HISTAMINE METABOLISM IN BRONCHIAL ASTHMA

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Histamine Metabolism in Bronchial Asthma

SUMMARY

This thesis is a study of the metabolism of endogenous and exogenous histamine in patients with bronchial asthma and control subjects. The effect of exogenous histamine on pulmonary ventilation in these subjects is also investigated.

A preliminary experiment in Part I confirms observations already published by Dr. R. W. Schayer in which the urinary metabolites of C^{14} histamine injected into guinea pigs are identified by paper chromatography and autoradiography. The principal metabolites are C^{14} 1-methyl, 4-imidazole acetic acid, C^{14} 1-ribosyl imidazole acetic acid and C^{14} 1-methyl histamine. The methods used in this preliminary experiment were developed and used to examine the urine extracts of patients admitted to hospital in status asthmaticus for the naturally occurring metabolites of histamine. The effect of treatment with corticotrophin gel on the urinary metabolites of histamine is also reported. Urine extracts from non allergic subjects were examined as controls.

Patients admitted in status asthmaticus were found to have the imidazole, urocanic acid in their urine extracts and no evidence of histamine or of the principal metabolite of histamine in man, 1-methyl, 4-imidazole acetic acid, or of the alternative metabolite 1-ribosyl imidazole acetic acid. In contrast the urine extracts of controls contained no urocanic acid and the histamine metabolite 1-methyl, 4-imidazole acetic acid was identified in significant quantity. Within 24 hours of starting corticotrophin treatment in the asthmatic subjects, the urocanic acid had disappeared and 1-methyl, 4-imidazole acetic acid could be identified in the urine extracts. Histamine itself was also identified in the urine extracts of half of the asthmatic subjects studied whilst on corticotrophin.

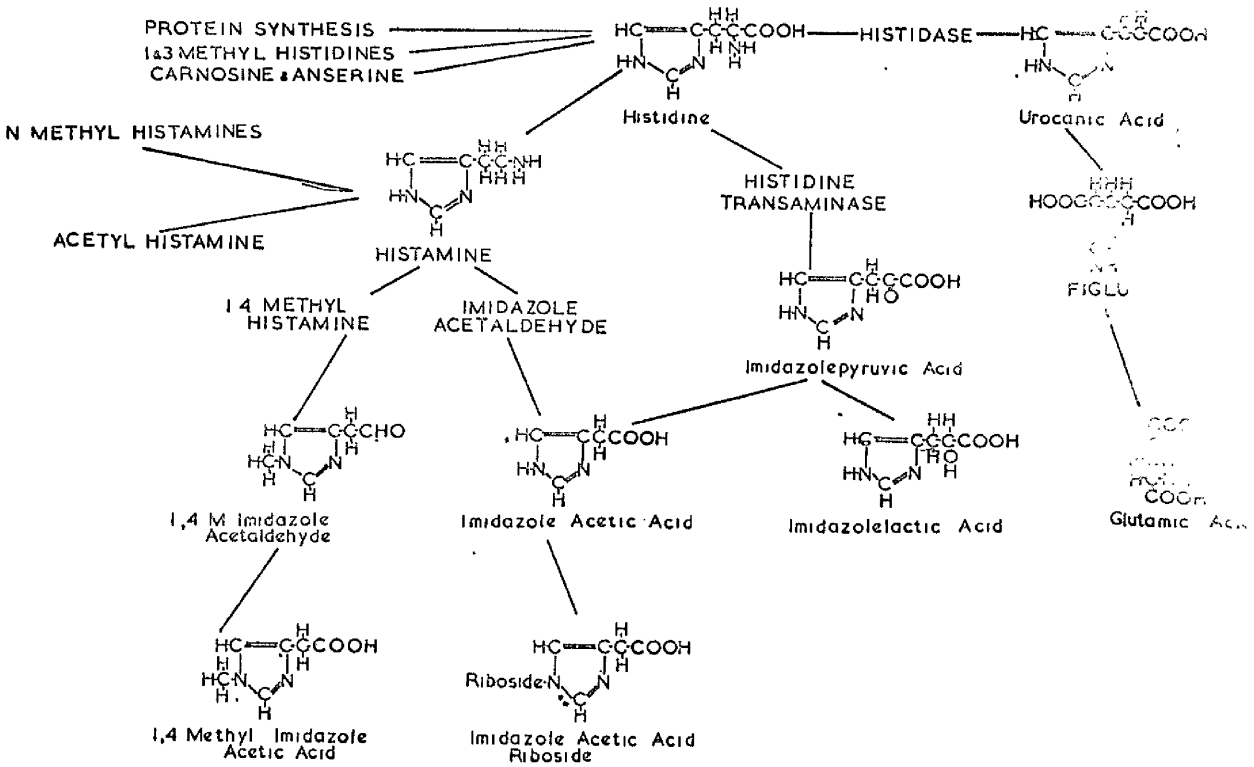
The significance of the observations in Part I is discussed and it is being suggested that in an attack of asthma histamine is being retained in the body and not made available for metabolism. This leads to a feed back via l-histidine and overloading the metabolic pathway from l-histidine via urocanic acid to glutamic acid. The

