problem are being carried out in this laboratory using inbred strains.

Previous work on the carmine storage in mice of inbred strains[9] demonstrated an inferior reticulo-endothelial storing ability in animals of the C3H strain as compared with C57 blacks. Although the origin and the significance of natural antibodies, such as the antisheep agglutinins studied in the present report, is not yet understood, there is considerable evidence in support of the part played by the reticulo-endothelial system in their production. In addition to the previously reported depression of the storing capacity of reticulo-endothelial tissues, the present study suggests impairment of the reticulo-endothelial system in relation to the production of natural antibodies in some mouse strains with high spontaneous mammary cancer incidence as compared with the resistant C57 black strain.

Summary. Heteroagglutinins for sheep erythrocytes were studied in the serum of 4 inbred mouse strains. Antisheep agglutinins were present much more frequently and in significantly higher titers in C57 black than in C3H, dba, and Marsh-albino strains. Some properties of the sheep agglutinins were studied. No such differences were found in the distribution of heteroagglutinins for human red cells in the different strains. The possible significance of these findings was discussed.

9 Stern, K., PROC. SOC. EXP. BIOL. AND MED., 1948, **67**, 315.

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Nature and Probable Origin of Conjugated Histamine Excreted After Ingestion of Histamine.

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Anrep and coworkers reported that a histamine derivative appeared in the urine of various animals and a human subject after oral administration of histamine. This histamine derivative was inactive when tested on the isolated guinea pig ileum, but after acid hydrolysis of the compound a typical histamine response could be elicited. Injection of histamine was not followed by increased excretion of the derivative. Liver or intestine were suggested as likely sites for the conjugation of the ingested histamine.[1] Rosenthal and Tabor recently made the important observation that the chemical behavior of the urinary histamine derivative resembled that of acetyl histamine [4(β-acetyl aminoethyl) imidazole].[2]

The purpose of the present investigation was to test whether or not the histamine derivative is actually identical with acetyl histamine and to discover the origin of the compound in the body. The experiments described below present evidence, obtained by paper chromatography, for the identity of the histamine derivative with acetyl histamine and show that this compound appears in the feces as well as in the urine after oral administration of histamine. It is further shown that histamine is rapidly acetylated when added to fresh but not when added to previously autoclaved feces. Lastly, some common fecal microorganisms are shown to produce traces of acetyl histamine from histamine. The evidence obtained suggests that ingested histamine is acetylated within the intestinal contents from which it is partially absorbed and excreted by the kidneys. The methods employed are modifications of those previously developed for the identification of histamine

1 Anrep, G. V., Ayadi, M. S., Barsoum, G. S., Smith, J. R., and Talaat, M. M., *J. Physiol.*, 1944, **103**, 155.

2 Rosenthal, S. M., and Tabor, H., *J. Pharm. and Exp. Therap.*, 1948, **92**, 425.

in blood.[3]

Separation of acetyl histamine from related substances. A mixture of 10 μg of histidine, 10 μg of histamine and 10 μg of acetyl histamine was applied to 2 paper strips (Strips 1 and 2, Fig. 1) and the individual substances to separate strips (Strips 3, 4 and 5, Fig. 1). The chromatograms were developed for 15 hours with butanol saturated with 10% ammonium hydroxide as the mobile phase and 10% ammonium hydroxide saturated with butanol as the phase at the bottom of the chamber. After brief drying, color was developed by drawing the strips through a solution prepared by mixing 10 ml of a solution of 0.125% p-bromoaniline in 0.1 N hydrochloric acid with 10 ml of 3.7% sodium nitrite solution and adding to this mixture 10 ml of a 20% sodium carbonate solution. Histidine, histamine and acetyl histamine appear as red bands which are well separated (Fig. 1). (A number of other histamine derivatives were chromatographed in a similar manner. All of those tested migrated much faster than acetyl histamine.)

Approximate Rf values (Fig. 1)
Distance compound moves along paper
Distance solvent moves

Histamine		Acetyl histamine	
Strip 1	.56	Strip 1	.71
" 2	.56	" 2	.71
" 5	.55	" 3	.71

The Rf values for histidine are difficult to estimate because of its slow migration rate with the solvent used.

