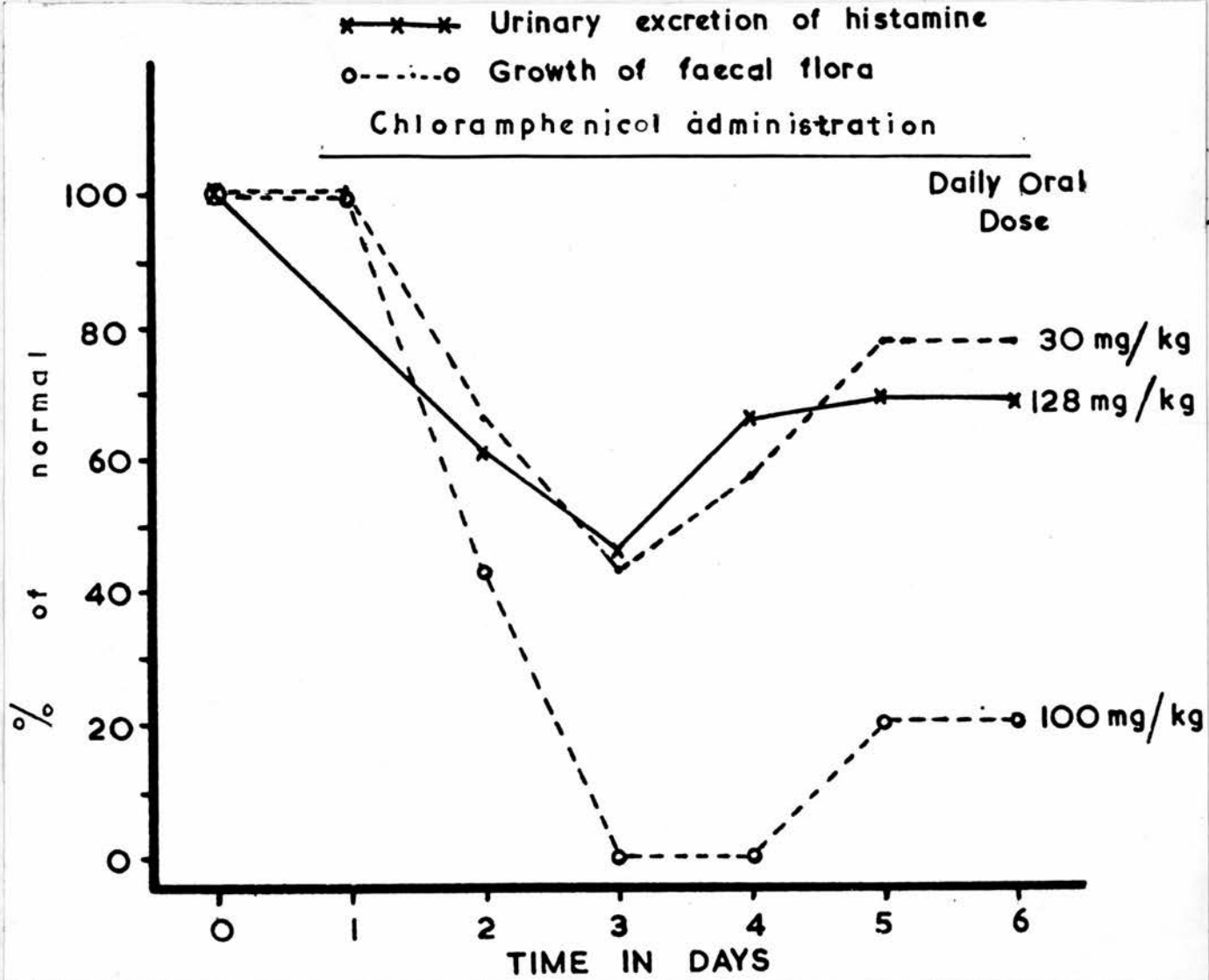histamine was observed. Histamine is rapidly

Fig. 20.