effect of corticotrophin gel is to release endogenous histamine and its metabolite 1-methyl, 4-imidazole acetic acid appears in the urine. At the same time urocanic acid disappears from the urine (see attached figure).

Part II is a comparison of the metabolism of infused C^{14} histamine dihydrochloride in asthmatic subjects with that in controls. The levels of radioactivity in the urine and serum of the two groups are compared in the 24 hours after the infusion and there is shown to be no significant difference in the two groups. The urinary and serum metabolites of C^{14} histamine are identified as C^{14} 1-methyl, 4-imidazole acetic acid and C^{14} 1-ribosyl, 4-imidazole acetic acid and again there is shown to be no significant difference in the rate of metabolism or in the metabolic pathway of C^{14} histamine in the two groups. During the infusion of C^{14} histamine there was diminished pulmonary ventilation in the asthmatic subjects as compared to the controls and this is shown to be statistically significant. This well known hypersensitivity of ventilation in asthmatic subjects to histamine is discussed in relationship to this study and to the

recently proposed hypothesis that histamine supersensitivity is due to a disturbed function of the alpha and beta receptors on bronchial smooth muscle. Finally, preliminary results are reported to show that alpha adrenergic receptor blocking drugs can alter histamine supersensitivity and inhibit the fall in ventilation in asthmatic subjects which follows an infusion of histamine.

HISTAMINE METABOLISM



"If a man will begin with certainties, he shall end in doubts; but if he be content to begin with doubts he shall end in certainties."

Francis Bacon (1561 - 1626)
in Advancement of Learning I, 8.

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Acknowledgements

I wish to acknowledge my debt to the late Dr. W. R. Snodgrass and to Dr. T. N. Fraser; the former first stimulated my interest in bronchial asthma and both encouraged me to have a research interest in this disease. The work described in this thesis was carried out whilst I was attached as Senior Registrar to the M.R.C. Clinical Research Unit at the Western Infirmary under the late Dr. J. Reid and the late Dr. B. Bronte-Stewart. As the main research effort of the M.R.C. Unit was not directed towards bronchial asthma or indeed respiratory diseases, Professor Sir Edward Wayne kindly arranged that I should have the necessary facilities and technical help.

At various times three technicians have assisted me with the work described, Miss Joan Munro, Miss Vivian Park and Mr. Robert Rae. Each worked with me soon after they had left school and I wish to thank them for the care, accuracy and enthusiasm they showed in their work. Mr. R. C. Dowell whilst a fourth year medical student received a summer grant from the M.R.C.

and during his holiday in 1965 assisted in the isotope investigations on the serum metabolites of C^{14} histamine.

My own knowledge of chemistry is limited and I am indebted to Dr. Charles Brooks of the Chemistry Department, Glasgow University for his constant advice and criticism on the chemical methods used.