Urinary excretion of the histamine derivative after feeding histamine to a dog. A normal urine sample was collected from a healthy male dog who was then given histamine diphosphate, equivalent to 200 mg of the free amine, dissolved in 250 ml of water by stomach tube. The dog was sedated with morphine and atropine prior to the administration of the histamine solution to avoid vomiting.[2] Freshly voided urine was collected thereafter at intervals up to 33 hours.

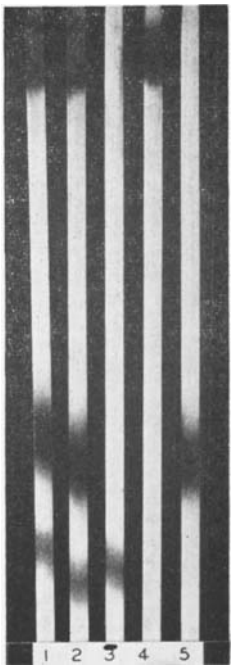

FIG. 1.
Separation of histidine, histamine, and acetyl histamine.
Strips 1 and 2: 10 μg histidine, histamine, and acetyl histamine applied as mixture.
Strip 3: 10 μg acetyl histamine.
Strip 4: 10 μg histidine.
Strip 5: 10 μg histamine.

From each sample of urine, 0.3 ml was diluted with water to 5 ml and 1.5 g of a salt mixture of 1 part $Na_3PO_4 \cdot H_2O$ and 6.25 parts anhydrous Na_2SO_4[4] was added. Two ml of butanol were added to the mixture and the tubes shaken for 20 minutes. After centrifugation, the butanol layer was applied to the paper strips by capillary pipettes.[5] Suitable controls were prepared by applying to strips extracts obtained from urine samples to which acetyl histamine and histamine had been added and by applying the pure substances to separate strips. The chromatograms were developed in the manner described in the foregoing experiment and uniformly showed bands in a position identical with that occupied by pure acetyl histamine on the control strips (Fig. 2). The chromatogram representing normal

[3] Urbach, K. F., PROC. SOC. EXP. BIOL. AND MED., 1948, **68**, 430.

[4] McIntire, F. C., Roth, L. W., and Shaw, J. L., *J. Biol. Chem.*, 1947, **170**, 537.

[5] Urbach, K. F., in press.

