THE URINARY EXCRETION OF HISTAMINE IN HEALTH AND DISEASE

A Thesis presented for the Degree

of

Doctor of Medicine

by

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INTRODUCTION

The pharmacological actions of histamine and its presence in the human body have been established for many years, but understanding of the role of histamine in physiological processes has not kept pace, mainly because the methods available have been inadequate for measurement of the small quantities of histamine present in body tissues and fluids.

The development by Barsoum and Gaddum (1935) of a suitable method for estimating histamine in the blood marked an important advance, while the discovery of the antihistamine drugs (Fourneau and Bovet, 1933) stimulated further investigation of the physiological actions of histamine.

In 1939, Ackermann and Fuchs isolated histamine from normal human urine, and subsequent work in Cairo and Edinburgh stressed the necessity for more sensitive techniques. Working in the Department of Pharmacology at Edinburgh, Roberts and Adam (1950) developed a chromatographic method by which, for the first time, the minute amounts of free histamine present in human urine could be measured with reasonable accuracy.

In this thesis, the method of Roberts and Adam is applied to the study of urinary histamine in various circumstances of health and disease. After a historical review, the thesis is divided into sections and where pertinent, the literature relating to each aspect

of histamine metabolism is reviewed at the beginning of the section. It is hoped that this arrangement will facilitate the clear exposition of the several separate investigations which the study comprises.

HISTORICAL REVIEW

1. Early experimental investigations.

The first extensive study of the metabolism of histamine was undertaken by Dale and Laidlaw in 1910. In the course of their experiments they investigated the urinary excretion of histamine in a cat by administering 150 mg. of the substance subcutaneously and collecting the urine during the next 24 hours. The urine gave a very intense Pauly's reaction (Pauly, 1904) but had no perceptible effect on the isolated uterus of a guinea-pig in doses of five ml. The authors concluded that histamine was not excreted as such in the urine but that it was probably present as a compound devoid of the characteristic physiological activity, although having the iminazole ring intact.

In 1913, Oehme carried out experiments on rabbits to discover whether histamine was excreted in the urine after intravenous infusion. He found that urine collected before the infusion of histamine had no stimulating effect on the guinea-pig uterus, whereas, in two out of three trials, urine secreted during the course of the infusion had an activity equivalent to that of a histamine solution of 1 in 5 million concentration.

Guggenheim and Loeffler (1916) confirmed these results and agreed with Oehme that the small amounts of histamine excreted under these experimental conditions could play no significant part in the inactivation of the amine. Being primarily interested

in detoxication mechanisms, neither Oehme nor Guggenheim and Loeffler pursued their investigations further. The latter authors did, however, attempt to identify the imidazole compound found by Dale and Laidlaw in the urine. They considered that histamine might be rendered inactive by conjugation, either as the acetyl or as the glycyl compound. They felt that small quantities of acetyl histamine could not readily be distinguished from other conjugated amines, such as glycyl phenyl ethylamine, but that if glycyl histamine were present, its activity could be measured, and would be increased 100 times by hydrolysis with hydrochloric acid. This effect was not found in their experiments on rabbits and they therefore concluded that conjugation played no part in the detoxication of histamine, which was probably accomplished by deamination and oxidation.

Koch (1913) isolated histamine by crystallization from the urine of three out of six dogs following parathyroidectomy, but owing to the nature of the extraction, was not able to express his results quantitatively. The urine was collected by drainage from the cages and, although acidified, was allowed to stand in an atmosphere which Koch admitted was often strongly ammoniacal. It is possible, therefore, that more histamine than usual was present as a result of faecal contamination or of bacterial action, as suggested later by Misrahy and Salama (1947), who were unable to demonstrate abnormal urinary histamine excretion in parathyroid-
:ectomized dogs.

No interest was taken in the subject of urinary histamine from

1916 until 1930, and in the latter year Feldberg and Schilf, in a comprehensive review of histamine metabolism, concluded that histamine was not excreted in the urine. In the same year, however, Best and McHenry (1930), during their study of histaminase, found 0.2 ug. of histamine per ml. in each of two specimens of dog's urine, which had been hydrolysed with hydrochloric acid and subsequently neutralized. The extracts were assayed on the atropinized cat's blood pressure and unfortunately no attempt was made to confirm that the activity measured was due to histamine. These workers found that the dog's kidney contained more histaminase than any other tissue except the intestine and considered it probable that the kidneys inactivated histamine rapidly by this means.

Three years later, Macgregor and Peat (1933) perfused dog kidney--lung preparations with blood and saline to which histamine had been added and found histamine in the urine excreted by the kidney. They suggested that the kidney had a twofold action on histamine, the greater part being inactivated by histaminase and the remainder being excreted in the urine.

Rose and Browne (1938) studied the fate of intravenously injected histaminein rats and found that, of the tissues studied, the kidney took up most histamine but, in commenting on the findings of Macgregor and Peat, they stated that in none of their own experiments had the rats secreted urine within three hours of the injection. Since the kidney of the rat, unlike that of the dog, does not contain large quantities of histaminase (McHenry and Gavin, 1931), it might have been expected on the hypothesis of Macgregor and Peat that more

histamine would have been excreted by the rat's kidney than by the dog's, but in the absence of urinary secretion this could not be confirmed.

2. Urinary histamine in normal human beings.

The excretion of imidazole compounds in normal human urine was investigated by Koessler and Hanke (1924a) during their extensive study of proteinogenous amines. Using a colorimetric method, they found that a normal adult excreted 120 to 220 mg. of imidazole compounds daily, but they did not discuss the possibility that histamine might form part of this total. Ten years later, Loeper, Lesure and Thomas (1934), using the same methods, measured imidazoles in the blood, urine and faeces of various patients and suggested that histamine was an important constituent of the group. Ungar and Pocoulè (1937), however, pointed out that they had no grounds for this suggestion, since their method only measured a mixture of imidazoles. Numerous specimens of normal human urine were examined by Ungar and Pocoulè, both by direct assay of the urine on guinea-pig intestine and by assay after extraction of the urine by the method of Barsoum and Gaddum (1935). Histamine was only detected if the urine had been exposed to the air for 24 hours or more, when an average of 0.15 mg. per litre was found, and these workers concluded that histamine did not exist in human urine.

As a result of the discovery of histamine in fresh mammalian liver and kidneys, Ackermann and Fuchs (1939) decided to re-open the question of its occurrence in normal urine. Making use of an adsorptive substance (Lloyd's reagent) with purification of the

eluate and assay on a strip of guinea-pig's jejunum, they obtained 117 ug. of histamine from ten litres of urine. To obtain larger amounts for identification, they treated a very large quantity of urine with ammoniated zinc hydroxide and subsequent purification, and thereby recovered 658 ug. of histamine from 1,000 litres of urine. From this they were able to crystallize 0.9 mg. of a picrate, which had the same melting point as histamine dipicrate. Although the amounts obtained were extremely small, this work established the presence of histamine in normal human urine.

In the following year, Werle and Effkemann (1940) reported the results of investigations of eight normal adults, four men and four women. They found that the urinary excretion of histamine ranged from 10 to 120 ug. per litre, measured by direct assay on the guinea-pig's intestine.

In 1944, Anrep, Ayadi, Barsoum, Smith and Talaat published an important paper on their investigations of animals in the Cairo zoo. Their method of extracting histamine from urine was based on the fact that both free and conjugated histamine are absorbed from urine by charcoal, from which they can be released by repeated washings with acid-alcohol. Free histamine can be measured in the unhydrolysed extract, while the activity after hydrolysis measures free and conjugated histamine. It was found that the urinary histamine of herbivorous animals was low, and almost all in the free form, while that of carnivora was high and 98 to 100% conjugated. Rats occupied an intermediate position ; on a diet of carbohydrate their excretion of histamine was low, about 10 ug.

a day, more than half in the free form, while when given meat the excretion rose to as much as 230 ug. a day, mostly conjugated.

A few normal humans were also investigated and found to excrete small amounts of free histamine and larger amounts of the conjugate. The greatest excretion was 2.4 mg. in 24 hours on a high meat diet, almost all this histamine being conjugated.

When conjugated histamine was given orally or subcutaneously to dogs, a high proportion of it was rapidly excreted in the urine unchanged. When free histamine was given by mouth, three to five per cent of the dose was excreted in the urine in the conjugated form ; after subcutaneous injection the urinary free histamine increased slightly, while the conjugate showed no significant change.

Although under the conditions of Anrep's experiments only a small proportion of injected histamine appeared in the urine, Alexander (1946) showed that when very large doses were used, so that the body was "saturated" with histamine, a much larger proportion was excreted. He found that ,whereas the histamine excretion of normal mice was very low, an average of 37.7% of an injected dose of 3.0 mg. appeared in the urine in the following 24 hours.

The increase in urinary conjugated histamine after dogs had been given histamine by mouth was confirmed by Rosenthal and Tabor (1948) and the same result was shown to occur in guinea-pigs, rats and rabbits. These authors used a colorimetric method for the determination of histamine, based on the diazo reaction given

by imidazole compounds ; they stated that no other substance giving the same colour reaction was detectable in body tissues, and that under the conditions of their experiments the method was specific for histamine, though it would not distinguish free from conjugated histamine by colour alone, nor could it be used for concentrations of less than 0.5 to 1.0 ug. of histamine per 5 ml of urine. The method therefore is of use only in experiments involving the administration of histamine, and is not sensitive enough to detect the quantities of histamine occurring normally in urine.

Using this method, Millican, Rosenthal and Tabor (1949) extended their investigations into the fate of oral histamine. They found that in mice and rats the administration of histamine orally was was followed by an increase in urinary histamine, mainly as free histamine in the first few hours with a relative rise in conjugated histamine later, this rise being much more marked in rats. In guinea-pigs, rabbits and dogs, nearly all the histamine excreted after oral administration was conjugated. This suggests that the high tolerance of rats and mice for histamine is not based on conjugation or destruction of histamine, since a high proportion of it is excreted in the urine in the free form.

In 1949, conjugated histamine was identified by Urbach as acetyl histamine (4(β-acetyl aminoethyl) imidazole). This identification was based on evidence obtained by paper chromatography and was confirmed chemically by Tabor and Mosettig (1949), who isolated crystalline acetyl histamine from the urine of dogs.

Adam (1950) confirmed and extended the work of Anrep and his

associates by studying the excretion of histamine given orally and by intravenous injection to normal human beings. After oral administration of 133 mg. of histamine to healthy adults, 0.17 to 1.0% of the dose appeared in the urine as conjugated histamine, and there was only a very slight rise in the excretion of free histamine. When 3.5 to 5.0 mg. of histamine were infused intravenously, 0.6 to 2.6% was excreted in the urine as free histamine and there was no appreciable rise in the conjugate. No increase in blood histamine during the infusion could be demonstrated, and Adam therefore concluded that histamine liberated in the body was more likely to be detected in the urine than in venous blood, probably because it was concentrated in the kidneys.

The method used by this author was a modification of Anrep's charcoal method and was not sufficiently sensitive to estimate the amounts of free histamine in normal human urine, since only concentrations of 0.1 ug. per ml. or more could be measured. Roberts and Adam (1950), therefore, developed a chromatographic method involving separation of free from conjugated histamine by adsorption of the former on a column of a cationic exchanger, Decalso, with subsequent adsorption of the conjugate on a column of charcoal and sand, and assay of the eluates separately. The recovery of free histamine by this means was only 67%, but this disadvantage was outweighed by the greater sensitivity of the method, making possible the accurate determination of free histamine in human urine.

Studies of five healthy men showed a mean excretion of 21.6 ug.

of free histamine (32.4 ug. corrected) in 24 hours, with a range of 12.1 to 41.4 ug. The mean excretion of conjugated histamine was 125 ug. in 24 hours (expressed as histamine) with a range of 16 to 727 ug. The relatively constant daily excretion of free histamine compared with the wide fluctuations of the conjugate suggested the possibility that free histamine in the urine represents the continuous formation or liberation of histamine in the tissues, while the conjugate depends mainly on the diet.

3. Urinary histamine in normal pregnancy.

Ungar and Dubois (1937) examined 107 specimens of urine from pregnant women and found histamine in 72 of the specimens in amounts ranging from 10 to 1600 ug. per litre (as histamine hydrochloride), the maximum frequency of histaminuria being in the middle trimester. Werle and Effkemann (1940), however, failed to confirm this work ; using the same method of direct assay, they investigated the urine of 21 women and found that pregnant women excreted the same quantity of histamine as non-pregnant women, the range being 30 to 130 ug. per litre in the former, and 30 to 120 ug. per litre in the latter.

Kapeller-Adler (1941) was also unable to find abnormal amounts of histamine in urine from pregnant women, while Rockenschaub (1953), using the more precise method of Roberts and Adam, reported that the excretion of free and conjugated histamine by ten normal pregnant women was within the range found in normal men. The evidence therefore seems conclusive that there is no increase in histaminuria in normal pregnancy.

OUTLINE OF THE PRESENT INVESTIGATION.

The data in this thesis are presented in three main parts. The first part describes the methods used and the preliminary tests carried out. The second part is concerned with the variations which occur in the excretion of histamine by healthy human beings. The third part is a study of the excretion of histamine in diseases in which a disturbance of histamine metabolism is suspected of playing a contributory role.

Throughout the thesis, the word " histamine " is used to describe the substance whose pharmacological activity is measured. Although there is no conclusive proof that the substance is histamine, its pharamcological properties and the results of confirmatory tests which will be described in Part I , leave little doubt of its identity. It is therefore considered justifiable to consider the substance as histamine, rather than to refer to it as " histamine-like activity ".

In some sections of the investigation, values are given for blood histamine as well as for urinary histamine. The histamine content of the blood was measured with the object of obtaining a more comprehensive picture of histamine metabolism in the conditions studied, and with the possibility in mind that a correlation might be found between the blood level and the amount of histamine excreted. That no such correlation was demonstrable is not surprising, since the Barsoum-Gaddum method does not distinguish free from conjugated histamine, nor does it measure

separately the histamine content of the various blood constituents. The values are included, however, as it is considered that they enhance the value of the data and also have some interest of their own.

All estimations of blood histamine, and of both free and conjugated histamine in the urine, were carried out in duplicate, with the exception of the urinary histamine estimations on healthy children and infants, and the recovery experiments. Each value is therefore the mean of duplicate samples. Where the values for duplicates were widely divergent, both were discarded, but this was necessary only three or four times in nearly two thousand estimations.

PART I. METHODS AND PRELIMINARY STUDIES.

A. Methods.

1. Collection of specimens.

Urine was collected in chemically clean bottles containing twice normal hydrochloric acid to ensure that the specimen was maintained at pH 5 or less. The urine was stored until extraction in a refrigerator at 2° to 4°C.

Urine was collected from infants by placing them on a metabolic bed and allowing the urine to pass through tubing strapped to the penis into a bottle below the bed. Paul's colostomy tubing of one inch width proved satisfactory for the purpose, being light and easily distensible.

Venous blood was drawn from an arm vein into a 10 ml. syringe moistened with heparin solution. A specimenwas immediately diluted in a white cell pipette with a solution of 0.1% phloxine in 50% propylene glycol for enumeration of eosinophils, according to the method of Randolph (1944).