Finally I must thank all those who willingly volunteered to take part in the experimental work described in this thesis and Miss Anne Sim for typing the manuscript.

INTRODUCTION

The History of Asthma and the development of Allergy and Anaphylaxis

The ancient literature contains several references to dyspnoea and although at that time there was no clear distinction between the various forms of respiratory distress, Aretaeus in the second century A.D. gave a graphic description of asthma.

The first Renaissance physician to write about asthma was Gerolamo Gordano (A.D. 1501-1576). A mathematician as well as a physician he was called from Italy to Edinburgh to treat John Hamilton, Archbishop of St. Andrews. For six weeks Gordano studied the Archbishop before diagnosing Asthma. His treatment included diet, exercise and purging to decrease the cough producing secretion of mucus in the throat and the substitution of a bed of unspun silk for one of feathers. Happily for Gordano, John Hamilton recovered, but only lived long enough to be hanged by the Scottish Reformers (Major 1954).

During the seventeenth century "spasmodic asthma" was finally differentiated from other forms of breathlessness, largely as a result of the writings of Baptisti van Helmont

(1607) and Willis (1681). In 1698 Sir John Floyer published his treatise on asthma, he distinguished asthma from emphysema and clearly assigned the cause of spasmodic asthma to "a contracture of the muscle fibres of the bronchi".

Bree (1811) in his book "Disordered Respiration" suggested that hypersensitivity to hair powder could be an aetiological factor in precipitating an attack of asthma. The concept of hypersensitivity as a factor in asthma was put on firmer clinical ground by the detailed observations of Salter (1859). He recognised that animal emanations might cause asthma and gave a full description of his own cat asthma. In addition he was the first to stress the hereditary factor in asthma which he was able to trace in more than 40 per cent of his cases.

The most prominent physicians of the 19th century realised that bronchial muscle spasm did not explain all the clinical and experimental observations. A favourite view was of some internal bronchial obstruction which was probably caused by oedema of the bronchial mucosa and responsible for the clinical picture. Further progress had to await the concept of allergy and anaphylaxis.

Allergy and Anaphylaxis

In 1894 Flexner reported that rabbits would withstand one injection of dog serum but would succumb to a second dose administered at a later date. Héricourt and Richet (1898) performed many experiments on induced hypersensitiveness in animals and showed that second innoculations of small doses of eel serum or toxins extracted from actinae caused violent symptoms and death. This phenomenon was termed anaphylaxis by Portier and Richet (1902). This work was expanded by others including Smith (1904) and Von Pirquet and Schick (1905). In 1905 the latter group described serum sickness, the combination of symptoms which frequently occurred in children five to ten days after an injection of therapeutic antiserum. The symptoms reappeared almost immediately and with great regularity after a second often minute injection of serum.

The term Allergie was introduced by Von Pirquet in 1906 and at first it simply implied an altered capacity to react. The English word Allergy was adapted from the German. Today, however, allergy is usually defined as a

specifically induced altered reactivity in which there is evidence of an underlying immunological mechanism. The word hypersensitivity is frequently used synonymously with allergy often when no immunological basis has been identified.

Anaphylaxis is a specific example of the hypersensitive state and occurs in certain animal species. First exposure to the antigen has little apparent effect, but on re-exposure to the antigen the animal has an immediate reaction which is characteristic for the particular species. The animal which is most exquisitely sensitive to anaphylaxis is the guinea pig. Almost a minute after an intravenous injection of the antigen in a previously sensitised guinea pig there is a marked restlessness and bristling of hair on the neck and head. This is followed by rubbing of the nose and often loud sneezing, the animal may jump and there is a slowing of the respiratory rate associated with violent respiratory efforts. In a few minutes respiration ceases but the heart continues to beat for several minutes longer. The pathology of the shocked guinea pig was described by Auer and Lewis (1910) the most important finding being acute hyperinflation of lungs or "emphysema" due to intense

constriction of the bronchi and bronchioles causing death from asphyxia.