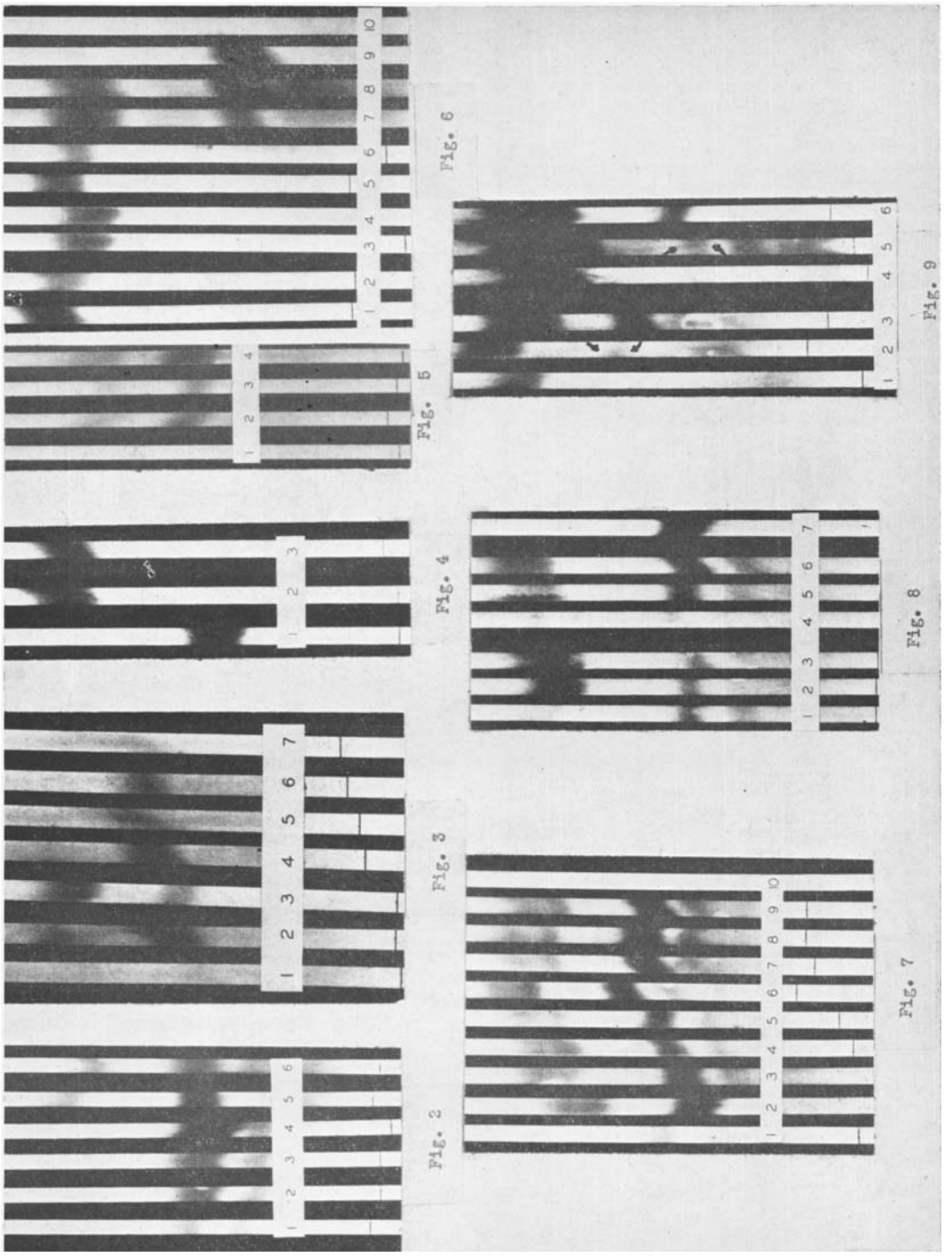

FIG. 2–9.

(All upper ends of the strips have been cut. The pencil lines on the lower ends of some of the strips indicate how far the solvent has advanced. On all chromatograms the upper bands are due to histamine and the lower due to acetyl histamine (histamine derivative) unless otherwise indicated).

FIG. 2—Chromatograms of dog urine after oral administration of histamine.

Strip 1—Normal urine.
" 2—Urine 22 hr after histamine administration.
" 3—Urine 33 hr after histamine administration.
" 4—Urine 33 hr after histamine administration, 20 μg acetyl histamine added.
" 5—Normal urine, 10 μg histamine and 10 μg acetyl histamine added.
" 6—Histamine and acetyl histamine (control).

FIG. 3—Chromatograms of human urine after oral administration of histamine.
Strip 1—Normal urine.
" 2—Urine 1½ hr after histamine administration.
" 3—Histamine and acetyl histamine (control).
" 4—Urine 3½ hr after histamine administration.
" 5—Urine 6 hr after histamine administration.
" 6—Urine 8 hr after histamine administration.
" 7—Urine 20 hr after histamine administration.

FIG. 4—Chromatograms of hydrolysis of acetyl histamine.
Strip 1—Acetyl histamine before hydrolysis.
" 2—Acetyl histamine after hydrolysis.
" 3—Histamine (control).

FIG. 5—Chromatograms of human stool after oral histamine administration.
Strip 1—Normal stool.
" 2—Stool 6 hr after histamine administration.
" 3—Stool 20 hr after histamine administration.
" 4—Normal stool plus acetyl histamine and histamine (control).

FIG. 6—Chromatograms of effect of histamine addition to bowel loops and bowel contents of a dog.
Strip 1—Washed ileum loop immediately after histamine addition.
" 2—Washed ileum loop incubated 4½ hr after histamine addition.
" 3—Ileum contents immediately after histamine addition.
" 4—Ileum contents incubated 4½ hr after histamine addition.
" 5—Washed colon immediately after histamine addition.
" 6—Washed colon incubated 4½ hr after histamine addition.
" 7—Colon contents immediately after histamine addition.
" 8—Colon contents incubated 4½ hr after histamine addition.
" 9—Acetyl histamine (control).
" 10—Colon contents before histamine addition.

FIG. 7—Chromatograms of effect of histamine addition to human stool.
Strip 1—Normal stool.
" 2—Histamine and acetyl histamine (control).
" 3-9—Stool immediately, 1 hr, 4 hr, 8 hr, 24 hr, 30 hr, and 55 hr after histamine addition and incubation.
" 10—Normal stool 24 hr after incubation.