2. Extraction of histamine from urine.

The method used for the determination of urinary histamine was that of Roberts and Adam (1950), whose paper contains full details of the technique.

The urine passed during a period of 24 hours is measured and a sample is filtered after adjusting the pH to 7.6.A prepared column of a cationic exchanger, sodium alumino-silicate (Decalso), is washed with distilled water and 50 ml. of the filtered urine are passed through the column. When about 30 ml. have passed,

10 ml. of the percolated urine are collected and set aside for determination of conjugated histamine. After the 50 ml. have percolated through the column, it is prepared with normal saline and absolute alcohol, and the free histamine adsorbed on the column is then eluted with strong ammonia, followed by ammoniated chloroform. The eluate is collected in a flask and evaporated to dryness under negative pressure in a water bath at 40°C. Finally, the residue is acidified with 3% acid alcohol and dried with absolute alcohol.

The 10 ml. of urine for conjugated histamine are passed through a column of charcoal and sand to adsorb the histamine, which is then eluted with 3% acid alcohol. The eluate is collected in a flask, dried, and boiled with 20% hydrochloric acid on a sand bath for one hour to hydrolyse the acetyl histamine. The hydrolysed specimen is evaporated to dryness and finally dried with absolute alcohol.

The dried samples can be kept in a dry atmosphere for an indefinite period, but were seldom kept for more than a few days in the present experiments.

3. Extraction of histamine from blood.

The method used was essentially that of Barsoum and Gaddum (1935) as modified by Code (1937).

Five ml. of blood are transferred by siliconed pipette to a test tube containing 10 ml. of 10% trichloracetic acid. After shaking and allowing to stand for one hour, the contents of the test tube are filtered by suction, the tube being washed with 10 ml. of

5% trichloracetic acid, and the washing added to the contents of the filter funnel. Five ml. of concentrated hydrochloric acid are added to the filtrate, which is boiled on a sand bath for 1½ hours and finally dried twice in the presence of alcohol, under reduced pressure in a water bath.

The dry specimen is extracted in 20 ml. of absolute alcohol, using three aliquots of 10, 5 and 5 ml., the extract is centrifuged, decanted into a flask, and finally evaporated to dryness in a water bath under negative pressure.

The normal range of values for blood histamine by this method is from 0.012 to 0.067 ug. per ml., with a mean value of 0.040 ug. per ml. (mean of 36 determinations on 12 healthy adults).

4. Assay of specimens.

The urine samples are taken up in warm saline or Tyrode's solution, 10 ml. being used for the free histamine and 5 ml. for the conjugated histamine. The blood samples are taken up in 5 ml. of Tyrode's solution diluted 3:1 with distilled water. The samples are carefully neutralized with sodium hydroxide solution and are centrifuged and decanted if necessary.

Assay is carried out on a strip of guinea-pig ileum suspended in a bath of Tyrode's solution containing atropine. The effect of the samples is compared with that of a standard solution of histamine acid phosphate. The histamine values for both free and conjugated histamine are calculated in terms of the base on the assumption that this represents 36.16% of the weight of the phosphate.

B. Preliminary studies.

1. Bacteriological tests.

During the tests on healthy children, samples of urine taken just before extraction were cultured bacteriologically. No growth occurred in the majority of cases but six showed a few colonies of bacteria, and five showed a moderate growth of E. Coli. In no case was the urine infected before collection and there was no evidence that the bacteria cultured had any effect on the values obtained for free or conjugated histamine.

2. Identification of histamine.

Mepyramine maleate in a concentration of 2×10^{-8} was added to the bath at the end of an assay and the effect noted on equiactive doses of the standard solution of histamine and of a urine extract. The fact that the rate of recovery from inhibition was the same for the extract as for the standard strengthens the evidence that the activity of the extract was due to histamine. A further test consisted in boiling an extract from the Decalso column for one hour in concentrated hydrochloric acid and comparing its activity with that of an unboiled extract of the same urine. The histamine equivalents agreed to within ten per cent.

These tests confirm the more extensive tests carried out by Roberts and Adam and constitute substantial evidence for the identity of the substance assayed. Conclusive proof is not possible short of complete chemical identification, and the activity cannot be differentiated from that of certain pharmacologically active

compounds closely related to histamine, such as the N-alkyl and N-dialkyl histamines (Roberts and Adam, 1950).

3. Recovery experiments.

In the experiments on normal children and on infants with gastro-enteritis, the Decalso was part of the batch used by Roberts and Adam in their original work, in which the mean recovery of free histamine in 116 experiments was 67% ± 1.1 (standard error of the mean). For the rest of the present work, the Decalso used was supplied by the Permutit Company of New York, and therefore recovery experiments were carried out, the results of which are presented in table I. The values for recoveries were derived by subtraction of the amount of histamine found in the control urine from that found in the same urine with histamine added. In half of the experiments, the free histamine had first been removed from the urine by passage through a Decalso column before addition of the histamine acid phosphate. The mean recovery of free histamine in 18 experiments was 66.5% ± 1.8.

A correction factor can be applied to the values for free histamine but all the results presented are uncorrected.

The charcoal used in all the experiments was part of the original batch used by Roberts and Adam and was prepared in Dr Adam's laboratory, so no further recovery experiments were considered necessary. Recovery of acetyl histamine by this method was 82% ± 2.4.

4. Storing of urine.

Since it was not practicable to extract urinary histamine every day during experiments lasting one or two weeks, a study

was made of the keeping qualities of histamine in urine. The results are shown in table II and from this it is seen that urine can safely be kept at pH 4 and 2°C. for at least 48 hours without significant alteration in the histamine content. Nevertheless, throughout the investigation, the urine was never kept longer than 24 hours from the completion of collection before extraction was started.

PART II. THE EXCRETION OF HISTAMINE IN HEALTH

1. Normal excretion by adults.

The daily excretion of histamine in the urine of eight healthy men was measured for three days, not necessarily consecutive, and the results are shown in table III. The mean excretion of free histamine was 17.7 ug. per 24 hours, the range being from 8.0 to 26.8 ug. per 24 hours. The mean excretion of conjugated histamine was 41 ug. per 24 hours, with a range from 13 to 149 ug. per 24 hours.

When the excretion of free histamine is expressed in terms of body weight, the mean daily excretion is 0.24 ug. per Kg. per 24 hours, with a range from 0.11 to 0.32 ug. per Kg. per 24 hours.

The greatest daily excretion of conjugated histamine in this series was 149 ug., but in the subsequent work higher values were frequently found in normal adults and occasionally very high values up to 900 ug. were recorded. Under normal conditions, no healthy adult excreted more than 26.8 ug. of free histamine in a 24 hour period, although Roberts and Adam gave 41.4 ug. per 24 hours as the upper limit of normal.

The values for both free and conjugated histamine in the urine of five healthy women fell within the normal range for men.

2. Normal excretion by children.

The daily excretion of histamine in the urine of 40 healthy male children was determined and the results are shown in table IV. The children were divided into four groups according to their body weight at the time of the collection. These groups correspond roughly to the following age groups : under one year, two to five years, six to nine years, and ten to twelve years.

All the children were in hospital or convalescent homes, and strictly speaking were convalescent ; the majority had recovered from minor respiratory infections ; a few had been admitted for investigation of symptoms for which no cause had been found ; others had healed fractures and were being kept in hospital for remedial exercises. Children with a history or family history of allergic diseases, and unduly fat or thin children, were not selected. The subjects of the investigation, therefore, can reasonably be regarded as healthy children.

It is evident from the table that as the body weight increases, so does the 24 hour excretion of free histamine. This relationship is less definite for the conjugate, the values for which are more widely scattered. As the average weight rose from 5.4 Kg. to 30.1 Kg., the amount of free histamine rose from 1.7 to 12.7 ug. per 24 hours.

When the excretion was calculated as ug. per Kg. body weight per 24 hours, the mean for the two heavier groups (0.42 ± 0.026) was significantly higher (P 0.05) than the mean for the two lighter groups (0.315 ± 0.023). Nevertheless, the results are

thought to justify the use of this method of calculation and to show that it is more likely to give consistent results than any other method.

Infants excrete only minute quantities of the conjugate ; this is consistent with the excretion that might be expected with a milk diet. Anrep and his associates (1944) found in the rat and the dog that diets of milk and casein had no effect on the excretion of conjugated histamine, whereas meat or histamine given by mouth greatly increased it. Again, it is possible that the bacterial flora of the alimentary tract of infants is poor in organisms that acetylate histamine.

3. The effect of variation in urine volume.

Anrep's group (1944) reported the results of experiments in which diuresis had been induced in dogs ; they concluded that the amount of histamine excreted in the urine was independent of urine volume. Throughout the present work, no relationship was noted between histamine excretion and urinary output, but to confirm the observation, a comparison was made between the amounts of histamine excreted on each of two consecutive days, the fluid intake being limited on the first day and large quantities of fluid being administered on the second day. The results recorded for five healthy men (table V) substantiate the statement that histamine excretion bears no relation to urine volume.

4. Diurnal-nocturnal variation.

Roberts and Adam (1950) collected urine from five men during three consecutive periods of eight hours, and found a greater average excretion during waking hours than during sleep, but differences were hardly big enough for definite conclusions to be drawn.

In table VI, the excretion of free histamine during the day is compared with that during the night in three men and one child. The collections were made over four consecutive days, and were twelve-hour rather than eight-hour specimens, since this period was more convenient and it was felt that any real difference between day and night excretion would be apparent. No significant difference was found between the mean excretion of free histamine during the day and the mean excretion during the night.

This series of experiments was carried out before the effect of diet on the urinary free histamine was appreciated. It is obvious from the data in Part II, section 5, that a large meal taken in the evening would increase the free histamine excreted during the night. It is possible that the greater part of the basal free histamine is excreted during the daytime, but this could only be determined with the subject fasting.

5. The effect of diet.

The observation made by Anrep and his associates (1944) that herbivorous animals excrete less histamine in the urine than carnivores directed their attention to the effect of diet on urinary histamine. They found that rats fed on a diet of starch, sugar and olive oil excreted very little histamine, more than half being in the free form, whereas a diet of meat resulted in an increase of both free and conjugated histamine in the urine, the latter being preponderant. The amount of histamine excreted on a meat diet varied considerably in different rats, and also in the same rat observed over a period of days, the variations being attributed to the different amounts of meat consumed.

When a large meat meal was given to a dog which had been deprived of food for 36 hours, conjugated histamine began to appear in the urine after 6 hours, reached a maximum at 14 hours, and then declined taking more than 72 hours to return to the fasting level. Experiments on other dogs gave similar results, the amount of histamine excreted varying with the amount of meat consumed.

Human subjects also excreted increased quantities of conjugated histamine when fed meat ; traces of free histamine found were too small to measure.

Feeding rats and dogs on food which contained no free or conjugated histamine, such as casein, egg albumen and milk, had no effect on the urinary excretion of histamine, while when histamine acid phosphate was given to dogs by mouth, about three to five per cent appeared in

the urine as conjugated histamine. When 4.5 mg. of histamine acid phosphate were given to a dog, 244 ug. of histamine appeared in the urine over 14 hours, whereas when 400 g. of meat containing 4.5 mg. of extractable histamine were given, 3794 ug. were excreted in the subsequent 45 hours.

From these experiments, Anrep's group concluded that the conjugated histamine in the urine probably derived from histamine in the meat, although they could not explain why a much larger proportion of histamine was recovered from meat than from histamine acid phosphate given by mouth.

Adam (1950) extended these experiments by giving histamine by stomach tube to human subjects, who were on an ordinary hospital diet and had been fasting for an unspecified period before the experiments were started. About 50% of the conjugated histamine which appeared in the urine after a single dose of histamine did so in the first six hours, thereafter excretion proceeded exponentially and was nearly complete in 24 hours. The total amount excreted varied between 0.17 and 1% of the dose administered.

Adam noted that the urinary free histamine increased slightly in the first six hours although the method he used was too inaccurate to estimate less than 100 ug. of histamine per litre of urine without considerable error, and the increased amounts found in the first six hour period were about one third of this concentration.

Since small quantities of both free and conjugated histamine in the urine can be accurately measured by the method of Roberts and Adam (1950), a study of the effect of changes of diet on

urinary histamine in man was undertaken, with the objects of determining more accurately the changes which occur in free histamine, and of confirming the previous findings for conjugated histamine.

Experiments.

A. The effect of changes in diet.

This group of experiments was carried out on five healthy adults, four men and one woman (subjects A to E). Each experiment started with a control period of three days, during which the subject ate a full mixed diet. The amount and type of food eaten each day was approximately the same, but was not rigidly fixed. During the second period of three days, no food whatsoever was eaten, only water or saline being permitted by mouth. On the seventh and eighth days, the diet consisted of bread and milk in any quantities desired, while on the ninth, tenth and eleventh days, 700 to 800 g. of meat were consumed daily. At no time was any restriction placed on the intake of water.

Throughout the period of eleven consecutive days, 24 hour urine specimens were collected and blood was drawn each morning at the same time for blood histamine estimations. The results are presented in tables VIIa and VIIb, and are shown graphically in figure 1.

B. Excretion following orally administered histamine with and without food.

The subjects were three healthy men (subjects A, F and G), who had fasted for at least 24 hours before each of the experiments, which were conducted at intervals of not less than three days. During the experimental period, the subjects drank 100 ml. of water or saline every hour to ensure an adequate output of urine, which

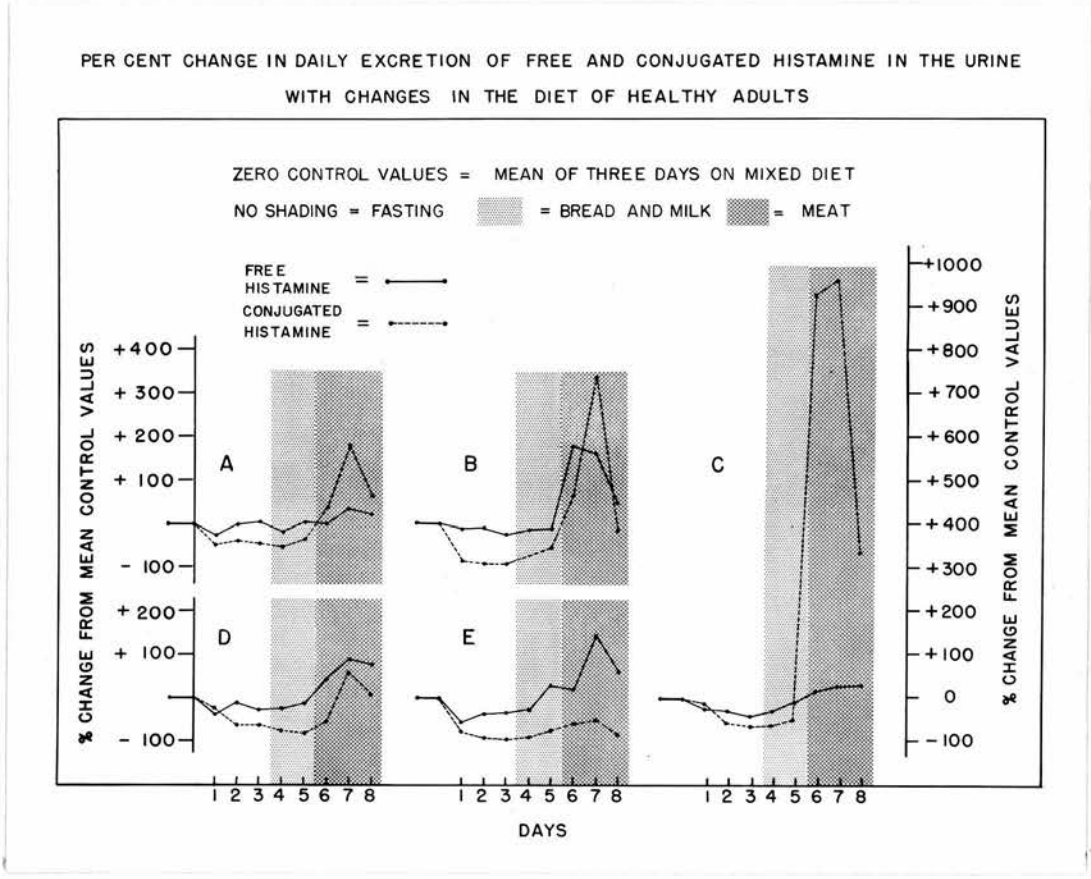
PER CENT CHANGE IN DAILY EXCRETION OF FREE AND CONJUGATED HISTAMINE IN THE URINE
WITH CHANGES IN THE DIET OF HEALTHY ADULTS
ZERO CONTROL VALUES = MEAN OF THREE DAYS ON MIXED DIET
NO SHADING = FASTING = BREAD AND MILK = MEAT
FREE HISTAMINE =
CONJUGATED HISTAMINE =
% CHANGE FROM MEAN CONTROL VALUES
+400
+300
+200
+100
-100
A
B
C
D
E
+200
+100
-100
+1000
+900
+800
+700
+600
+500
+400
+300
+200
+100
0
-100
% CHANGE FROM MEAN CONTROL VALUES
1 2 3 4 5 6 7 8
DAYS

Figure 1.