In anaphylaxis four distinct pharmacologically active substances have been identified in the tissues as a result of the antigen - antibody reaction. These are histamine, slow reacting substances (S R S - A), 5-hydroxytryptamine and the polypeptide bradykinin.

The mechanism by which these agents are released has yet to be fully worked out even in the much studied guinea pig but it is known that antibody must first become absorbed on to the tissues. This absorption is a property of the 7 S globulins which include the specific antibody. The sequence of events associated with the interaction of antigen and antibody appears to be in two stages and requires both calcium and an enzyme not unlike chymotrypsin.

The fundamental mechanism of anaphylaxis would appear to be the same in different species although the symptoms and their severity vary markedly from one kind of animal to another. The main reason for this is that the pharmacologically active substances liberated differ in nature and quantity in different animals and the species susceptibility to each of these substances shows considerable

variation. Rats, for example, can tolerate vast quantities of histamine and are highly resistant to anaphylaxis. In the rabbit, on the other hand, death from anaphylaxis is caused by intense constriction of the pulmonary artery and embarrassment of the pulmonary circulation due to aggregation of platelets in their lungs and the liberation of 5-hydroxytryptamine from these platelets. Considerable amounts of histamine are liberated but the rabbit is quite resistant to this.

Anaphylaxis in Man and its relationship to Asthma

Acute systemic anaphylaxis in man is fortunately rare but it does occasionally follow parenteral administration of even small amounts of antigen in sensitised subjects, for example penicillin. The symptoms and signs commonly begin with itching of the scalp and tongue, flushing of the skin and urticaria appears. In addition there is difficulty in breathing due to bronchial spasm.

The first definite suggestion that human hypersensitiveness (hay fever) was related to anaphylaxis as seen in animals was made by Wolff-Eisner (1906). Meltzer (1910, a & b), influenced by the work of Auer and Lewis in his laboratory, independently suggested that asthma was a phenomenon of local anaphylaxis in man.

The most familiar form of local anaphylaxis is hay fever. Symptoms are produced when the antigen (usually grass pollen) comes into contact with the mucous membrane of the nose or with the conjunctiva. Another example is asthma especially the type in which some causative factor, such as pollen, feathers, animal dander or house dust can be identified as the precipitating agent of the bronchial

obstruction. From the earliest writings it has been a matter of common observation that a tendency to asthma, infantile eczema and hay fever runs in families. There is substantial evidence for a hereditary basis although its exact nature has not been defined. For example Crawford (1936) reported on allergic manifestations in a family at Leamington Spa in five generations and by 1958 Dr. Barbara Crawford had observed urticaria in the sixth generation. Others have reported on the hereditary basis of this type of hypersensitivity (Cooke and Vanderveer, 1916, and Coca, 1927) which does not strictly conform to Mendalian laws and the best explanation to date is that put forward by Weiner, Zieve and Fries (1936) suggesting two allelomorphic genes are responsible, one being allergic and giving rise to three genotypes.

Clinical hypersensitivity of this type with a hereditary predisposition was termed Atopy by Coca and Cooke (1923) and such hypersensitivity is now known to be associated with the presence in the blood of specific antibodies to the inciting agent. These antibodies have special qualities and are known as atopic or reaginic antibodies.

Definition of Asthma

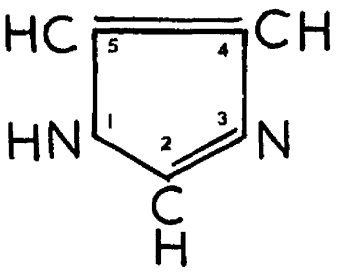
Over the years, the word "asthma" has acquired a variety of meanings. Some employ the word to denote the physical signs of bronchial obstruction and thus it is used synonymously with bronchospasm. This leads to asthma being described as a feature of many diseases in which rhonchi are audible including bronchiolitis and emphysema; indeed some physicians still call acute pulmonary oedema cardiac asthma. I am sure it is important to limit the use of the word asthma to a condition which may be defined as intermittent or continuous episodes of dyspnoea in which the obstruction to air flow is principally expiratory and due to bronchial and bronchiolar obstruction and is reversible. There is frequently a history or a family history of allergic conditions such as hay fever or atopic eczema. The symptoms often date from childhood and the subject's blood may contain reaginic antibodies. Where the bronchospasm occurs with the development of chronic bronchitis, the condition should be regarded as a variant of chronic bronchitis rather than a type of asthma and it is worth quoting Rackemann, who, writing in