FIG. 8—Chromatograms of effect of autoclaving human stool on acetyl histamine formation.
Strip 1—Autoclaved stool.
" 2—Autoclaved stool immediately after histamine addition.
" 3—Autoclaved stool incubated 4½ hr after histamine addition.
" 4—Nonautoclaved stool.
" 5—Nonautoclaved stool immediately after histamine addition.
" 6—Nonautoclaved stool incubated 4½ hr after histamine addition.
" 7—Acetyl histamine (control).

FIG. 9—Chromatograms of effect of addition of histamine to pure cultures of *Aerobacter aerogenes* and *E. coli*.
Strip 1—Aerogenes culture immediately after histamine addition.
" 2—Aerogenes culture incubated 40 hr after histamine addition.
" 3—Histamine and acetyl histamine (control).
" 4—*E. coli* culture immediately after histamine addition.
" 5—*E. coli* culture incubated 4½ hr after histamine addition.
" 6—Histamine and acetyl histamine (control). (Traces of acetyl histamine at arrows. On Strip 5 at least 2 unidentified bands appear below the acetyl histamine).

urine shows the presence of a small amount of the histamine derivative (Strip 1, Fig. 2).

Approximate Rf values (Fig. 2)

Histamine		Urinary histamine derivative		Control acetyl histamine	
Strip 5	.55	Strip 1	.73	Strip 5	.74
" 6	.57	" 2	.73	" 6	.73
		" 3	.73		

Urinary histamine derivative with acetyl histamine added	
Strip 4	.73

Urinary excretion of the histamine derivative after ingestion of histamine by a human subject. After collecting a sample of normal urine, a gelatin capsule containing 100 mg histamine diphosphate was given by mouth to a human subject (K.F.U.). Urine was collected at frequent intervals after the ingestion

of histamine. Since the amount of conjugate present was small, the size and purification of the samples had to be modified: to 20 ml of urine were added 6 g of the salt mixture described. The samples were extracted twice with 10 ml of butanol. The combined butanol extracts were aerated until free from ammonia and were then passed through cotton succinate.[4] After passage of the butanol extracts, the cotton succinate was washed with 2 ml of acetone which was followed by elution with 2 ml of 0.5 N H_2SO_4, followed by 2 ml H_2O. The combined H_2SO_4-H_2O eluate was neutralized with 10 per cent NaOH and 1.5 g of the salt mixture of Na_3PO_4 and Na_2SO_4 was added. The resulting solution was extracted with 2 ml of butanol, which was then applied to the paper strips as in the previous experiment. Control strips were prepared as in the foregoing experiment. The amounts of histamine derivative appearing on the chromatograms are much smaller than those observed in the previous experiment. This is partly explained by the smaller amount of histamine employed and partly by the failure of cotton succinate to absorb acetyl histamine quantitatively.[2] Moreover, Anrep has reported that man, after the oral administration of histamine, does not excrete as much of the histamine derivative in the urine as does the dog.[1] It is of interest to note that free histamine, as well as histamine derivative, appears in the human urine early after the histamine administration (Strip 2, Fig. 3).

Approximate Rf values (Fig. 3)

Histamine		Control acetyl histamine		Histamine derivative	
Strip 3 (control)	.53	Strip 3	.69	Strip 2	.69
Strip 2	.53			Strip 4	.68
				Strip 5	.68
				Strip 6	.70

Hydrolysis of acetyl histamine to histamine. The foregoing experiments provide evidence that the substance excreted in the urine of dog and man after histamine ingestion is acetyl histamine. In order to obtain evidence for the identity of the compound described by Anrep[1] with acetyl histamine, a sample of synthetic acetyl histamine was hydrolized under the conditions used by Anrep for the hydrolysis of the urinary compound to histamine. Six mg of acetyl histamine were dissolved in 5 ml of water and 1 ml concentrated hydrochloric acid. 0.01 ml (10 μg) of the solution was applied to paper strips both before and after boiling the solution for one and one-half hours. A control strip was prepared by applying 10 μg of histamine. Chromatograms were prepared as in the first experiment, and are shown in Fig. 4. It is evident that acetyl histamine is easily converted to histamine under the conditions employed. A trace of acetyl histamine was still present after the hydrolysis, possibly because of the relatively large amount of acetyl histamine employed. The approximate Rf values of pure histamine (Strip 3) is 0.55 and of histamine obtained from the hydrolysis of acetyl histamine (Strip 2) 0.55.