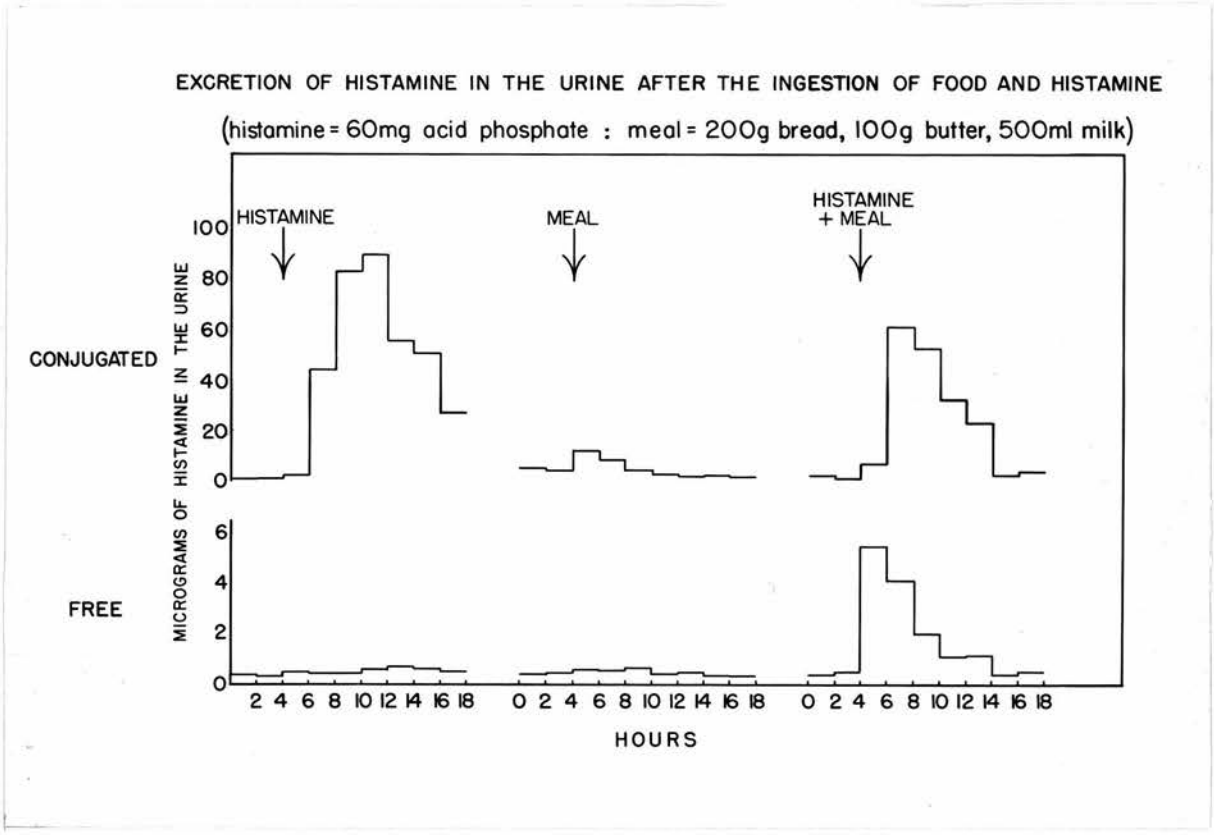
EXCRETION OF HISTAMINE IN THE URINE AFTER THE INGESTION OF FOOD AND HISTAMINE
(histamine = 60mg acid phosphate : meal = 200g bread, 100g butter, 500ml milk)
HISTAMINE
MEAL
HISTAMINE + MEAL
CONJUGATED
FREE
MICROGRAMS OF HISTAMINE IN THE URINE
100
80
60
40
20
0
6
4
2
0
2 4 6 8 10 12 14 16 18
0 2 4 6 8 10 12 14 16 18
0 2 4 6 8 10 12 14 16 18
HOURS

Figure 2.

was collected every two hours. Two control specimens were obtained before giving the test meal, and at least seven samples of urine were collected after the meal. The test meals were as follows :-

i. 60 mg. of histamine acid phosphate in 100 ml. of water.

ii. A meal consisting of bread (200 g.), butter (100 g.) and milk (500 ml.).

iii. Meals i. and ii. taken together.

The results of these experiments are contained in tables VIIIa, VIIIb and VIIIc, and those for subject A. are also shown in figure 2.

C. Other experiments.

A number of additional experiments were carried out on subject A., the general plan of these being the same as in group B. above.

i. A test dose of 60 mg. of histamine acid phosphate was given by stomach tube ; the results were similar to those obtained by giving the same dose by mouth. In this experiment, the urine was collected at hourly intervals for six hours following the dose, in order to ascertain more precisely the time of appearance of histamine in the urine.

ii. A second experiment consisted in holding 5 mg. of histamine acid phosphate in 5 ml. of water in the mouth for five minutes, this being repeated six times in half an hour, making a total of 30 mg. After each five minut interval, the contents of the mouth were ejected so that none of the dose was swallowed.

iii. Lastly, 500 g. of broiled steak were given as the test meal.

The results of this group of experiments are contained in table IX.

Comment.

The experiments of group A. show that a man deprived of food excretes less free histamine in the urine than he normally does ; the small, relatively constant amount excreted daily during starvation may be regarded as his basal level of excretion. The extra free histamine excreted on an ordinary diet represents about one quarter of the basal amount (mean of five experiments = 26.5%), while the consumption of a large amount of meat raises this proportion to about one half (mean of five experiments = 51.5%).

The urinary conjugated histamine falls to a low level during starvation and rises on a diet of meat, but a given quantity of meat may result in the excretion of different amounts of conjugated histamine by different people, and even by the same person on successive days (in table VIIa, compare days 9, 10 and 11 for subject B ; on each day the same amount of meat was consumed).

Experiment Ciii. shows that both free and conjugated histamine begin to appear in the urine within two hours of a meat meal and reach a maximum by the fourth to fifth hours. The response to meat in this subject was poor and was partly obscured by the fact that 24 hours of starvation had not reduced the urinary conjugate to the usual low level.

The blood histamine during the period of starvation was lower than the control level in three of the five subjects of group A, but the fall was only pronounced in one (table VIIb., subject A).

Experiments Ci. and Cii. show that no absorption of histamine occurs from the mouth, and therefore the doses of histamine in group B. experiments were simply swallowed rather than given by

stomach tube. The test dose of 60 mg. of histamine acid phosphate was chosen for the experiments of groups B. and C. because it represents approximately the greatest amount of extractable histamine found by Anrep's group in one Kilogram of meat.

When histamine is given by mouth to a fasting subject, large amounts of conjugated histamine appear in the urine during the third hour after administration, and increase rapidly during the fourth and fifth hours. By 12 hours, 1.7% of the dose (mean of three experiments) has been excreted as conjugated histamine. None of the dose appears in the urine as free histamine.

A meal of low histamine content, such as bread and milk, causes no change in the excretion of free histamine and only a small rise in the conjugate. This statement must be qualified, however, since it has been observed that after a period of a day or more on a bread and milk diet, conjugated, and occasionally free, histamine may increase in the urine. This is difficult to explain, and one can only assume that the new diet in some way alters conditions in the alimentary tract, so that more histamine is formed or absorbed.

When histamine is added to a low-histamine meal, there is an increase in conjugated histamine comparable with that produced by histamine alone, and a sharp rise in free histamine within the first two hours. The source of this extra free histamine is not known. It may come from the histamine in the meal, either because the process of digestion facilitates the absorption of free histamine or because the conjugating mechanism is fully occupied with the products of digestion, and conjugation of the histamine is not therefore complete.

It seems unlikely, however, that any free histamine coming from the gastro-intestinal tract would escape inactivation in the liver with its great reserve capacity, which should be more than adequate to deal with the products of a single meal.

Assuming then that this free histamine does not come from the histamine in the food, it can only come from histidine in the food or from histamine released in the body. It is unlikely that the decarboxylation of absorbed histidine causes the increase, since a similar result would be expected with the meal alone. It is possible, therefore, that the extra free histamine is the result of liberation of histamine in the body. On this view, the histamine would presumably reach the general circulation from the parenteral tissues, since any free histamine coming from the gastro-intestinal tract, whether from the food or liberated in the wall, would be conjugated in the liver.

A possible explanation of the results obtained is to be found in current concepts of gastric secretion. It is believed that the consumption of food causes the release of a hormone, gastrin (Kumarov, 1942), which stimulates the parietal cells of the stomach to produce gastric juice. Histamine is probably the intermediary between the hormone and the cells (Emmelin and Kahlson, 1944) and it is likely that such a histamine-liberating hormone would act both on the stomach wall and on distant tissues, thus accounting for the free histamine in the urine.

The fact that neither histamine alone, nor a low-histamine meal alone, will produce a rise in urinary free histamine such as occurs

when they are given together, might mean that they have a synergistic action ; Kim and Ivy (1933) found such a synergism between the secretagogues and the vasodepressor substances of liver extract. This might in part account for the fact that meat, which contains a relatively large amount of histamine, strongly stimulates gastric secretion and also increases the excretion of free histamine in the urine.

It is concluded that conjugated histamine in the urine is derived from the alimentary tract, partly from histamine in the food and partly from histamine liberated in the wall of the stomach and intestine or formed in the lumen by decarboxylating bacteria, while small quantities of histamine liberated from distant tissues may account for the extra free histamine in the urine resulting from the ingestion of food. Most of the liberated histamine will, of course, be destroyed by enzymes in the body, the urinary excretion merely representing that portion which escapes destruction.

6. The effect of cortisone.

Review of the literature.

The close relationship between histamine metabolism and the suprarenal glands has been recognized since 1920, when Dale found that the resistance of cats to histamine was reduced to a very low level after adrenalectomy. This was confirmed later in dogs by Banting and Cairns (1926) and in rats by Crivellari (1927). The lowered resistance was attributed to adrenaline insufficiency by Wyman (1929) and to cortical insufficiency by Perla and Marmorston-Gottesmann (1931), but the work of Ingle (1937) and others showed that both secretions were important in maintaining resistance to histamine. Of the two, however, the cortex appears to be of greater importance, since Komrad and Wyman (1951) have shown that ten days after transplant of cortical tissue to adrenalectomized rats, the tissue is capable of secreting sufficient hormone to meet the severe demands of an acute stress in addition to the daily needs of the animal. The fact that a completely normal response to histamine administration was not obtained suggests that the medullary secretion does play some part in normal protection against stress, although the authors point out that other factors, such as altered blood supply, may in part account for the relative inefficiency of the cortical transplants.

The mechanism of the protection afforded by cortisone is not clearly understood. Saunders (1951) found that whole adrenal extract protected adrenalectomized mice against anaphylactic shock, whereas desoxycorticosterone did not. He felt that this supported the view

that the neoglycogenic hormones protect against the effects of increased capillary permeability. Halpern, Benacerraf and Briot (1952) showed that in mice neither cortisone nor adrenaline could alone restore the resistance of adrenalectomized mice to normal, but that cortisone and adrenaline together could do so. They suggested that the decreased tolerance to histamine of the adrenalectomized mouse is related to the increased haemoconcentration caused by the histamine, and that cortisone and adrenaline protect the animal by increasing the resistance of the vascular bed to the toxic effect of histamine on small vessels. Ingle and Nezamis (1953), on the other hand, found that cortisone did not significantly affect the resistance of rats to histamine given by continuous intravenous injection.

Rose and Browne (1938) found a retardation in the rate of disappearance of injected histamine from the blood and tissues after adrenalectomy in rats, which they attributed to a decreased ability to destroy histamine. Wilson (1941) suggested that an increase in the initial blood level might in part explain the decreased rate of disappearance, since he found that adrenalectomy in rabbits produced a rise in blood histamine, which was restored to normal by cortical extract. The work of Marshall (1943) confirmed that of Rose and Browne, since he showed that after adrenalectomy in rats, the histamine content of the whole body, except the stomach and intestine, was significantly increased.

Karady, Rose and Browne (1940) showed that there was a diminution of histaminase in the rat's lung after adrenalectomy, which could be restored by adrenal cortical extract. They thought that this might

play a part in the decrease of resistance to histamine following adrenalectomy, but that it could not be the whole explanation since in the rat histamine inactivation takes place mainly in the liver and kidney, neither of which contain histaminase.

In 1951, Carlsten and Wood reported a rise in histaminolytic activity in the thoracic duct lymph of adrenalectomized cats, which was reversed in five out of ten animals by the infusion of adrenal cortical extract. These authors speculated that the histaminase shown to disappear from the lung by Karady and his associates might pass into the tissue lymph, because of some increase in the permeability of cell membranes following adrenalectomy. They also suggested the possibilities that cortical hormone is a competitive antagonist of histamine, or that loss of cortical hormone may set free some histaminase activator.

Valette and Huidobro (1952) found a rise in histaminolytic activity in the blood and intestine following adrenalectomy in rats, which was abolished by cortisone. They reconciled this finding with the decreased resistance to histamine after adrenalectomy by suggesting that, since certain tissues in the rat which contain no histaminase do inactivate histamine, adrenalectomy may inhibit this inactivation, and the rise in histaminase amy simply be a response to the increased histamine in the blood and tissues.