1947 stated "When asthma begins after the age of 40, it should be considered as due to factors other than Allergy until proved otherwise."

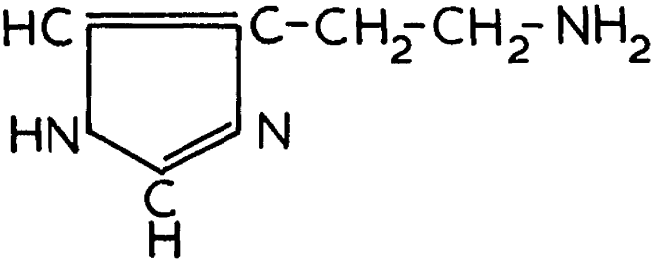
Histamine and Anaphylaxis

Histamine was synthesised by Windaus and Vogt in 1907 and its natural occurrence in ergot was reported by Barger and Dale (1910) and also by Kutscher (1910). In the same year the pharmacological actions of histamine were described by Dale and Laidlaw (1910). Its formation from the amino acid l-histidine by decarboxylation as the result of the action of putrefactive bacteria in the human intestine was reported by Ackermann (1910) and Mellanby and Twort (1912). Histamine is 4(β amino ethyl) imidazole (Figure 1 A & B). Its principal pharmacological effects are on the vascular system, the smooth muscle and the endocrine glands. Histamine causes dilation of the capillaries with altered permeability which leads to fluid loss and oedema. The direct stimulant action on smooth muscle is well seen in bronchiolar constriction. This is independent of innervation and not inhibited by atropine. In addition histamine stimulates salivary and mucous secretion and precipitates a sharp rise in the gastric secretion of free acid.

In 1910 Dale and Laidlaw called attention to the general similarity between the symptoms of anaphylaxis and



A



B

FIGURE 1.

Figure 1.

A. The imidazole ring to show numbering of ring.

B. Histamine or 4- (β-amino ethyl) imidazole.

the symptoms produced by histamine injection but at that time there was little further evidence to support this. However the part played by histamine could not be overlooked when it was realized that animal tissues contained sufficient histamine to provoke symptoms if it could be released. Further, those organs which contained the largest amounts of histamine were the ones most intimately involved in the anaphylaxis.

Sir Henry Dale made his historic indictment of histamine in anaphylaxis in 1929 and since then much evidence has slowly accummulated to support his conclusion. The most important recent evidence has been based on the observations of Riley and West (1953) that the granules in mast cells are the site of most of the histamine in the body. The release of histamine from mast cell granules following the antigen-antibody reaction in anaphylaxis has been described by several authors (Mota, 1957, Austin and Humphrey, 1961. Bareus and Chakravarty, 1960).

It is now generally accepted that the release of histamine from mast cell granules is the source of one of the more important pharmacologically active substances released in anaphylaxis. Acute urticaria is usually present

in anaphylaxis and Adam, Hunter and Kinnear (1950) have demonstrated an increased output of free histamine in the urine of men during acute urticaria.

The introduction of the antihistamine drugs (Halpern, 1942) and their benefit in hay fever and urticaria in man seemed to confirm, at least for the clinician, the part played by histamine release in these conditions. In striking contrast was the ineffectiveness of antihistamine drugs in bronchial asthma. Although this lack of response to antihistamine drugs is unexplained it should be remembered that antihistamine drugs do not inhibit the very powerful effect of histamine on gastric acid secretion. In these two situations the receptor sites for histamine do not seem to be accessible to the currently available antihistamine drugs.