Comparison of the effect of chloramphenicol on
the growth of faecal flora in children and on the
urinary excretion of histamine in the rat.

absorbed from the alimentary canal and excreted in
the urine in mice. (2). Thus it is clear that
the drugs must have been present and exerting their
effects on the flora of the lower part of the ileum,
whence histamine is most readily absorbed, for at
least two hours by the time that any effect had
been observed in the urinary histamine excretion.
It has been shown in the case of aureomycin that
it is excreted in the bile within two hours of
intravenous injection. (50). A high concentration
would therefore rapidly be attained in the lumen
of the lower intestine even if all the drug which
had been administered orally was absorbed in the
stomach and duodenum. The doses of the drugs
which were used were approximately the same, weight
for weight, as the maximum effective doses used in
human beings. For this reason, the smaller dose
of chloramphenicol probably had a maximal effect
on the bacterial flora of the alimentary canal.
Increasing the dose would not be expected to have
any greater effect. Increasing the dose, however,
might result in a more pronounced effect on the
surviving bacteria after administration had stopped.
Provided that each of the antibiotics affected the
histamine-producing organisms, it would be expected
that the effects on the urinary excretion of

histamine would be the same with each of them. It
is suggested that the diminished concentration of
histamine in the wall of the small intestine
following the administratione of chloramphenicol
was due to a decrease in the production of histamine
by the surviving bacteria in the intestine, with
consequent diminished absorption from the lumen of
the canal. Administration of the antibiotics
reduced the urinary excretion of histamine to a
minimum figure of 50% of normal. This reduction
also, it is suggested, is a result of diminished
absorption of histamine from the lumen of the
intestinal canal, with consequent reduction in the
excretion of histamine in the urine.

The increase in the excretion of conjugated
histamine which was observed after the oral
administration of histamine acid phosphate, together
with the absence of any change after its parenteral
administration in the present experiments, supports
a similar observation in experiments in man (1),
and makes it probable that the formation of the
conjugate is associated with the presence of free
histamine in the alimentary canal. The fact that
the oral administration of chloramphenicol decreased
the excretion of conjugated histamine supported
this conclusion very strongly. It has been suggested

that the conjugation of histamine occurs in the intestinal lumen as a result of the activity of the intestinal bacteria, and is then absorbed through the wall and ultimately excreted in the urine in the conjugated form (47). Conjugation does not occur in the kidney of the cat (10), but it has been concluded as a result of observations on the pigeon liver _in vitro_, that conjugation occurs in this organ (33). The present experiments do not exclude this possibility, but they strongly support the suggestion that conjugation occurs in the intestinal lumen as a result of the activity of the micro-organisms, and make it clear that this function occurs under ordinary conditions in the normal animal.

These observations therefore suggest that about 50% of the free histamine excreted in rats' urine is formed as a result of bacterial activity within the lumen of the intestine, and that a similar proportion of the conjugated histamine is absorbed from the lumen of the intestine into the wall, whence it ultimately passes into the urine. They do not exclude the possibility of the formation of histamine or its conjugate within the tissues of the rats. It has been shown that the injection of histadine in guinea pigs results in an increase in

the excretion of histamine in the urine. (22, 42).
Thus it may be concluded that the urinary histamine
arises as a result of the animal's own metabolic
activity as well as from bacterial activity in its
intestine.

If the diet of dogs is changed from one of
carbohydrate to one of meat, there is an increase
in the urinary excretion of histamine. This
increase cannot wholly be accounted for by the
histamine contained in the meat. (3). However,
changing the diet in the dog from one consisting
of a balanced ratio of carbohydrate, fat and
protein, to one high in protein and containing no
carbohydrate, causes considerable qualitative
and quantitative changes in the bacterial flora
of the intestine. (46). It is suggested that the
change in the bacterial medium of the alimentary
canal occasioned by the change of diet, might
stimulate the histamine-producing power of the
bacterial population of the intestine. This
suggestion is supported by the fact that a change
in the excretion of histamine did not begin to
occur until eight hours after the administration
of the meat, and reached a maximum in fourteen
hours. These changes in the excretion occurred
at the same times as the changes in the excretion

of histamine by the rats following administration
of the drugs; that is, the meat produced a maximum
excretion in the dog at the same time that the
excretion reached its minimum in the rat after the
administration of drugs. The increased bacterial
formation of histamine after administration of the
meat may explain the origin of the increase in
the urinary excretion of histamine for which there
was previously no explanation.