Some doubt has been cast on the significance of much of the foregoing experimental work by the report of Schayer, Smiley and Kennedy (1952). Using radio-active histamine, they found no significant difference in the rate of histamine destruction between

adrenalectomized and sham-operated mice. They were able to use smaller doses of histamine than Rose and Browne, so that this may explain the discrepancy between the two reports, but the authors felt that participation of the adrenal cortex in histamine inactivation under physiological conditions was questionable.

Recent interest in substances which liberate histamine in the body has resulted in new evidence of the association between the adrenal cortex and histamine metabolism. The polyoxyethylene derivative of sorbitan monolaureate (Tween 20) causes liberation of histamine with a resulting fall in blood pressure ; if a second injection is given 24 to 48 hours later, there is a second though somewhat smaller fall in blood pressure. Goth and his co-workers (1951) have shown that if dogs are given cortisone continuously, the second but not the first fall in blood pressure is prevented, though such dogs still respond to injected histamine with a fall in blood pressure. The authors infer that cortisone prevents accumulation of histamine in the tissues following release, though it does not prevent the release of pre-formed histamine. Schayer, Kennedy and Smiley (1953b) have suggested a possible mechanism is by inhibition of histidine decarboxylase, thus preventing accumulation of bound histamine in the tissues.

There have been comparatively few studies of the effect of the suprarenal hormones on blood and urinary histamine. Reference has been made above to Wilson's observations on the blood histamine of rabbits. Staub (1946) showed that physiological amounts of adrenaline administered to human subjects caused a rise in blood histamine to

as much as 0.15 ug. per ml. in ten minutes from the start of injection, so that the rise in blood histamine observed after adrenalectomy is probably due to loss of the cortical hormone.

Herbert, de Vries and Rose (1950) reported no change in blood histamine following the administration of adrenocorticotropic hormone, but their report was based on a single observation in one patient with eosinophila and is therefore not conclusive.

Schayer, Kennedy and Smiley (1953b), in experiments with radio-active histamine, demonstrated three radio-active peaks on paper chromatograms of the urine ; they showed that peak three, which corresponds to unchanged histamine, was increased after administration of cortisone.

The only reports of the effects of cortical hormones on urinary histamine excretion in man have been those of Rose and his associates and of Grob, and since these deal with patients with asthma, they will be considered in Part III with the diseases of allergy.

Much of the literature on the histamine-adrenal relationship is confused and contradictory, and unjustifiable conclusions have sometimes been drawn by applying the results of experiments on one animal to another species. The possible effects of cortisone on histamine metabolism which have been suggested and for which there is some evidence, are three in number. 1. Cortisone may prevent the increased capillary permeability produced by histamine ; 2. Cortisone may increase the rate of destruction of histamine in the tissues ; 3. Cortisone may prevent re-accumulation of histamine in the tissues after it has been released. It has been shown repeatedly that

cortisone does not prevent the union of antibody with antigen, nor does it prevent the release of histamine.

Experiments.

In view of the close relationship between histamine metabolism and the suprarenal glands, it appeared possible that the adminstration of cortisone might elicit abnormal responses in individuals with disorders of histamine metabolism not otherwise apparent. As a preliminary, therefore, an investigation of the effect of cortisone on blood and urinary histamine in healthy adults was undertaken.

Three 24 hour specimens of urine were collected from each subject as controls, and then 200 mg. of cortisone acetate (Cortone acetate, Merck) were given daily for three days, in doses of 50 mg. every six hours by mouth. Urine was collected throughout this period and for several days afterwards. No restrictions were placed on the diet, but the subject was asked to eat approximately the same quantity and variety of food each day.

The results are shown in table Xa., while figure 3. shows the percentage change in free and conjugated histamine in the urine from the mean control levels. The mean percentage rise in the excretion of free histamine during the period of cortisone administration is 25% with a range of +43% to -7%, the fall of 7% in one subject (subject 2) being caused by control levels which were unusually high for this individual, whose excretion had been observed at intervals over a year, and averaged 21.3 ug. per 24 hours. There is usually a fall to a lower level after stopping cortisone, but in some cases the rise persists for a short time after stopping administration.

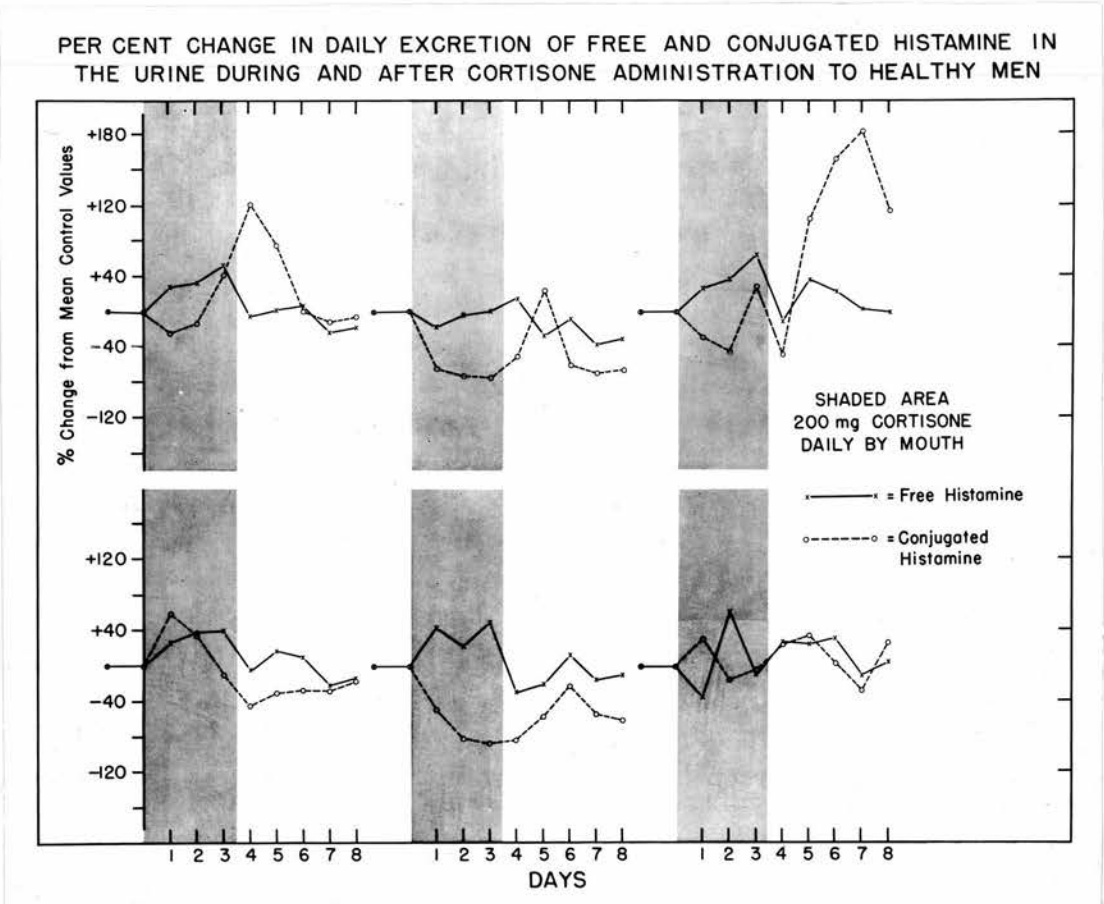
PER CENT CHANGE IN DAILY EXCRETION OF FREE AND CONJUGATED HISTAMINE IN THE URINE DURING AND AFTER CORTISONE ADMINISTRATION TO HEALTHY MEN
% Change from Mean Control Values
+180
+120
+40
-40
-120
SHADED AREA
200 mg CORTISONE
DAILY BY MOUTH
= Free Histamine
= Conjugated Histamine
1 2 3 4 5 6 7 8
DAYS

Figure 3.

No consistent pattern is seen for the excretion of conjugated histamine, although there is a tendency for it to fall during cortisone administration and to rise after stopping the hormone. This effect is only seen when the control level is high, and is not constant.

The rise of free histamine appears to be a direct effect of the cortisone and cannot be explained by an increased excretion of potassium, since the Decalso method eliminates potassium. To make quite certain, however, direct estimations of the potassium content of the extracts were made after the assay ; no difference was found between the control extracts and the extracts of urine passed during the administration of cortisone.

Each morning, at the same hour, usually 9.30 a.m., venous blood was drawn for the estimation of blood histamine. The results are recorded in table Xb. and figure 4. In all cases, there is a fall in blood histamine in response to cortisone, sometimes to very low levels, followed by a gradual return to normal levels after stopping the hormone.

Comment.

It is apparent from the foregoing experimental evidence that increased urinary excretion cannot account for the protective action of cortisone against the effects of histamine, although it may play a minor role. The facts that the blood histamine fell and that the conjugated histamine sometimes fell during cortisone administration suggest that histamine was transferred from the blood to the tissues and this may be the reason for the increased tolerance of histamine.

The work of Schayer, Smiley and Kennedy (1952) would indicate

that the histamine is held rather than destroyed in the tissues and the rise in urinary conjugated histamine which occurred in some cases after stopping cortisone might be considered as evidence in support of this view.

7. The effect of isonicotinic acid hydrazide.

Certain synthetic anti-tuberculous drugs have been shown to reduce the activity of enzymes and particularly of diamine oxidase (Zeller et al., 1952). When one of these drugs, isonicotinyl hydrazine, was injected into a rat twenty minutes before the injection of radio-active histamine, there was an increase in the excretion of unchanged histamine, shown by an increase in peak 3 on the paper chromatogram of the urine, and a reduction in peak 1, which represents the product of diamine oxidas activity (Schayer, Kennedy and Smiley, 1953a).

On the basis of these observations, it seemed worth while to investigate the effect of oral isonicotinic acid hydrazide on the urinary excretion of histamine in man.

Experiment.

After a control period of three days, isonicotinic acid hydrazide (Cotinazin, Pfizer) was given by mouth to a healthy adult male, in a dose of 100 mg. every eight hours for three days. Estimations of blood and urinary histamine were made daily and the results are recorded in table XI.

Comment.

No effect of the drug on blood or urinary histamine was observed. Possibly if a larger dose had been used some change might have been demonstrated, since Zeller's group (1952) found that the concentration of the drug required to inhibit mammalian diamine oxidase in vitro was higher than the ordinary therapeutic concnetration. In the absence of a definite effect and in view of the toxic properties of the drug, no further experiments were undertaken.

PART III. THE EXCRETION OF HISTAMINE IN DISEASE

1. Diseases of the liver.

The role of the liver in histamine metabolism.

Today, nearly fifty years after the discovery of the extremely potent physiological properties of histamine, the mechanism of inactivation of the amine in the body is still not clearly understood. The greater part is probably broken down by enzyme systems, while some is rendered inert by conjugation, but where these processes occur is not certain.

It was natural that interest should first turn to the liver as the site of many other detoxicating processes. Dale and Laidlaw (1911) obtained some evidence of the disappearance of histamine perfused through the liver, but found that the limit of destructive power was quickly reached. This work was confirmed by Meakins and Harington (1922) who, however, pointed out that the results of these perfusion experiments might not be applicable to the intact animal. In experiments on cats with Eck fistulae, injection of histamine into the caecum produced a marked fall in blood pressure not seen in normal cats. The authors concluded that the liver must play some part in the detoxication of histamine, possibly by providing a capillary "cushion" and thereby preventing the too rapid entry of histamine into the general circulation. Ivy (1924), however, introduced histamine into the stomach of dogs with Eck fistulae without producing symptoms, while Koessler and Hanke (1924b) found that histamine injected into the portal vein of dogs, so that it had to pass through the liver

before reaching the general circulation, caused marked systemic effects comparable with those produced on direct injection into the saphenous vein. They concluded, therefore, that the liver took little part in the detoxication of histamine.

More recent experimental work shows that the liver does in fact remove histamine from the blood (Trethewie and Gaffney, 1951 ; Anrep, Barsoum and Talaat, 1953), but the importance of this action under physiological conditions is not known.

The enzyme which destroys histamine was called histaminase by Best and McHenry (1930). Later work by Zeller (1938), however, suggested that the enzyme is not specific for histamine but attacks other diamines and should therefore be termed diamine oxidase. Tabor (1951) showed that the oxidation of histamine by diamine oxidase resulted in the formation of imidazole acetic acid, which has been found in the urine, although it is probably an intermediate and not a final product of histamine metabolism (Tabor, Mehler and Schayer, 1953). It has been shown that this is the main method of destruction of histamine in the rat, but that in the mouse and the cat, the major role is played by an unknown enzyme which has been designated " histamine-metabolizing enzyme II ", (Schayer, 1952 ; Schayer, Kennedy and Smiley, 1953b).

The main source of diamine oxidase in the rat is probably the intestine, whereas the site of activity of the enzyme II in the mouse appears to be the liver (Schayer, Kennedy and Smiley, 1953a). The site of action and the relative importance of the two enzyme systems in man are not known.

Inactivation of histamine by conjugation can take place both in the intestine and in the liver. Urbach (1949) first identified conjugated histamine as acetyl histamine and showed that histamine was rapidly acetylated when added to fresh human faeces. This evidence, together with the fact that pure cultures of E. Coli could produce conjugated histamine from histamine, led him to conclude that histamine is conjugated by bacteria in the intestine, though conjugation in the liver as well was not excluded.

Trethewie and Day (1949) showed that a bound form of histamine could be liberated from the dog's liver by snake venom, and later Trethewie and Gaffney (1951) found conjugated histamine in the perfusate from livers after histamine had been injected into the portal vein. That conjugation is not confined to the intestine is virtually certain from the work of Millican (1953) who found that acetylation still occurred after removal of the entire intestinal tract in rats.

Histamine injected parenterally is apparently not conjugated in the tissues, since no acetyl histamine appears in the urine (Anrep et al., 1944 ; Adam, 1950), and it is therefore probable that histamine is conjugated in the liver but that in the intact body conjugation also takes place in the intestine.

Histamine metabolism in liver disease.

In view of the evidence reviewed above that the liver plays a part in the inactivation of histamine, it is reasonable to suppose that diseases of the liver may cause abnormal metabolism of histamine. The only extensive investigation of histamine metabolism in liver disease was carried out by Chambon and Berthier (1945) who measured the level of blood histamine in patients with various types of liver

disease. They found that marked hepatic insufficiency or cirrhosis was often accompanied by high blood histamine values, sometimes as high as 0.3 ug. per ml., but they were unable to relate the severity of liver damage to the level of histamine in the blood.

One of the most distressing symptoms of liver disease is the severe pruritus which so often accompanies jaundice. This symptom is rarely encountered in haemolytic jaundice but occurs characteristically in the obstructive type, so that it has been attributed to the return of some constituent of the bile to the blood (Watson and Hoffbauer, 1946). The bile salts have been most suspect since they are retained to the greatest degree in obstructive jaundice, but no definite evidence has been produced and the administration of bile salts to jaundiced patients does not aggravate the itching (Rosenthal, 1934). Attempts have been made to correlate the level of bile acids and salts in the blood with the degree of pruritus, but without success (Rowntree, Greene and Aldrich, 1927 ; Brulé and Cottet, 1942).