Histamine and Asthma

As already observed Meltzer (1910) suggested that bronchial asthma can be considered a form of hypersensitivity in man and a manifestation of localised anaphylaxis. In anaphylaxis histamine is rapidly released and produces pharmacological effects. This suggested that the possibility of histamine release or abnormal histamine metabolism should be looked for in patients with asthma.

The evidence for disturbed histamine metabolism in bronchial asthma is much less clear than in anaphylaxis and often indirect in nature. In 1935 Barsoum and Gaddum published a method to estimate histamine in the blood and using this method or modification of it, several authors have reported on blood histamine levels during an attack of asthma. Cerqua (1936) reported on eight patients with bronchial asthma who had a definite increase (18-30 mg. histamine per 100 ml. blood) during an attack of asthma but who had normal levels (7-10 mg. histamine per 100 ml.) in quiescent periods. Similar findings have been reported by Jacquelin (1937) and Parrot (1938). Rose (1941)

studied the alterations in the blood histamine levels in patients with bronchial asthma. In 30 cases of asthma he reported wide fluctuations in blood histamine concentration. In 9 cases the levels were consistently above and in 3 consistently below the normal range of 2.5 to 7.5 mg. histamine per 100 ml. This was in striking contrast to the remarkably constant levels found in 50 normal persons observed over a period of months. These observations confirmed the findings previously reported by Code and MacDonald (1937). The concentration of histamine in the blood could not be related to the degree of clinical symptoms in the asthmatic subjects or to the progress of the disease but it should be remembered that respiratory function tests of ventilation were not being used at that time to assess airways obstruction. In a further communication Rose, Rusted and Fownes (1950) measured the concentration of free histamine in systemic arterial and pulmonary mixed venous blood (pulmonary artery) during an induced attack of asthma. They reported that systemic arterial levels of histamine were higher than pulmonary mixed venous levels and that both were higher than in

non-asthmatic controls.

Schild, Hawkins, Mongar and Herxheimer (1951) showed that human bronchial rings from a case of grass pollen asthma constricted when challenged with pollen antigen and that histamine was liberated into the organ bath.

On the other hand Reisser (1937) and Howarth and MacDonald (1937) were unable to detect any significant change in the concentration of histamine in the blood of patients during an asthmatic attack. Further, Mitchell, Logan, Peters and Henderson (1954) using a much improved bio-assay method to measure free histamine carried out a careful study of asthmatic children and showed that in the intervals between attacks the free histamine output in the urine was within the normal range, whereas in an acute attack of asthma the concentration of free histamine in the urine tended to decrease.

Whilst investigating the effect of intravenous histamine in man, Weiss, Robb and Ellis (1932) reported that this procedure frequently precipitated an asthmatic-like attack in patients with asthma. This observation was further investigated by Curry (1946) who confirmed that histamine diphosphate (0.2 mgm. histamine base I.M. or 0.02 mgm.

given I.V.) caused a rapid fall in vital capacity within 2-5 minutes with full recovery by 30 minutes. These doses of histamine had little effect on the vital capacity of 10 normal subjects or on 10 patients with a history of severe allergic tendencies but no asthma.

Curry went on to demonstrate that antihistamine preparations given I.V. had almost a complete protective effect against both the systemic and bronchoconstriction effects of parenteral histamine although an orally administered antihistamine was less effective in protecting against bronchoconstriction. Essentially similar results have been reported by Bouhuys, Jonsson, Lichtneckert, Lindell, Lundgren, Lundin and Ringquist (1960) who investigated the effect of I.V. histamine and inhalation of histamine on pulmonary ventilation in man. Intravenous histamine diminished ventilation in asthmatic subjects but had no effect on the ventilation of non asthmatic subjects. In contrast inhalation of histamine affected ventilation in all subjects tested although a much higher concentration of histamine inhalation (x 50) was required with non asthmatic subjects to diminish pulmonary ventilation.