The present experiments suggest that histamine
was absorbed through the intestinal wall in both
the free and conjugated forms in animals which
were entirely normal apart from the fact that the
bacterial production of histamine in the intestinal
lumen had been reduced. Thus it is concluded
that absorption of histamine normally occurs
through the intestinal wall in the rat, when
conditions are such that histamine metabolism in
the alimentary canal is normal or is returning
towards the normal state. These findings
therefore suggest that the result of the alimentary
canal is to increase the quantity of histamine
in the body. This might seem incompatible with
the conclusion that histamine is excreted into
the alimentary canal. (43). This conclusion was
based on experiments in which compound 48/80 was

injected into anaesthetised cats. Freely
diffusible histamine under these conditions was
presumably present in abnormally high concentrations
in the intestinal mucosa. The experiments recorded
above involved comparatively little interference
with the animal, and are thought to portray
histamine metabolism under normal conditions. These
experiments do not exclude the possibility that
histamine may be excreted through the gastric wall,
later to be absorbed in the intestine, or that
histamine may be inactivated in the intestinal wall.
(10). Nor do they exclude the possibility that
some excretion may occur through the intestinal
wall whent the concentration of histamine in the
tissues is high.

Adrenaline caused a delayed release of histamine,
which continued for five hours or longer after
injection with the larger doses. The maximum
increase in the excretion was 190% of normal, with
a dose of 370 μg./kg., a small effect when compared
with the other releasers. There was a delay of a
half to one hour before any response became apparent;
thereafter, the course of the release resembled
that due to octylamine. The injection of adrenaline
caused haematuria and vasoconstriction, which
however, disappeared before the histamine excretion

returned to normal. The vasoconstriction would probably be responsible to some extent for the slowness in the increase in the histamine excretion, since the local vasoconstriction would cause a delay in the distribution of adrenaline to its target organs. The injection of adrenaline also caused temporary oliguria. This would reduce the excretion of histamine, and probably allow increased destruction of histamine in the tissues of the rat. (3). It is thus probable that the excretion of histamine which actually occurred was less than might have occurred in the absence of oliguria. That is, adrenaline is probably capable of releasing more histamine than it actually did in these experiments.

The doses of adrenaline used were large when compared with those used by some of the other workers, (8, 23, 44, 35), but all these gave it by continuous infusion. The smallest dose which was found to be effective, namely 37 μg./kg., caused toxic effects in the rats; it released histamine in four of the six rats in which this dose was investigated. Therefore it appears that, even if, in the intact animal and in high doses, adrenaline causes a release of histamine, it is not a liberator of histamine under physiological

conditions. Adrenaline in these experiments was therefore not found to be a histamine liberator with the characteristics of propamidine and compound 48/80. The release of histamine which it did cause could probably be explained by the toxic effects which it had on the experimental animal.

The perfusion of the lungs of white rats with adrenaline causes the release of histamine into the perfusion fluid. (23). Even when the fluid did not contain any haemolysed cells, it contained histamine within 10 minutes of starting the perfusion. The release of histamine observed in the present experiments may have originated in the same way. In these circumstances this release of histamine probably occurred because large doses of adrenaline were used, and the experiments were continued for eight hours. Both these factors would increase the chance of detecting histamine release in the intact animal by a weak liberator which also had other actions, including that of vasoconstriction. It is possible that the prolonged release of histamine was observed in the cat for the same reason (8); it may also explain why a physiological release of histamine was not observed in the human being with smaller

doses of adrenaline. (35).

Histamine liberators caused the release of histamine in measurable quantities into the urine of the rat under conditions which were physiological apart from the administration of a water load. The experiments therefore gave a quantitative measure of the way in which a normal animal reacts to a histamine liberator, and provided a method of comparing the effects of different liberators under the same experimental conditions.