Rosenthal (1929) discussed the possibilities that the disordered liver might produce abnormal metabolites causing itch, or that lack of bile might favour the formation or absorption of itch-producing substances in the gastro-intestinal tract. He considered that histamine was most likely to be the substance involved since it was liberated in many conditions and had been shown to cause itching (Eppinger and Gutmann, 1913).

It has been suggested (Rothman, 1941) that the liberation of histamine cannot be responsible for itching which occurs without

visible changes in the skin, since histamine in a dilution of 1:1,000,000 still causes whealing but not itching. It is possible, however, that the effect of histamine may be intensified by other factors, and it seems unjustifiable to exclude the possibility on the basis of such an experimental observation.

Gate, Pellerat, Badel and Murat (1944) investigated the blood histamine in a number of patients with itching skin conditions and found that it was often abnormally high, though this was by no means a constant finding. The difficulty inherent in such an investigation is the fact that the threshold to itch varies greatly from person to person, and even in the same person at different times of the day, as has been shown by Cormia (1952).

Itching caused by various drugs has been attributed to histamine. McIntosh and Paton (1949) found that trypanocidal diamidines, such as stilbamidine, liberate considerable quantities of histamine when given intravenously in the usual therapeutic dosage, and thought that this might account for the transient itching which so commonly occurs after administration of these drugs. Feldberg and Paton (1951) showed that opium alkaloids release histamine from the skin and suggested that the pruritus experienced by the opium addict is the result of this action.

Recent experimental evidence indicates that pruritus in jaundice may be caused in a similar way. Injection of the bile salt, sodium tauroglycocholate, into the isolated perfused skin of a cat has been shown to release up to 15% of the total skin histamine (Schachter, 1952). Anrep and Barsoum (1953) reported that ligature of the common bile duct of dogs was followed by a rise in blood histamine, which

they considered to be due to retention of bile by the liver with a consequent release of histamine, as has been shown to occur on perfusion of the liver with sodium glycotaurocholate (Anrep et al., 1953). The liberation of histamine by bile salts may also account for the temporary relief experienced by asthmatic patients when they develop jaundice (Gorin, 1949).

In spite of the suggestive evidence outlined above, little work has been done to establish a causal relationship between histamine and the pruritus of jaundice. Chambon and Berthier (1945) estimated the blood histamine in 12 cases of jaundice of various origins and could find no correlation between the severity of the jaundice and the level of histamine in the blood, although in mild cases the histamine values were within the normal range. They did not, however, attempt to relate the blood histamine levels to the degree of itching of the skin.

Cortisone and liver disease.

Since cortisone has been shown to alter the metabolism of histamine its effect in disorders of the liver is of interest. On the whole, both ACTH and cortisone have proved disappointing in the treatment of liver disease. Butt, Comfort, Power and Mason (1950) reported that physical findings and liver function tests were essentially unchanged following cortisone therapy and this has been the general experience since (Haven, Myerson and Carroll, 1952). A decrease of serum bilirubin has sometimes been found (Gyorgy and Bloemle, 1951) and there have been a few reports of diminution in pruritus as a result of treatment with ACTH or cortisone (Flink and Williams, 1952 ; Haven, Myerson and Carroll, 1952), although the general impression is that these hormones have little anti-pruritic effect.

Clinical investigation.

A study was made of the blood and urinary histamine in patients with cirrhosis of the liver, and in patients with the pruritus of jaundice.

Patients with cirrhosis of the liver. Single blood and urinary histamine determinations were made on six patients with cirrhosis. All of the patients had the typical history and physical signs of advanced portal cirrhosis, and were in a decompensated state with ascites. The results obtained are presented in table XIIa.

The effect of cortisone was studied in two of the patients who had high levels of histamine in the blood. After a control period of two days, 50 mg. of cortisone were given every six hours for three days, during which daily histamine determinations were made on the urine and blood. Throughout the investigation, the patients were on a standard diet containing a measured amount of protein. The results appear in table XIIb., and are shown graphically in figure 5.

Single blood determinations were made in two patients in the terminal stages of cirrhosis, both being in deep coma at the time. Values of 0.017 and 0.010 ug. per ml. respectively were obtained.

Patients with the pruritus of jaundice. The 17 patients studied were suffering from obstructive jaundice, in most cases due to stricture of the bile passages or to malignant disease. At the time of drawing the blood, each patient was assigned to one of two groups, designated mild and severe respectively. The nine patients in the mild group all had a recent history of pruritus, but had little or no itching when the blood was drawn, while the severe

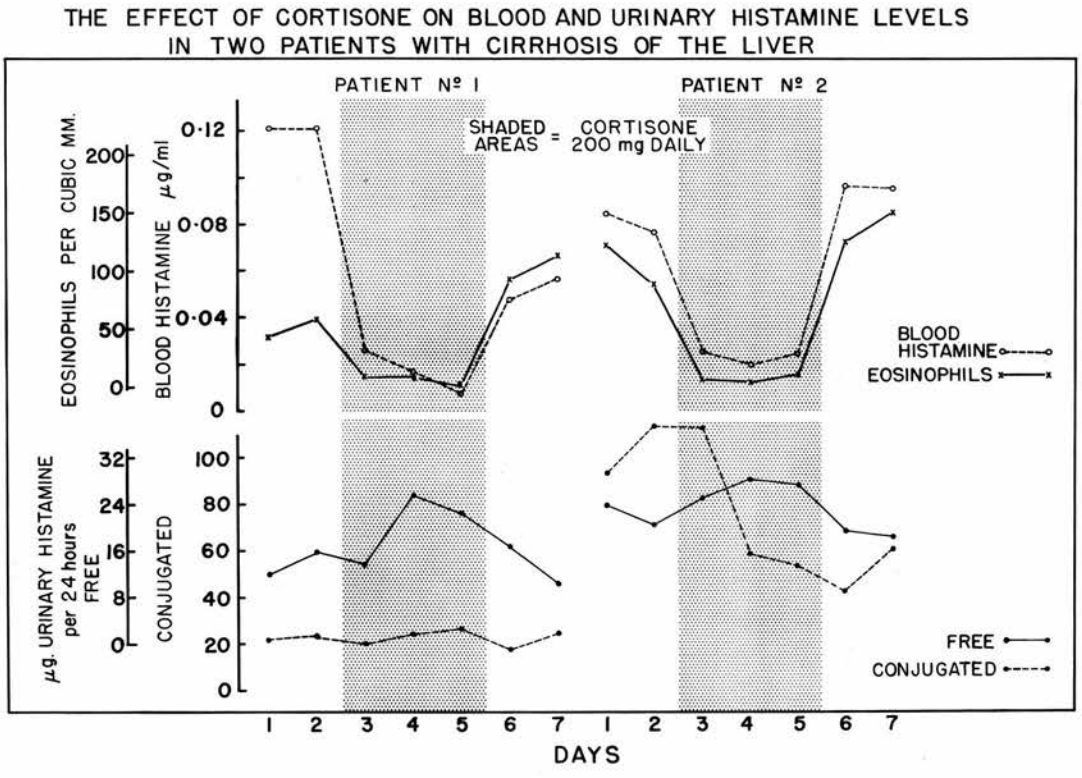
THE EFFECT OF CORTISONE ON BLOOD AND URINARY HISTAMINE LEVELS
IN TWO PATIENTS WITH CIRRHOSIS OF THE LIVER
PATIENT Nº 1
PATIENT Nº 2
SHADED AREAS = CORTISONE 200 mg DAILY
EOSINOPHILS PER CUBIC MM.
BLOOD HISTAMINE µg/ml
µg. URINARY HISTAMINE per 24 hours
FREE
CONJUGATED
BLOOD HISTAMINE
EOSINOPHILS
FREE
CONJUGATED
DAYS

Figure 5.

group of eight patients complained of moderate to severe pruritus at that time.

Urine was collected during the 24 hours preceding the venepuncture. The results of this investigation are contained in table XIII.

Cortisone was given to one of the patients with severe itching ; the blood histamine fell from 0.067 to 0.025 ug. per ml., but the itching persisted. After a few days without specific treatment, during which the symptom was very troublesome, the patient was given methyl testosterone, which resulted in almost complete relief. Daily blood and urinary histamine determinations during the first three days of treatment showed no significant alterations from the pre-treatment levels.

Comment.

In the patients with cirrhosis, the daily excretion of free histamine ranged from 8.5 to 20.9 ug., with a mean of 15.4 $\pm$ 2.3 ug., which does not differ significantly from the mean excretion of free histamine in the urine of normal adults.

The mean blood histamine was 0.066 $\pm$ 0.013 ug. per ml., with a range from 0.037 to 0.121 ug. per ml. Although only two of the six values were above the range of normal, the mean for the group was significantly ($p < 0.001$) higher than the normal mean of 0.040 $\pm$ 0.002 ug. per ml (mean of 36 estimations on 12 adults). The group is small, but the results confirm those of Chambon and Berthier (1945), although the very high values reported by these authors were not obtained.

In contrast, it is interesting that very low values for blood histamine were recorded in the two patients who were in the terminal

stages of cirrhosis.

There was no obvious clinical difference between the mild and severe groups of jaundiced patients, apart from the degree of itching, nor did they differ significantly in the depth of jaundice, as estimated by the mean value for serum bilirubin.

The mean 24 hour excretions of free histamine were 13.5 $\pm$ 2.6 and 15.7 $\pm$ 3.7 ug. for the mild and severe groups respectively. These means do not differ significantly from one another nor from the mean excretion of normal adults.

The mean blood histamine for the mild group was 0.052 $\pm$ 0.007 ug. per ml. and for the severe group was 0.078 $\pm$ 0.009 ug. per ml. The difference between these means is just significant ($p < 0.05$) and both are significantly higher ($p < 0.02$ and < 0.001 respectively) than the mean normal values. Only two of the values in the mild group fell outside the normal range, but five of those in the severe group were at or above the upper limit of normal found in this study (0.067 ug. per ml.).

There is, therefore, a relationship between the degree of pruritus experienced in jaundice and the level of histamine in the blood, in that severe itching is usually accompanied by an increase in blood histamine. High values were found without itching in cirrhosis, and therefore it appears that only in jaundiced patients is an increase in blood histamine associated with itching. Moreover, the high blood histamine does not seem to be the direct cause of the pruritus, since no relief was obtained when the level was lowered by means of cortisone. It is possible that a high content of histamine in jaundiced tissues

causes the itching, and that the raised blood level merely reflects the high tissue level. If cortisone lowers the blood level by causing the histamine to pass into the tissues from the blood, as has been suggested, this would explain the lack of effect of the hormone on the pruritus.

The relief of itching by the use of methyl testosterone has been reported by Sherlock and Lloyd Thomas (1952), who commented that the rationale of its use was not known. The lack of effect on blood and urinary histamine levels suggests that it does not act by altering the metabolism of histamine.

In conclusion, in both cirrhosis and jaundice with pruritus, high blood histamine values were found with normal excretion of histamine in the urine. These results suggest that the high blood levels were not due to the liberation or retention of histamine in the plasma, at least in the free state, since in such circumstances, increased urinary excretion would be expected. It is possible, of course, that histamine was retained in the blood because the kidneys themselves failed to excrete it, but it would appear more likely that the high values were due to a disturbance of liver function resulting in an increase in bound histamine in the blood, probably in the cellular elements.

2. Infantile gastro-enteritis.

The theory that the symptoms of gastro-enteritis in infants are caused by the absorption of toxic bacterial products formed in the alimentary tract has been advanced repeatedly (Jeans and Marriott, 1947 ; Ivy, 1948) and the evidence has been reviewed recently by Kerpel-Fronius (1948). Mellanby (1916) was the first to suggest that histamine might be one of these toxins, since it was known that the intestine contained bacteria which could decarboxylate histidine in culture. Many such decarboxylating organisms have since been identified in human faeces, but there is no evidence that they occur in significant numbers in the intestinal contents of infants with gastro-enteritis (Gale, 1940, 1944).

The histamine which is normally present in human faeces (Myhrman and Tomenius, 1939) is probably largely destroyed by histaminase in the intestinal mucosa and some is absorbed after conjugation but produces no symptoms because it is inert. The intestinal wall presents a barrier to the passage of free histamine (Dworetsky and Code, 1951) and if any is absorbed it is probably conjugated in the liver, so that it is unlikely that any absorbed histamine reaches the circulation in the free state, at least in normal circumstances. If, on Mellanby's view, histamine causes toxic effects after it is absorbed, it must be assumed that appreciable quantities of free histamine reach the circulation unchanged, either because unusually large amounts are formed in the intestine, or because it is no longer conjugated or destroyed. Such agents as chloroform and alcohol, when applied to the intestinal mucosa,

promote the absorption of histamine, and it is conceivable, on this analogy, that inflamed gut, as compared with healthy gut, allows histamine to pass more readily.

There has been little or no experimental evidence in support of the theories outlined above. Boyd (1923) found that extracts of intestinal mucous membrane from cases of acute intestinal intox-:ication contained a substance which produced diarrhoea and symptoms of circulatory failure when injected into animals. She also reported that portal blood from these cases was very toxic to animals and considered that a circulating toxin resembling an amine base was the cause of intestinal intoxication in infants (Brown and Boyd, 1923). Neumann (1949) claimed that antihistaminic drugs had a beneficial effect in gastro-enteritis and maintained that this supported the histamine theory, but there has been no confirmation of his results.

Adam (1950) has shown that histamine infused intravenously is more easily detected in the urine than in the blood, probably because it is concentrated in the kidneys. Hence the passage of free histamine from the gut into the general circulation in amounts large enough to produce the symptoms of gastro-enteritis would be expected to increase the amount excreted in the urine.

Clinical investigation.

The urine of ten infants suffering from acute gastro-enteritis was examined for histamine. The disease was of the endemic type and at the time of collection every infant was passing frequent, loose, green stools and shows signs of dehydration. The infants

were given only Hartmann's solution, by mouth or by intravenous drip, during the period of collection, and either chloramphenicol or sulphadiazine by mouth. In each case a second 24 hour collection of urine was made one week after the first. By this time all the infants were on normal milk feeds and were passing normal stools.

The results obtained are shown in table XIV. The first five of these infants received chloramphenicol during the acute stage of the illness ; the next four, sulphadiazine ; and the last infant, no drug treatment. When the histamine equivalent for the conjugate was less than 0.01 ug. per ml., the amount of conjugate is shown as less than the value calculated from the threshold dose of the standard solution of histamine. These values therefore varied with the sensitivity of the strips of ileum used in the assays. In calculating the mean, it is assumed that no histamine was present in these samples.

Comment.

The difference between the amount of free histamine excreted in the acute stage of gastro-enteritis and the amount excreted by the same infant after recovery was not significant. The results therefore do not support the view that the absorption of histamine from the alimentary tract is a cause of symptoms in infantile gastro-enteritis.

3. Allergic diseases.

Histamine in human allergy.

There is abundant evidence that histamine is released during anaphylactic shock and many attempts have been made to show that allergic reactions in man are the result of a similar occurrence. It is now generally conceded, however, that histamine cannot be the sole cause of the manifestations of allergy and indeed may be merely a by-product of the antigen-antibody reaction.