The consensus of reported work on histamine in bronchial

asthma is suggestive that at least in some cases there is a disturbance of histamine metabolism as compared to normal, but what this disturbance is remains obscure. The observations of Rose and Mitchell are not necessarily contradictory if during an acute attack of asthma histamine is retained in the body with a corresponding fall in the urinary output of free histamine. Further, all workers are agreed on the hypersensitivity of the asthmatic subject to injected histamine as compared to the non asthmatic subject.

Histamine is a highly active pharmacological substance and it is probable the body has an efficient enzymatic system to inactivate and metabolise it, the free histamine being measured representing only a small fraction of the total metabolic turnover. In recent years Schayer and his associates (1959) have shown this to be the case.

Recent developments in histamine metabolism

The in vivo pathway of histamine catabolism has been distinctly clarified in the past few years and we now have a better understanding of the fate of histamine in man and animals.

Schayer and Karjala (1956) have shown that when minute amounts of C^{14} ring labelled histamine are injected into guinea pigs, paper chromatograms prepared from the urine extracts collected in the first few hours after injection of the isotope give three radioactive spots. The first is a conjugate of imidazole acetic acid with ribose; 1-ribosyl, 4-imidazole acetic acid (Karjala, Turnquest and Schayer, 1956). The second is 1-methyl, 4-imidazole acetic acid (Karjala and Turnquest, 1955) and the third is 1-methyl histamine (1-methyl, 4(β-amino ethyl)-imidazole) together with a small quantity of the original C^{14} histamine (Rothschild and Schayer, 1958) (Figure 2).

Schayer and Cooper (1956) have reported on the metabolism of minute amounts of ring labelled C^{14} histamine injected intradermally in man. Almost 100 per cent of the administered radioactivity could be recovered in the