Doses of the histamine liberators were used, which it was found would produce a release of histamine into the urine comparable to that produced by the intraperitoneal injection of a dose of histamine, which could be easily assayed, and which produced no toxic effects in the rats. The injection of a dose of histamine provided not only a standard quantitative, but a standard qualitative response also. This is shown in Fig. 5 and Table 12. A standard quantitative response is the histamine release into the urine in excess of the control value, and is expressed as a percentage of the control value. A standard qualitative response is defined in terms of the period prior to the maximum release of histamine, and of the total period of release. The response

caused by the injection of a suitable dose of
histamine has been taken as a standard response
with which the effects of different drugs on the
excretion of histamine in the urine could be
compared. It was assumed that a liberator with
the same type of qualitative response as that
produced by histamine itself, acted by releasing
histamine in the same way and in a form resembling
that in which the injected histamine passed
through the tissues into the urine. That is, the
liberator must have passed rapidly to the target
organs, and must have caused an immediate release
of histamine, which was quickly absorbed into the
blood, and excreted in the urine in the same
fashion as was the injected histamine. A quick
and transient rise in the excretion of histamine
was the result; the excretion returned to the
control value in one, or at the most, two hours.
It has been shown that three hours after the
intravenous injection of histamine in the rat, the
histamine content of all the tissues except the
blood and kidney had returned to normal. In the
blood and kidney, the histamine values after three
hours were small compared to what they were
following the injection, and in both, the decrease
was most rapid in the first hour after the injection.

(40). This observation justifies the measurement
of the urinary excretion of histamine, following
the injection of histamine, as a standard qualitative
response. Injected histamine can be detected in
the plasma in a physiologically active form. (9).
The injection of propamidine and other histamine
liberators causes a fall in blood pressure in the
cat and the liberation of histamine into its
plasma. (28). Thus it appears that in both types of
experiment, physiologically active histamine is
released into the plasma before it passes into
the urine. The difference between the experiments
lies in the source of the histamine. It is clear
that this difference in the cases of propamidine
and compound 48/80 did not affect the time over
which the urinary excretion of histamine occurred
in the present experiments. It is a characteristic
of histamine liberators to produce a delayed fall
in the blood pressure of the cat 15 - 20 seconds
after the injection of the liberator. (28). A
short delay such as this in response to the
histamine liberator could not be detected in these
experiments. A delay of more than half an hour
before the response occurred, and a prolongation
of the action of the liberator was detected with
adrenaline and the mono-alkylamines.

The relatively constant excretion of histamine
in each half hour period permitted even small
increases in the excretion to be observed under the
experimental conditions employed. Although the
excretion might vary considerably between individual
rats, the mean excretion from two or more rats
remained almost the same during each experiment,
and from day to day. This supports the results of
Emmelin (9), who found little variation in the
plasma histamine of the rat over long periods. Thus,
comparisons between the effects of different
liberators in the experiments performed on different
days could justifiably be made. Doses of histamine
liberators were used comparable to those administered
in the perfusion of cat's skin. (11). These doses
caused the liberation of histamine in the whole
animal; the concentration of this liberated
histamine in the urine provided a solution which
could be assayed easily with the relatively simple
methods which were employed.

Propamidine and compound 48/80 both caused a
transient and rapid release of histamine. Compound
48/80 was considerably more efficient than
propamidine in liberating histamine. Responses
comparable to that produced by an injection of
20 µg./kg. of histamine were caused by a dose of

108.

50 μg./kg. of compound 48/80, or a dose of
propamidine six times as great as this.

Octylamine and decylamine also caused an increased
excretion of histamine in the urine. However, the
course of release with these two drugs differed
from that produced by the injection of histamine.
Both the alkylamines caused a prolonged release of
histamine in the urine which was maintained at a
raised value for several hours in the case of
octylamine and for one hour in the case of decylamine,
before gradually declining. A dose of 100μg./kg. of
octylamine, in fact, caused a continuous increase in
the excretion of histamine for five and a half hours.
Octylamine was a much more efficient releaser of
histamine than decylamine. A dose of 500 μg./kg. of
the latter elicited only a small and brief release
of histamine, although the course of the release
was similar with both drugs.