In their original work on the physiological actions of histamine, Dale and Laidlaw (1910) were careful to state that the immediate symptoms of the anaphylactic reaction were not entirely, although to a large extent, those of poisoning with histamine. However, as a result of the demonstration that histamine is present in the body (Barger and Dale, 1911) and is liberated in anaphylactic shock in the dog (Gebauer-Fulnegg and Dragstedt, 1932) and in the guinea-pig (Bartosch, Feldberg and Nagel, 1932) it became widely accepted that the symptoms of anaphylaxis were due to the release of histamine, although some authorities never subscribed to this idea (Dale, 1929).

Serious doubts were cast on the histamine theory by reports that the blood histamine fell in anaphylactic shock in the horse and calf (Code and Hester, 1939) and in the rabbit (Rose and Weil, 1939), but the first definite evidence that histamine was not the sole cause of symptoms was the demonstration by Jaques and Waters (1940) that heparin was responsible for the very prolonged coagulation time of blood in canine anaphylaxis, a feature which had been difficult to explain on the histamine theory. In the decade following this

discovery, it became apparent that a number of metabolic products of cell injury might contribute to the anaphylactic reaction, among those suspected being acetyl choline, adenosine, lysocithin, potassium and various cell enzymes. That histamine takes part in anaphylaxis is certain, but the extent to which it contributes to the symptoms and signs is still unknown (Dragstedt, 1950).

The opinion of Lewis (1927) that liberation of the H-substance was of fundamental importance in protein sensitivity in man led to investigation of the role of histamine in human allergy. Certain indirect evidence of the part played by histamine appeared in 1929, when Weiss, Robb and Blumgart reported that injections of histamine caused dyspnoeic attacks in patients with bronchial asthma, and Kalk reported that skin whealing in dermatographia was accompanied by a rise in gastric acidity similar to that produced by histamine. The first direct measurement of histamine, however, was the work of Cerqua (1936), who studied the blood histamine in six patients with urticaria and eight patients with asthma, and reported a rise in blood histamine during acute attacks, with a fall to normal between attacks. Haworth and Macdonald (1937) also reported that the blood histamine was higher than normal in asthma, their patients being cotton workers suffering from cotton-dust asthma. They found that the blood histamine levels were much more variable in the asthmatics than in the control group of healthy students.

Randolph and Rackemann (1941) measured the blood histamine in acute attacks of asthma ; the mean level was 0.088 ug. per ml., compared with a normal mean of 0.045 ug. per ml., while in one

patient with a very severe attack, the blood histamine rose to 0.16 ug. per ml. Gate, Pellerat, Badel and Murat (1944) found an elevated blood histamine level in all of seven patients with allergic eczema, and in three out of five patients with urticaria.

In contrast with these reports, Rose (1941) found that, although the blood histamine of asthmatics showed wider fluctuations than normal, there was no correlation between the level of histamine in the blood and the onset and disappearance of symptoms. He found that the blood histamine was normal in 23 patients with urticaria and that the only acute condition associated with a change in blood histamine was angioneurotic oedema, in which the level consistently fell during an attack. Rose (1940) also reported that he could detect no rise in blood histamine following the subcutaneous injection of 1 mg. of histamine diphosphate in ten patients, although symptoms of intoxication were produced.

A possible explanation of the contrdictory results obtained by different authors is the demonstration by Serafini (1948) that extensive skin whealing produced in dermatographic and asthmatic patients resulted in a rise in blood histamine in one to two minutes, followed by a rapid fall to normal levels by three to five minutes. Of interest also is the recent work of Hawkins, Mongar and Schild (1951), who showed that histamine is released from the isolated lungs and bronchi of an asthmatic individual by a pollen extract to which he was sensitive. On the other hand, Rose, Rusted and Fownes (1950), in studies on arterial and mixed venous blood obtained by cardiac catheter from asthmatic patients, found that the blood

histamine levels were higher in asthmatics than in their normal controls, but that no rise accompanied attacks of asthma induced by ragweed or other agents.

Urinary histamine in allergic diseases.

Since changes in body histamine are more likely to be reflected in the urine than in the blood stream (Adam, 1950), investigations of urinary histamine in allergic diseases might be expected to yield valuable information. Comparatively little such work has, however, been carried out.

Using a modification of Anrep's charcoal method of extraction, Adam, Hunter and Kinnear (1950) investigated the urinary excretion of histamine in urticaria. They found that the mean daily excretion of free histamine in nine cases was significantly higher than normal, the increase being mainly due to two patients with severe acute urticaria, who excreted an average of 104 and 65 ug. per 24 hours respectively. Though the results were inconclusive, they supported the conception that histamine is either liberated or newly formed in urticaria.

In a preliminary report on the effect of adrenocorticotropic hormone (ACTH) in asthma, Rose, Pare, Pump and Stanford (1950) referred to unpublished work by Rose, in which he had found large amounts of histamine in the urine of asthmatics, whereas little or none was present in non-allergic patients. The effect of ACTH in a group of six patients with severe chronic asthma, not in acute status, was studied by Rose and his associates, and a complete remission or a definite improvement occurred in all the patients

within 48 hours. A marked to complete disappearance of histamine from the urine was recorded concurrently, except in one patient in whom remission was not complete and whose urinary histamine increased after ACTH.

No values for histamine were given in this paper, but in a previous paper read to the first clinical ACTH conference in 1949, Rose (1950) referred to one patient who excreted 900 to 2800 ug. of histamine per day in the seven days before treatment, and 13 to 400 ug. per day in the six days after treatment. In all cases the histidine increased in the urine at the same time.

In the same year, Rose and his co-workers published the results of an investigation on a series of 15 patients with allergic complaints, mainly asthma ; in most, a moderate to marked increase of urinary histamine followed the administration of ACTH, with a return to normal as symptoms subsided. From 4 to 7 mg. per 24 hours were excreted during ACTH therapy, compared with 0.5 to 0.7 mg. before treatment, and in two cases amounts equal to 200 mg. were excreted during ACTH administration (Rose, Pare, Pump, Stanford and Johnson, 1950).

In 1951, these apparently conflicting results were clarified in a paper describing 18 balance studies on patients with various forms of hypersensitivity (Rose, Pare, Pump, Stanford, Mackenzie, and Venning, 1951). Every patient had a moderate to marked excretion of histamine in the urine during the control period, and following ACTH this usually increased, although not invariably. There was a general decrease in the output as therapy continued, and in the majority

of cases the post-treatment period was characterized by a reduction in the excretion of histamine, as compared with pre-treatment levels. The values given for the amounts of histamine excreted by two of the patients were lower than those previously reported by these authors, the control values ranging from less than 50 ug. to about 700 ug. in 24 hours, and the values on ACTH treatment from less than 50 ug. to about 2800 ug. in 24 hours (compared with 4 to 7 mg. in the previous report). In five control subjects the authors noted that the output was in the neighbourhood of 50 ug. per 24 hours, and they also stated that asthmatic patients had been observed to excrete amounts equal to 250 mg. during a 24 hour period.

The reports of Rose and his associates have been considered at some length because they are widely quoted in the literature. In none of their papers do these authors state the methods they used for extracting histamine, and it is possible that the great activity found in their extracts of urine from asthmatic patients was due to potassium.

In 1952, Grob published the results of an investigation into the renal excretion of histamine and histidine in man. Using the colorimetric method of Rosenthal and Tabor (1948), he found that the excretion of histamine in a group of 15 patients comprising nine asthmatics, one patient with cold allergy, two with lupus erythematosus, one with rheumatoid arthritis and two with myasthenia gravis, was not significantly different from normal, and that the urinary excretion of histamine bore no relation to the severity of asthma or to the appearance of extensive urticaria. Grob treated 14 of his patients with ACTH or cortisone and found a transient

increase in histaminuria in 10 patients, no change in three, and a decrease in one.

In spite of the fact that the method of Rosenthal and Tabor is relatively insensitive, the average daily excretion of histamine in nine normal subjects was stated by Grob to be 7.6 mg. in 24 hours, with a range of 4.3 to 15.4 mg. (expressed as the free base). Since the greatest daily excretion found in a normal man by Roberts and Adam (1950) was less than one mg. of total histamine, while Anrep's group (1944) reported that the maximum excretion by a man on a high meat diet was 2.4 mg. in 24 hours, it seems probable that Grob was measuring substances other than histamine, since the method he used is not specific for that substance and he reported no confirmatory tests.

It should be noted that the work of both Rose and Grob dealt with the total excretion of histamine in the urine and did not differentiate free from conjugated histamine. Since the conjugate varies so greatly from day to day, estimations of the total urinary histamine can only have a limited value.

Cortisone and allergy.

Since 1949 there have been many papers describing the beneficial effects of cortisone in allergic conditions. Evans and Rackemann (1952) analyzed a number of these reports and found that 62% of 116 patients with chronic asthma refractory to ordinary treatment were completely relieved by cortisone, while a further 24% obtained moderate relief. Cortisone produces similar effects in hay fever and other allergic conditions, although the benefit is

usually only temporary.

Experimentally it has been shown that cortisone does not prevent the release of histamine which accompanies hypersensitivity reactions (Carryer and Code, 1950), nor has it any protective action against anaphylactic shock or histamine poisoning in guinea pigs (Dworetzky, Code and Higgins, 1950 ; Landau, Nelson and Gay, 1951), although large doses reduced the mortality from anaphylaxis in mice (Nelson, Fox and Freeman, 1950). There is some evidence that cortisone delays antibody formation (Bjorneboe, Fischel, and Stoerk, 1951 ; Germuth, Oyama and Ottinger, 1951 ; Hayes, 1953), but since a varying degree of suppression has been found by different authors, it is probable that the hormone affects different types of antibody in different ways (Evans and Rackemann, 1952).

In any event, the antibody effect is unlikely to be of major importance in the therapy of allergic states (Fischel, 1952) and the exact mechanism of action of cortisone is unknown, although probably the non-specific inhibition of the inflammatory reaction, produced by a decrease in vascular permeability and a reduction of local phagocytic activity, contributes to the clinical improvement. If there is a single common action of cortisone which will explain all its manifold effects, it may well be a modification of the reactivity of mesenchymal tissue (Thorn et al., 1953).

Clinical investigation.

Free and conjugated histamine were measured in the urine of 24 children who had had frequent severe attacks of asthma or pollinosis (hay fever) or both. In 14 of the children, the urine was collected

during the interval between attacks when no symptoms were present, while in 10 the collection was made as soon as possible after the onset of an acute attack. The attacks of pollinosis were in most cases due to sensitivity to ragweed pollen ; the only drugs used in treatment were adrenaline and sedatives as required. The results of this investigation are shown in tables XVa and XVb.

One child was followed through a course of monthly injections for ragweed desensitization. Urine for histamine determinations was collected before the start of the course and for the 24 hours immediately following each injection. In spite of moderately severe local reactions, no significant change occurred in the urinary histamine, which throughout the course remained within the same range (9.7 to 14.2 ug. of free histamine and 12 to 48 ug. of conjugated histamine per 24 hours).

The effect of adrenocortical hormones on the urinary excretion of histamine was studied in six patients with acute allergic attacks. After a control period of 24 hours, during which the patient received only adrenaline if necessary, 150 to 200 mg. of cortisone or 80 mg. of hydrocortisone were given daily by mouth for three days. The histamine content of the urine was determined daily and the results appear in table XVI.

All the patients had acute symptoms sufficient to justify the use of cortisone, but some were more severely affected than others and are graded accordingly in the table. The first and fourth patients listed are the same individual who had two attacks of pollinosis from different types of pollen (elm and ragweed) at four months' interval. The attack caused by elm pollen was very

severe, with profuse lacrimation, swelling of mucous membranes, and scattered rhonchi in the chest, the rhinitis being the most troublesome feature. This patient, who was a member of the medical staff, volunteered to withhold treatment for 24 hours in spite of acute distress, to enable control observations to be made. The results of the administration of cortisone during this attack are shown graphically in figure 6.

The hormones produced a remission of symptoms in all cases, usually within 12 hours of starting treatment, although sometimes mild manifestations persisted for a day or two longer.

Comment.

The mean excretion of free histamine by children in the interval between allergic attacks is 0.38 ± 0.03 ug. per Kg. body weight per 24 hours, with a range of 0.21 to 0.57 ug. per Kg. During acute allergic attacks, the mean excretion is 0.31 ± 0.04 ug. per Kg., the range being from 0.16 to 0.52 ug. per Kg. The difference between these means is not significant and the ranges for both groups fall within the range for normal children. The amounts of conjugated histamine in the urine of the two groups are also within the normal range.

These results do not substantiate the claims of Rose and his associates that abnormally large quantities of histamine are excreted in the urine of allergic patients.

The data on the effect of the adrenocortical hormones are not sufficiently conclusive for a definite statement to be made. In the first patient, who was far more severely affected than the other

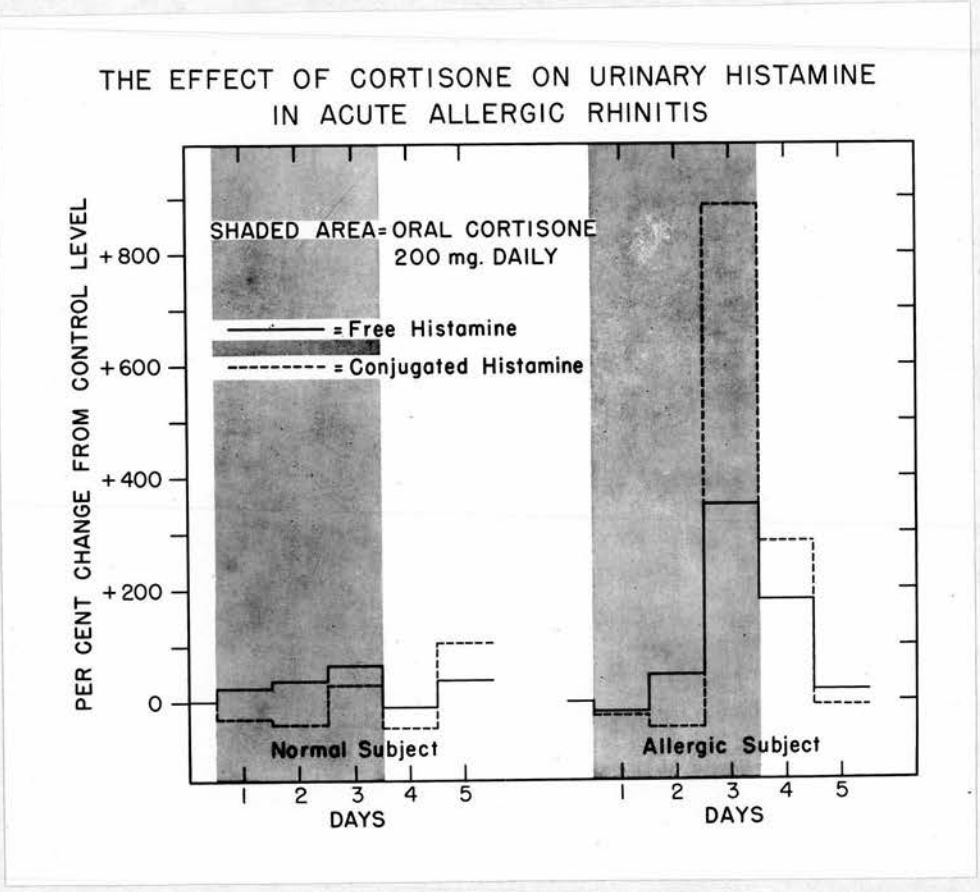
THE EFFECT OF CORTISONE ON URINARY HISTAMINE
IN ACUTE ALLERGIC RHINITIS
PER CENT CHANGE FROM CONTROL LEVEL
+800
+600
+400
+200
0
SHADED AREA = ORAL CORTISONE
200 mg. DAILY
= Free Histamine
= Conjugated Histamine
Normal Subject
Allergic Subject
1 2 3 4 5
DAYS
1 2 3 4 5
DAYS

Figure 6.

five, there was a dramatic outpouring of both free and conjugated histamine in the urine, the increase of free histamine preceding that of the conjugate by 24 hours. An abnormal increase in free histamine also occurred in the third patient, while in the second and fourth patients the increase was marked, though probably not abnormally so. The changes in urinary free histamine were only very slight in the mildly affected patients. Apart from those in the first patient, the changes in conjugated histamine were no greater than the normal variations without cortisone.