Excretion of the histamine derivative in human feces after oral administration of histamine. The previous experiments presented evidence for the identity of the urinary histamine derivative with acetyl histamine. Because of the possibility that the histamine derivative might also appear in the feces, as well as the urine after feeding histamine, 3 stool samples were collected before, 6 and 20 hours after the administration of the histamine to the human subject. To 1 g (wet weight) of each sample was added 10 ml water. A fourth sample was prepared by adding 20 μg of each histamine and acetyl histamine to 1 g of the stool sample collected before the histamine administration. All samples were shaken, filtered and 3 g of the previously mentioned salt mixture was added to the filtrates which then were twice extracted with 10 ml portions of ether. This was followed by extraction with 2 ml of butanol which were passed through cotton succinate. The elution of the cotton succinate and the preparation of the chromatogram shown in Fig. 5 were carried out as in the third experiment. Strip 1 (normal stool) shows no band. Strip 2, representing the stool sample obtained 6 hours after the ingestion of histamine, shows a pronounced acetyl histamine band below and a weak histamine band above. Strip 3, corresponding to the stool sample taken 20 hours after the ingestion of histamine, shows a weaker acetyl histamine band and only a very

faint trace of histamine. Strip 4 represents normal stool with histamine and acetyl histamine added.

Approximate Rf values (Fig. 5)

Histamine		Histamine derivative in stool		Control acetyl histamine	
Strip 2	.57	Strip 2	.71		
Strip 3	.57	" 3	.72		
Strip 4	.57			Strip 4	.70

Formation of the histamine derivative after addition of histamine to isolated intestinal loops and bowel contents of a dog. The appearance of the histamine derivative in feces, described in the previous experiment, could be due to two different mechanisms. Either the histamine derivative is formed inside the intestine or histamine is absorbed from the intestine, changed and re-excreted into the contents of the bowels. Since the histamine derivative had not been observed in the urine after the injection of histamine,[1] the first possibility was assumed to be more likely. Since it was, therefore, of interest to investigate whether the derivative is formed through interaction of histamine with the intestinal wall or with intestinal contents, the intestines were removed from a freshly killed dog and the contents were removed from segments of colon and ileum which were then washed clean and tied. The loops were filled with 10 ml of saline containing 1 mg of histamine per ml. To the intestinal contents 1 mg histamine per gram was added after removal of 1 g samples. All preparations were incubated at 37°C and 1 ml or 1 g samples were withdrawn after appropriate intervals. Each sample was diluted to 20 ml with water. The mixture was shaken and centrifuged and chromatograms were prepared from 1 ml of the supernatant by diluting to 5 ml, adding salt mixture, ether and butanol extraction and cotton succinate purification as described in the previous experiments. The chromatograms illustrated in Fig. 6 show that the histamine derivative was formed rapidly in the contents taken from the colon, but not in the washed loops in which histamine disappeared without formation of acetyl histamine. In the contents taken from the ileum traces of the histamine derivative were formed.

Approximate Rf values (Fig. 6)

Control acetyl histamine		Histamine derivative	
Strip 9	.74	Strip 7	.75
		" 8	.75

Formation of the histamine derivative after addition of histamine to human feces. The preceding experiment suggested intestinal contents as a site for the conversion of histamine. It was, therefore, of interest to investigate if human feces could convert histamine in a similar manner. Freshly passed human feces were divided into 2 samples. To one sample 1 mg/g histamine was added and 1 g portions were taken immediately from the control and histamine-containing feces. Both samples were then incubated and 1 g portions were withdrawn at convenient intervals. Chromatograms were prepared as described in the previous experiment and are shown in Fig. 7. Strip 1 (normal stool) shows a small amount of the histamine derivative present before histamine addition. Strip 2 represents 10 μg acetyl histamine and 10 μg histamine added to normal stool as a control and the following strips represent stool samples taken at various times after the histamine addition. It is of interest to note the appearance of an unidentified band (Rf 0.81) after 30 and 55 hours incubation. Strip 10 represents normal stool after 24 hours incubation.