The observation that decylamine is a less
efficient releaser than octylamine contrasts with
that of Mongar and Schild (34), who found that
decylamine was the more active releaser from
minced guinea pig lung. Other workers (29) found
that octadecylamine was a most efficient releaser
of histamine from the cells of rabbits' blood, but
that dodecylamine had no histamine releasing activity

at all. However, these workers found that the
release was complete in one or two minutes if
sufficient of the releaseing agent was present.
Their observation is in agreement with that of
Mongar and Schild, who found that the release was
complete in ten minutes if the concentration of
releasing agent was sufficient.

The differences in the histamine-releasing
properties of the mono-alkylamines observed in the
present experiments from those made by the other
workers may partly be due to species specificity.
The activity of the alkylamines as histamine
releasing agents has been shown to differ in all
the reported work. The experiments have been
performed on rabbits' blood corpuscles, minced
guinea pig lung, and intact rats. In the last
two species it is agreed that octylamine acts as
an efficient releasing agent, though contrasting
results were obtained with decylamine. In the
first two species contrasting results have been
obtained concerning the efficiency of dodecylamine.
MacIntyre, Roth and Sproull (29) state that "a
shortening of the aliphatic chain by only two
carbon atoms decreases the histamine release potency,"
and also that some of the alkylamines have the
power to inhibit histamine release. In their

experiments, they found that good inhibition
occurred with dodecylamine, but that the 12-carbon
chain was not long enough for good histamine
releasing action. It is possible that species
differences might accentuate the differences in the
histamine releasing properties of the different
mono-alkylamines, even though their chain lengths
differed by only two carbon atoms.

Mongar and Schild (34) compare the histamine
releasing properties of compound 48/80 and the
mono-alkylamines. They found the latter to be much
more efficient releasing agents. The present
observations are in agreement with this, when the
total release by compound 48/80 and octylamine are
compared. However, the release by compound 48/80 is
transient, and that by octylamine prolonged, and
this makes comparison between the histamine releasing
properties of the compounds difficult. This
difference might be due to other physiological
effects produced by octylamine on the rats. A
gradual release from the injection site might occur,
resulting in a gradually increasing effect on the
histamine stored in the surrounding tissues.
However, no signs of haematuria or other toxic effects
were observed in the rats, If the effect on the
course of the histamine release was due to any other

physiological actions of the drug, they were not visible during the experiments. Octylamine caused a continuing release of histamine and, whatever the reason, this kind of release was also caused by decylamine in a considerably larger dose, and appeared to differ from the explosive type of release characteristic of compound 48/80 and other histamine liberators. (11).

Mongar and Schild observed that compound 48/80 appeared to be a more active releaser than the momo-alkylamines when their activity was compared by their ability to produce the triple response in human skin. It is clear that the activity of the mono-alkylamines in releasing histamine differs considerably between species, and with different methods of assessing their power to release h histamine; and it appears that comparisons between these compounds and other histamine liberators with regard to their histamine releasing properties should be made with caution.

SUMMARY.

It has been shown that the urinary excretion of histamine provides a sensitive measure of the histamine metabolism in the rat. There was a constant daily excretion of histamine which had a diurnal rhythm. The excretion was not related to the volume of urine passed, but it did appear to vary in relation to the rat's rhythm of activity throughout the twenty four hours. About half of the free histamine and the same quantity of the conjugated histamine excreted in the urine was formed as a result of bacterial activity within the alimentary canal. It is rapidly absorbed into the walls of the canal, whence it passes into the blood and tissues of the body. Thence it passes in a physiologically active form, resembling the form in which orally administered or injected histamine passes through the body, into the urine. It has been found possible to study two stages in the metabolic path of histamine. The formation of histamine in the alimentary canal is a distinct stage in its normal metabolism, and can be influenced by drugs which affect the bacteria producing it. The passage of histamine through the tissues of the animal reflects the part of the metabolism of histamine directly under the

influence of the animal itself. This part of the
metabolism of histamine is controlled to some
extent by the presence of adrenaline in the tissues,
and is affected by the injection of different
histamine liberators in various ways.

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