The results of this investigation suggest that during acute allergic episodes, free histamine is liberated in the body by the action of cortisone, in amounts roughly proportional to the severity of the attack. If this were so, the symptoms of the acute attack might be due to the retention of free histamine in the tissues, and the release of this histamine would explain the clinical improvement obtained by treatment with cortisone. Alternatively, cortisone may act by depressing the activity of histaminase, thus allowing a larger proportion of the free histamine formed to be excreted in the urine. This is mere speculation, however, and no conclusion can be drawn from the evidence at present available.

4. Other diseases.

Reports on the excretion of histamine in the urine in diseases other than those reviewed above are scanty. Adam, Hunter and Kinnear (1950) measured the urinary histamine in a group of ten patients with various non-allergic diseases, such as pneumonia, anaemia, diabetes and intestinal disorders. There was no significant difference in the excretion of either free or conjugated histamine between this group and a group of ten normal subjects.

The blood histamine is known to be markedly elevated in chronic myelocytic leukaemia, and Pearce and Valentine (1950) therefore investigated the urinary histamine content in this condition. They reported that the mean excretion of ten patients did not differ from that of 25 normal adults ; however, they did not measure the excretion over a period of 24 hours but only for one hour with the patient fasting, so that their findings require confirmation.

During their research into the effects of ACTH in asthma, Rose, Pare, Pump, Stanford and Johnson (1950) investigated five patients with rheumatoid arthritis, and found that ACTH therapy produced little or no change in the histamine excretion of four of the patients, while in the fifth a moderate increase was observed. They did not make it clear whether the control values were different from values found in normal subjects, but presumably they were not.

A single observation on the common cold is recorded. Rose, Pare, Pump, Stanford, Mackenzie and Venning (1951) found that in a normal subject who usually excreted about 50 ug. of histamine in 24 hours, the development of a cold caused an increase to 1,000 ug. in 24

hours, but that this rapidly disappeared as the patient recovered from his cold. However, one of the healthy subjects in the present work developed an upper respiratory infection while his urinary histamine was being measured daily, and no effect on his output was observed.

There have been a number of reports of abnormal excretion of histamine in eclampsia, although the conclusions reached have not been in agreement.

Revoltella (1930), using the colorimetric method of Koessler and Hanke (1919), examined various tissues from eclamptic patients and concluded that in eclampsia and pre-eclampsia, abnormally high quantities of histamine were constantly present in the placenta, the maternal and fetal blood, various fetal organs, and in the maternal urine. Although there is no good evidence that he was really measuring histamine, his conclusions regarding the urine were confirmed by Kapeller-Adler (1941). Having shown that the normal histidinuria of pregnancy is markedly diminished in severe pre-eclampsia, she investigated the urine of such patients to see whether histamine was excreted instead of histidine. By precipitating large quantities of urine with ammoniated zinc hydroxide, after the method of Ackermann and Fuchs (1939) and subsequently extracting with amyl alcohol and sulphuric or hydrochloric acid, she was able to isolate 2.7 mg. of histamine dipicrate from 32 litres of urine from two women with severe toxaemia, whereas 39 litres of normal pregnancy urine yielded no detectable quantity of histamine.

Recently Rockenschaub (1953) investigated the urine of 20 women with pre-eclampsia, using the method of Roberts and Adam. He found that in mild pre-eclampsia, the excretion of free and conjugated histamine was within the range for normal pregnant women, but that in moderately severe pre-eclampsia, both free and conjugated histamine were reduced in amount, in some cases to a very low level.

No definite statement can therefore be made on the excretion of histamine in eclampsia, although the results reported by Rockenschaub are likely to be most accurate since he used the relatively sensitive method of Roberts and Adam.

SUMMARY AND CONCLUSIONS

Histamine in the urine in health.

Free histamine is excreted in the urine of normal men in amounts varying from 8.0 to 26.8 micrograms per 24 hours, as determined by the method of Roberts and Adam. For any one individual, the range of excretion from day to day is much smaller. Expressed as micrograms per kilogram of body weight per 24 hours, the range is from 0.11 to 0.32 micrograms, with a mean value of 0.24 micrograms. These values are comparable with those reported by Roberts and Adam, though the range is a little lower.

The excretion of free histamine by normal women is within the range found in normal men. Children excrete an average of 0.37 micrograms per kilogram of body weight in 24 hours ; the range is from 0.13 to 0.63 micrograms per kilogram, the higher rates being in the older age groups.

The amount of free histamine in the urine is independent of the urine volume, and no difference has been found between night and day excretions, though these are influenced by the time of meals.

On a normal diet, about one fifth of the free histamine in the urine is the result of the ingestion of food, while when large amounts of meat are eaten, this fraction is increased to one third.

When histamine is given by mouth to a fasting man, there is no increase in free histamine in the urine. When it is given with a meal of bread and milk, however, there is a rise in urinary free

histamine, which does not occur when the meal is given alone. The origin of this free histamine is not known. It is probably formed outside the alimentary tract and the suggestion is made that it is the result of the action of histamine-liberating hormones on the peripheral tissues.

The oral administration of cortisone results in an increase in free histamine in the urine. Isonicotinic acid hydrazide had no effect on urinary histamine in the one subject studied.

Conjugated histamine in the urine depends on the diet consumed. The excretion varies widely from day to day, the usual range in normal men and women being from 13 to 149 micrograms in 24 hours, although values up to 900 micrograms may occur. The range of values for conjugated histamine excreted by children is similar to that found in adults ; infants, however, excrete very little.

During starvation, the conjugate in the urine falls to a low level in the course of two to three days ; on a diet of meat it rises, the greatest amount excreted in a period of 24 hours being 2.3 micrograms.

Between one and two per cent of an oral dose of histamine is excreted as conjugated histamine in the 12 hours following the dose, the excretion usually starting in the third hour.

Cortisone has no consistent effect on urinary conjugated histamine although when the amount excreted is large, it may be reduced by cortisone and may increase considerably after stopping the hormone.

Histamine in the urine in disease

A study has been made of the histamine content of the urine and

blood in diseases of the liver, and of the urine alone in infantile gastro-enteritis and allergic diseases. In none of these conditions are abnormal quantities of histamine excreted in the urine in ordinary circumstances.

In cirrhosis of the liver and in obstructive jaundice, the blood histamine level is often high. Pruritus occurring in jaundice is usually accompanied by an increase in blood histamine without a corresponding increase in the excretion of histamine in the urine.

Cortisone increases the urinary free histamine and lowers the blood histamine in cirrhosis, the general effect being the same as in healthy adults.

The data indicate that there is a disturbance of histamine metabolism in liver disease, but that no excess of free histamine is formed or liberated as a result, since none appears in the urine.

Infants excrete the same quantities of histamine in the acute stages of gastro-enteritis as in the convalescent stages, and there is therefore no evidence that histamine absorbed from the intestine is a cause of symptoms in this disease.

Children with asthma and hay fever excrete normal amounts of histamine in the urine, both during acute attacks and in the intervals between attacks. The reports by Rose and his associates that large quantities of histamine are excreted in the urine in asthma have not therefore been confirmed.

The administration of cortisone or hydrocortisone to patients with acute allergic reactions results in an increase in urinary free histamine, which in the more severe cases is considerably

greater than the increase produced in normal persons. The evidence is inconclusive but suggests that in acute allergic attacks the body contains an excess of free histamine, which is released by the action of cortisone.

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It is a special pleasure to acknowledge my debt to Dr Henry Adam, whoes encouragement and helpful advice were a constant source of inspiration.

TABLES I TO XVI

Urine at pH 8	Histamine (µg.) added to 50 ml of urine	Percentage recovery
Filtered only	2.5	78.8
		63.2
		56.0
	5.0	61.4
		65.0
		60.0
	10.0	63.7
		51.6
		61.9
Passed through Decalso column	2.5	80.0
		74.0
		72.8
	5.0	60.7
		69.0
		63.1
	10.0	75.0
		68.0
		73.3

Mean percentage recovery	=	66.5
Range	=	51.6 - 80.0
Standard deviation	=	7.8
Standard error of the mean	=	1.8

TABLE I

Recoveries of histamine added to urine

Free histamine (µg.) in 50 ml. of urine			Conjugated histamine (µg.) in 10 ml. of urine		
in fresh urine	after 48 hours	after 96 hours	in fresh urine	after 48 hours	after 96 hours
0.96	1.00	1.00	1.70	1.43	1.75
1.10	1.17	1.33	1.25	1.19	1.25
0.84	0.91	1.05	0.71	0.81	0.81
0.60	0.50	0.65	1.62	1.87	1.75
1.43	1.36	1.50	0.90	0.89	0.90
0.81	0.80	0.80	0.50	0.48	0.50
1.30	1.20	0.86	0.20	0.21	0.21
0.50	0.51	0.42	0.55	0.53	0.53
0.74	0.66	0.66	0.71	0.71	0.71
		Mean			
0.92	0.90	0.92	0.90	0.90	0.93

TABLE II

The histamine content of nine samples of urine

before and after storage at pH 4 in a refrigerator

MEN

Subject	1		2		3		4		5		6		7		8	
	Histamine in the urine in ug. per 24 hours															
	Free	Conj.	Free	Conj.	Free	Conj.	Free	Conj.	Free	Conj.	Free	Conj.	Free	Conj.	Free	Conj.
Day 1	11.7	34	14.0	25	18.3	18	20.0	22	25.5	149	21.9	19	22.7	29	8.0	16
Day 2	17.2	29	10.9	99	23.5	26	19.0	18	26.8	103	24.0	24	22.2	48	8.9	16
Day 3	14.4	52	8.9	79	21.0	18	19.9	32	23.2	38	16.1	13	18.1	98	8.5	20
Mean	14.4	38	11.3	64	20.9	21	19.6	24	25.2	97	20.7	19	21.0	45	8.5	17

WOMEN

Subject	1		2		3		4		5	
	Histamine in the urine in ug. per 24 hours									
	Free	Conj.	Free	Conj.	Free	Conj.	Free	Conj.	Free	Conj.
	18.7	32	17.2	128	21.7	69	15.3	62	18.2	76

	Number of estimations	Number of subjects	Urinary free histamine (ug. per 24 hours) Mean	Range	Urinary conjugated histamine (ug. per 24 hours) Mean	Range
Men	24	8	17.7	8.0 - 26.8	41	13 - 149
Women	5	5	18.2	15.3 - 21.7	73	32 - 128

TABLE III. Histamine in the urine of healthy men and women.

I			II			III			IV	
Body weight (Kg.)	Urinary histamine µg./ 24 hours		Body weight (Kg.)	Urinary histamine µg./ 24 hours		Body weight (Kg.)	Urinary histamine µg./ 24 hours		Body weight (Kg.)	Urinary histamine µg./ 24 hours	
	Free	Conj.		Free	Conj.		Free	Conj.		Free	Conj.
2.3	0.3	2	13.2	3.6	3	18.6	11.4	33	28.2	7.2	9
3.7	0.8	3	14.1	6.1	13	20.9	7.6	24	28.2	8.1	28
4.5	1.3	6	14.1	7.8	14	21.8	6.1	9	28.2	12.6	14
5.0	1.5	3	14.3	3.3	31	22.3	9.3	15	28.6	14.5	16
5.0	2.4	<2	14.5	3.7	7	22.7	10.6	5	29.0	11.5	24
6.1	2.8	5	15.2	2.5	17	22.7	12.3	14	29.5	9.8	15
6.4	1.3	<2	16.4	4.5	5	24.5	6.8	93	29.5	18.5	20
6.7	1.1	<3	16.4	5.7	8	24.5	10.8	15	31.8	14.5	46
6.8	2.7	4	16.8	8.1	14	26.4	8.5	25	33.6	13.6	120
7.3	2.4	<6	17.3	5.5	47	26.4	11.0	9	34.9	16.5	114
						Mean					
5.4	1.7	2.3	15.2	5.1	15.9	23.1	9.4	24.2	30.1	12.7	40.6

Excretion of free histamine in µg./ Kg./ 24 hours				
Group	I	II	III	IV
Mean	0.30	0.33	0.42	0.42
Range	0.13 - 0.48	0.16 - 0.55	0.28 - 0.61	0.25 - 0.63

TABLE IV. Histamine in the urine of healthy children, grouped according to body weight.

ject	LOW FLUID INTAKE			HIGH FLUID INTAKE		
	Urine volume in 24 hours (ml.)	Free histamine in 24 hours (µg.)	Conjugated histamine in 24 hours (µg.)	Urine volume in 24 hours (ml.)	Free histamine in 24 hours (µg.)	Conjugated histamine in 24 hours (µg.)
1	520	9.0	21	1780	9.6	20
2	540	22.7	29	2440	22.2	48
3	1080	17.3	31	3770	16.0	85
4	610	13.2	17	3500	15.7	26
5	625	8.9	16	4100	8.5	20
an	675	14.2	23	3120	14.4	40

TABLE V

The effect of variations in urine volume on the urinary content of histamine.

Day	Male aged 5 years		Male aged 32 years		Male aged 25 years		Male aged 30 years	
	8 a.m. to 8 p.m.	8 p.m. to 8 a.m.	8 a.m. to 8 p.m.	8 p.m. to 8 a.m.	8 a.m. to 8 p.m.	8 p.m. to 8 a.m.	8 a.m. to 8 p.m.	8 p.m. to 8 a.m.
1	3.4	2.4	5.9	4.8	9.0	8.5	5.8	8.2
2	2.1	2.6	5.2	5.9	5.0	6.4	5.6	6.7
3	2.4	2.0	3.9	4.1	6.5	5.9	7.7	6.6
4	3.4	3.6	4.4	3.6	4.9	5.5	5.7	7.7
Mean	2.8	2.6	4.8	4.6	6.3	6.1	6.2	7.3

TABLE VI

A comparison between day and night excretion of free histamine

(values represent urinary free histamine in µg. per 12 hour period)

Subject		A.		B.		C.		D.		E.	
		Histamine in the urine in µg. per 24 hours									
Day	Diet	Free	Conj.	Free	Conj.	Free	Conj.	Free	Conj.	Free	Conj.
1	mixed	7.5	22	17.2	567	11.7	34	21.2	79	16.5	185
2	"	8.2	22	18.0	900	17.2	29	16.4	101	18.2	101
3	"	7.8	22	17.4	127	14.4	52	16.3	61	16.8	99
4	water	5.6	11	15.3	75	10.9	33	10.8	60	7.2	26
5	"	7.5	13	15.6	40	10.1	16	15.4	30	10.5	10
6	"	8.1	12	12.5	42	7.9	13	12.7	28	11.0	6
7	bread and milk	6.2	10	14.7	75	9.8	14	13.2	18	12.1	9
8	"	8.1	14	14.8	235	12.8	19	15.0	14	22.2	9
9	meat	7.6	30	48.5	856	17.1	394	25.4	34	20.4	50
10	"	10.2	61	45.4	2311	18.4	406	33.8	127	41.5	60
11	"	9.5	36	26.1	435	18.6	168	31.8	85	27.1	17

TABLE VIIa. Group A diet experiments.