Approximate Rf values (Fig. 7)

Control acetyl histamine		Histamine derivative	
Strip 2	.72	Strip 3	.71
		" 4	.70
		" 5	.70
		" 6	.70
		" 7	.71
		" 8	.72
		" 9	.72

The influence of autoclaving human feces on the formation of the histamine derivative. The previous experiment showed that histamine, when added to fresh human feces, is converted effectively to acetyl histamine. In order to test whether the reaction is due to a biological process or due to a chemical reaction not requiring living matter, a freshly passed human stool specimen was divided into 2 portions, one of which was autoclaved at 200 lb pressure for 20 minutes. After taking 1 g samples from the normal and autoclaved portions, 1 mg histamine per gram of stool was

added to each portion. One gram samples were withdrawn immediately after mixing the histamine into the stool and again after 4 hours incubation. The chromatograms shown in Fig. 8 were prepared as described above. The autoclaved sample, before the addition of histamine, shows a faint band, due to the histamine derivative which does not increase in intensity after incubation with histamine (Strips 1, 2, 3). The bands due to the histamine derivative increase markedly in color intensity on incubation of the non-autoclaved sample after addition of histamine (Strips 4, 5, 6). Pure acetyl histamine was applied to Strip 7 as a control.

Approximate Rf values (Fig. 8)

Control acetyl histamine		Histamine derivative	
Strip 7	.71	Strip 1	.73
		" 2	.73
		" 3	.73
		" 4	.72
		" 5	.71
		" 6	.71

Attempts to convert histamine to acetyl histamine with pure cultures of E. coli and Aerobacter aerogenes. The experiment showing the formation of the histamine derivative made it appear likely that conversion of the histamine was due to fecal organisms. Histamine, was, therefore, added to a pure culture of *E. coli* and of *Aerobacter aerogenes* incubated in a simple glucose-salt medium.[6] Samples of the cultures were withdrawn at intervals and chromatograms, shown in Fig. 9, were prepared in a manner similar to that described in the previous experiments. Both organisms appear to form acetyl histamine in small quantities from added histamine. In addition, the organisms apparently are able to produce other unidentified substances from histamine which give colored bands with the color reagent employed.

Approximate Rf values (Fig. 9)

Control acetyl histamine		Acetyl histamine formed in culture	
Strip 3	.70	Strip 2	.71
" 6	.71	" 4	.70

Discussion. The experiments presented provide evidence that the histamine derivative recoverable from urine and feces of dog and man is acetyl histamine.* This substance is probably identical with the histamine derivative described by Anrep.[1] The fact that acetyl histamine is formed from histamine in normal but not in autoclaved feces and the ability of common fecal organisms to form traces of acetyl histamine in pure culture suggests that the observed acetylation of histamine in feces may be due to bacteria. The fact that *E. Coli* and *Aerobacter aerogenes* in pure culture are not able to produce acetyl histamine as effectively in quantities comparable to those obtained from feces may be due to several obvious differences in the conditions obtaining in stool samples and in simple culture media. Other fecal organisms may be able to acetylate histamine much more effectively. The possibility that histamine may also be acetylated elsewhere in the body, *e.g.* the liver, has been suggested and is not excluded by the present experiments. It should be noted that on occasion small quantities of acetyl histamine and histamine were observed in normal stool and urine samples.

Conclusions. 1. The observation that a histamine derivative appears in the urine of dog and man after oral histamine administration is confirmed.

2. The histamine derivative can be effectively separated from histamine by paper chromatography.

3. The histamine derivative is identical with acetyl histamine as shown by paper chromatography.

4. Acetyl histamine is readily formed from histamine added to feces of dog and man.

5. It is suggested that histamine is acetylated in the intestinal contents to acetyl histamine which is partially absorbed from the intestinal tract and excreted in the urine as well as in the feces.

[6] Kohn, H. I., and Harris, J. S., *J. Pharm. and Exp. Therap.*, 1941, **73**, 343.

* Dr. Tabor and Dr. Rosenthal have isolated and crystallized the urinary histamine derivative. The melting point and chemical analysis characterize the compound as acetyl histamine. (Private communication).