The effect of changes in diet on the urinary excretion of histamine

Subject		A.	B.	C.	D.	E.
Day	Diet	Blood histamine in µg. per ml.				
1	mixed	0.030	0.044	0.045	0.067	0.042
2	"	0.029	0.048	0.046	0.052	0.045
3	"	0.029	0.044	0.050	0.062	0.047
4	water	0.012	0.040	0.050	0.044	0.030
5	"	0.012	0.045	0.052	0.042	0.039
6	"	0.020	0.044	0.054	0.047	0.034
7	bread and milk	0.021	0.042	0.046	0.044	0.034
8	"	0.023	0.047	0.048	0.042	0.034
9	meat	0.027	0.048	0.050	0.043	0.032
10	"	0.027	0.044	0.052	0.048	0.036
11	"	0.029	-	0.054	0.049	-

TABLE VIIb. Group A diet experiments

The effect of changes in diet on the level of histamine in the blood

Subject	A.		F.		G.	
	Micrograms of histamine in the urine					
Two hour period	Free	Conj.	Free	Conj.	Free	Conj.
6 - 8 a.m.	0.36	0.5	1.11	1.7	0.57	2.6
8 - 10	0.33	0.8	1.08	1.3	0.86	2.4
	60 mg. of histamine acid phosphate by mouth					
10 - 12	0.50	2.5	1.01	2.9	0.87	13.1
12 - 2 p.m.	0.44	44.0	0.98	35.6	0.49	61.6
2 - 4	0.44	83.2	1.06	151.1	0.67	58.1
4 - 6	0.59	89.6	0.96	138.7	0.49	60.0
6 - 8	0.70	55.8	0.86	157.3	0.51	29.0
8 - 10	0.64	51.2	0.85	118.4	0.65	15.6
10 - 12	0.54	27.2	0.97	25.0	0.55	12.4

TABLE VIIIa. Group B diet experiments

The urinary excretion of histamine

before and after an oral dose of histamine

Subject	A.		G.	
	Micrograms of histamine in the urine			
Two hour period	Free	Conjugated	Free	Conjugated
6 - 8 a.m.	0.40	5.7	0.65	1.8
8 - 10	0.44	4.2	0.41	1.6
	Bread and milk meal			
10 - 12	0.61	12.7	0.58	1.8
12 - 2 p.m.	0.57	9.1	0.47	2.7
2 - 4	0.67	4.7	0.48	3.4
4 - 6	0.42	3.1	0.52	3.4
6 - 8	0.47	2.1	0.39	2.8
8 - 10	0.37	2.3	0.46	5.5
10 - 12	0.35	1.9	0.45	3.4

TABLE VIIIb. Group B diet experiments

The urinary excretion of histamine

before and after a bread and milk meal

(meal = bread 200 g. ; butter 100 g. ; milk 500 ml.)

Subject	A.		F.		G.	
	Micrograms of histamine in the urine					
Two hour period	Free	Conj.	Free	Conj.	Free	Conj.
6 - 8 a.m.	0.39	2.8	1.09	0.8	0.39	2.7
8 - 10	0.52	1.9	1.13	0.6	0.38	1.5
	Bread and milk meal + histamine by mouth					
10 - 12	5.50	7.0	2.80	3.9	0.76	3.8
12 - 2 p.m.	4.13	61.6	4.54	62.5	1.38	9.2
2 - 4	1.96	53.1	2.29	103.2	1.06	25.7
4 - 6	1.10	32.5	2.02	134.3	0.72	21.0
6 - 8	1.15	23.3	0.86	172.8	0.47	12.6
8 - 10	0.38	3.0	0.86	39.1	0.44	12.5
10 - 12	0.45	4.0	0.91	44.7	0.34	11.6

TABLE VIIIc. Group B diet experiments

The urinary excretion of histamine before and after an oral dose of histamine accompanied by a bread and milk meal

(Histamine = 60 mg. of histamine acid phosphate .

Meal = bread 200 g. ; butter 100 g. ; milk 500 ml.)

Hourly period	Histamine in the urine (ug.) Free	Conj.	Two hourly period	Histamine in the urine (ug.) Free	Conj.	Free	Conj.	Free	Conj.
			6 - 8	0.64	0.9	0.39	1.7	0.45	11.2
			8 - 10	0.58	0.6	0.40	1.8	0.35	12.0
60 mg. of histamine acid phosphate by stomach tube						Histamine held in the mouth		500 g. meat	
10 - 11	0.32	0.9	10 - 12	0.59	1.6	0.51	3.3	0.73	21.2
11 - 12	0.27	0.7							
12 - 1	0.22	11.6	12 - 2	0.58	56.6	0.47	5.1	2.04	25.5
1 - 2	0.36	45.0							
2 - 3	0.22	61.9	2 - 4	0.50	93.1	0.41	4.1	1.54	20.5
3 - 4	0.27	31.2							
			4 - 6	0.64	21.5	0.41	2.7	1.47	13.2
			6 - 8	0.48	46.6	0.42	2.3	0.63	8.8
			8 - 10	0.77	34.1	0.50	1.6	0.49	5.5
			10 - 12	0.61	27.7	0.50	1.2	0.57	2.6
			12 - 2	0.44	11.3				
			2 - 4	0.44	13.0				
			4 - 6	0.52	8.5				
			6 - 8	0.44	3.9				
			8 - 10	0.38	5.9				

TABLE IX. Group C diet experiments

The urinary excretion of histamine in a fasting adult (subject A) -

i. Before and after a dose of histamine by stomach tube.
ii. Before and after holding histamine in the mouth for 30 minutes.
iii. Before and after a meal of broiled steak.

		Histamine in the urine in micrograms per 24 hours											
Day	Oral cortisone	free	conj.	free	conj.	free	conj.	free	conj.	free	conj.	free	conj.
1		24.0	24	25.5	149	21.0	18	20.8	23	9.5	25	19.0	18
2		16.1	13	26.8	103	18.9	20	18.0	46	10.7	52	19.9	32
3		20.6	15	23.2	38	21.6	54	16.1	17	12.4	153	19.6	29
4	200 mg.	25.8	13	20.9	35	25.9	22	23.2	46	15.7	39	12.8	34
5	200 mg.	26.8	15	24.2	26	28.0	17	25.3	39	13.4	14	31.4	22
6	200 mg.	30.9	24	25.4	25	33.9	40	25.6	26	16.4	11	17.7	25
7		19.2	37	29.2	47	18.3	16	17.6	16	7.7	13	24.9	33
8		20.7	30	18.5	120	28.1	64	21.4	20	8.7	33	24.6	35
9		21.8	17	22.8	39	25.5	85	20.3	21	12.3	60	25.9	27
10		15.6	15	15.8	30	21.4	95	14.2	21	9.4	35	17.5	19
11		16.7	16	17.8	32	20.4	67	15.9	24	9.8	30	20.7	33

TABLE Xa

The effect of cortisone on the urinary excretion of histamine

Subject No.		1	2	4	5	6
Day	Oral cortisone	Histamine in the blood in micrograms per ml.				
1		0.036	0.040	-	0.025	0.043
2		0.037	0.040	0.035	0.029	0.054
3		0.037	0.040	0.032	0.025	0.047
4	200 mg.	0.025	0.023	0.020	0.006	0.033
5	200 mg.	0.024	0.021	0.017	0.007	0.031
6	200 mg.	0.025	0.031	0.012	0.010	0.031
7		0.035	0.043	0.022	0.022	0.040
8		0.037	0.038	0.022	0.026	0.035
9		0.037	0.032	0.025	0.027	0.031
10		0.035	0.031	0.027	0.027	-
11		-	-	0.029	-	0.037

TABLE Xb

The effect of cortisone on the level of histamine in the blood

Day	Isonicotinic acid hydrazide by mouth	Blood histamine µg. per ml.	Urinary histamine in µg. per 24 hours	
			free	conjugated
1		0.032	10.6	46
2		0.030	9.7	56
3		0.026	11.3	63
4	300 mg.	0.029	13.2	165
5	300 mg.	0.026	10.6	49
6	300 mg.	0.025	10.4	52
7		0.030	9.2	48

TABLE XI

The effect of isonicotinic acid hydrazide on blood and urinary histamine

Patient	Urinary histamine ug. per 24 hours Free	Conjugated	Blood histamine ug. per ml.
1	13.9	28	0.121
2	20.9	104	0.081
3	19.0	100	0.037
4	20.5	147	0.067
5	8.5	227	0.051
6	9.5	54	0.040
Mean	15.4	110	0.066

TABLE XIIa.

The histamine content of the blood and urine of six patients with cirrhosis of the liver.

Day	Oral cortisone	Patient No. 1 Urinary histamine ug./ 24 hrs Free	Conj.	Blood histamine ug./ ml.	Patient No. 2 Urinary histamine ug./ 24 hrs Free	Conj.	Blood histamine ug./ ml.
1		12.0	22	0.121	24.0	94	0.085
2		15.8	24	0.121	20.6	114	0.077
3	200 mg.	13.6	20	0.026	25.2	113	0.026
4	200 mg.	25.6	25	0.017	28.4	59	0.020
5	200 mg.	22.6	27	0.007	27.4	54	0.025
6		16.9	18	0.048	19.7	43	0.097
7		10.6	25	0.057	18.5	61	0.096

TABLE XIIb

The effect of cortisone on blood and urinary histamine in two patients with cirrhosis of the liver.

	MILD						SEVERE				
Patient	Serum bilirubin mg. per 100 ml. Direct	Indirect	Urinary histamine ug./ 24 hrs Free	Conj.	Blood histamine ug./ ml.	Patient	Serum bilirubin mg. per 100 ml. Direct	Indirect	Urinary histamine ug./ 24 hrs Free	Conj.	Blood histamine ug./ ml.
1	3.7	1.5	25.5	44	0.050	10	12.9	3.1	39.1	140	0.130
2	14.0	4.7	23.6	169	0.044	11	1.2	0.3	7.2	20	0.100
3	6.1	2.1	6.1	23	0.030	12	6.6	3.4	17.6	56	0.057
4	11.6	3.6	9.1	149	0.045	13	2.0	0.8	14.8	74	0.053
5	10.1	2.8	6.4	54	0.054	14	13.6	4.2	17.5	6	0.061
6	5.5	1.5	10.4	24	0.042	15	11.9	2.5	7.9	18	0.083
7	6.6	1.6	8.5	19	0.032	16	18.0	4.0	14.7	15	0.071
8	5.2	1.8	10.5	26	0.100	17	17.0	3.7	6.9	153	0.067
9	13.6	3.5	21.2	35	0.068						
	Mean						Mean				
	8.5	2.6	13.5	59	0.052		10.4	2.7	15.7	60	0.078

TABLE XIII

The histamine content of the blood and urine of seventeen patients with pruritus and jaundice.

Infant	Free histamine in µg. per 24 hours		Conjugated histamine in µg. per 24 hours	
	Acute	Convalescent	Acute	Convalescent
1	1.0	2.4	<2	<10
2	0.5	1.2	3	< 4
3	2.8	3.2	<6	< 8
4	0.7	0.7	<3	9
5	0.5	1.2	1	2
6	1.0	0.6	<5	< 5
7	1.0	2.2	3	5
8	0.6	0.6	2	3
9	1.2	0.9	18	< 5
10	1.6	3.3	9	< 3
Mean	1.1	1.6	3.6	1.9

TABLE XIV

Urinary excretion of histamine in the acute and convalescent stages of infantile gastro-enteritis

Histamine in the urine
ug. per 24 hours

Body weight in Kg.	FREE Total	FREE per Kg. of body weight	CONJUGATED Total
18	7.3	0.40	44
20	8.4	0.42	15
24	9.3	0.39	49
25	14.2	0.57	39
27	8.7	0.32	56
29	14.0	0.48	9
29	7.5	0.26	8
31	11.0	0.35	34
31	17.8	0.57	18
32	11.6	0.36	16
32	6.7	0.21	19
40	10.7	0.27	47
41	17.3	0.42	11
50	17.0	0.34	26
Mean			
30.6	11.5	0.38	27.9

TABLE XVa

Histamine in the urine of children in the quiescent phase between acute allergic episodes

Histamine in the urine
ug. per 24 hours

Body weight in Kg.	FREE Total	FREE per Kg. of body weight	CONJUGATED Total	Acute condition
14	3.7	0.26	29	Asthma
25	7.0	0.28	31	Asthma
34	5.4	0.16	13	Ragweed pollinosis
36	14.0	0.39	39	Asthma
38	16.4	0.43	29	Ragweed pollinosis
38	19.7	0.52	14	Asthma
40	7.0	0.17	70	Asthma
43	15.4	0.36	41	Ragweed pollinosis
45	15.8	0.35	31	Ragweed pollinosis
50	10.6	0.21	74	Ragweed pollinosis
		Mean		
36.3	11.5	0.31	37.1	

TABLE XVb

Histamine in the urine of children in acute allergic attacks

Patient	Nature of attack	Day	Treatment	Urinary histamine ug. per 24 hours Free	Conj.
Male aged 38	Very severe elm pollinosis	1	none	25.8	212
		2	cortisone	21.2	161
		3	cortisone	38.1	113
		4	cortisone	116.6	2096
		5	none	72.7	817
		6	none	31.1	197
Male aged 16	moderately severe asthma	1	none	19.7	14
		2	cortisone	21.4	19
		3	cortisone	32.5	26
		4	cortisone	29.0	30
		5	none	25.6	27
Female aged 22	moderately severe ragweed pollinosis	1	none	25.5	38
		2	hydrocortisone	67.3	23
		3	hydrocortisone	35.5	20
		4	hydrocortisone	26.9	19
Male aged 38	moderately severe ragweed pollinosis	1	none	13.6	37
		2	hydrocortisone	29.3	61
		3	hydrocortisone	30.4	64
		4	hydrocortisone	30.2	147
Male aged 12	mild ragweed pollinosis	1	none	9.1	17
		2	cortisone	11.1	20
		3	cortisone	10.5	13
		4	cortisone	7.9	9
Male aged 11	mild ragweed pollinosis	1	none	9.8	23
		2	cortisone	9.4	20
		3	cortisone	14.3	36
		4	cortisone	14.3	24

TABLE XVI

The effect of cortisone and hydrocortisone on the urinary excretion of histamine in acute allergic attacks

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