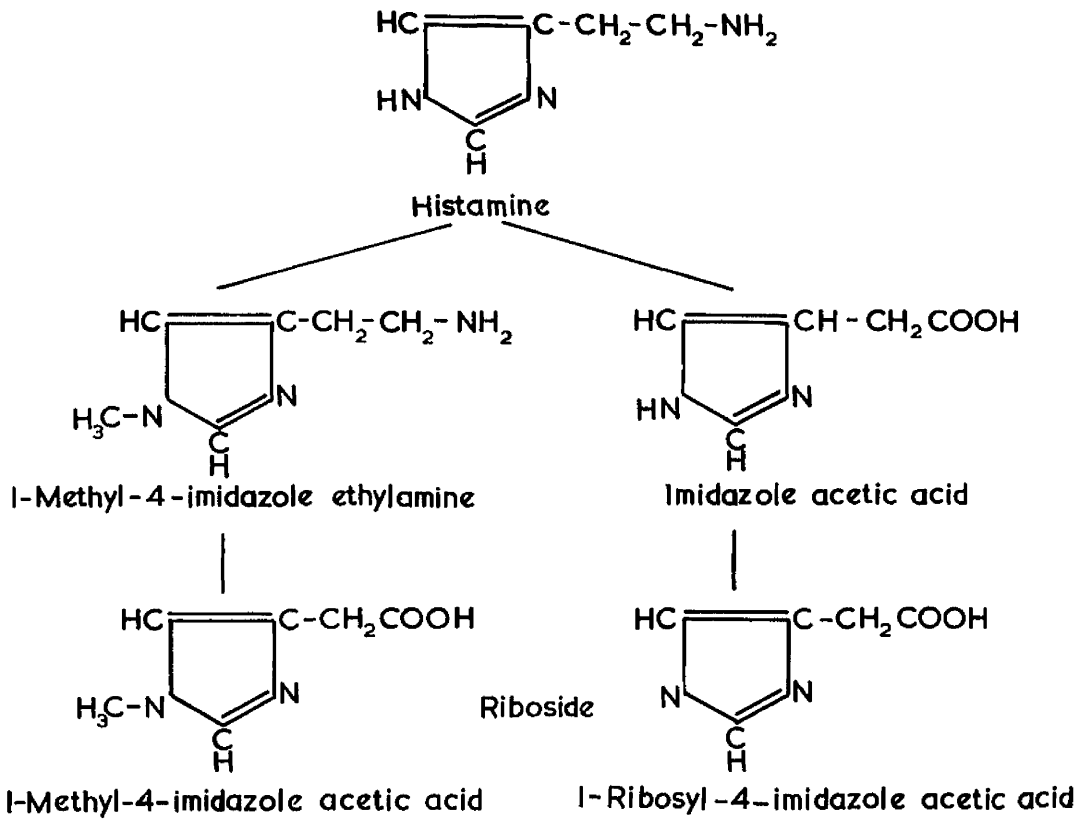


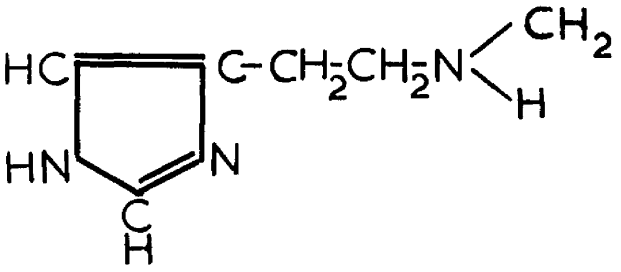
FIGURE 2.

Figure 2. The metabolites of exogenous C^{14} ring labelled histamine. There are two pathways:

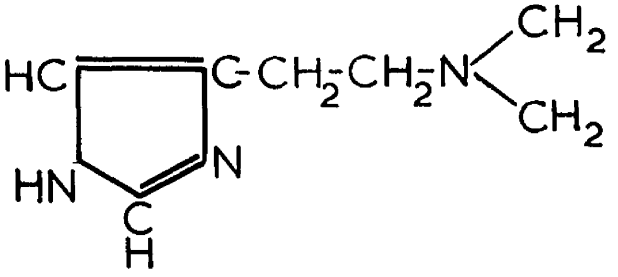
1. Oxidation to form imidazole acetic acid and its riboside.
2. Ring methylation at the 1 position to form 1-methyl histamine and 1-methyl, 4-imidazole acetic acid.

urine in 24 to 48 hours. Quantitative analysis for C^{14} histamine and its metabolites in the urine collected in the six hours after the injection accounted for 81 per cent of the total radioactivity. Only 2-3 per cent was recovered as the original C^{14} histamine. The principal metabolite recovered was 1-methyl, 4-imidazole acetic acid which accounted for 42-46 per cent of the recovered radioactivity. Imidazole acetic acid and the riboside of imidazole acetic acid accounted for 27-33 per cent and 1-methyl histamine for 4-5 per cent of the recovered radioactivity. Schayer was unable to account for more than 84 per cent of his recovered radioactivity and it may be that N-methyl and N-dimethyl histamine recently identified in human urine (Kapeller-Adler and Iggo, 1957,) accounts for some of the unidentified metabolites (Figure 3).

These observations have shown that the principal metabolite of histamine in man is 1-methyl, 4-imidazole acetic acid. The enzyme required for methylation in the 1-position of the imidazole ring, imidazole N-methyl transferase has been studied by Brown, Tomchick and Axelrod (1959). This enzyme is widely distributed in brain, stomach and lung and



A. N-Methyl histamine



B. N-dimethyl histamine

FIGURE 3.

Figure 3. The N-methyl and N-dimethyl metabolites of histamine identified in human urine by Kapeller-Adler and Iggo (1957).

appears to be specific for histamine as no other imidazole served as substrate. From the isotope studies with C^{14} histamine, 1-methyl, 4-imidazole acetic acid appears to be a specific metabolite of histamine in man and if it could be identified would provide a useful measure of histamine catabolism. In addition observations on it in asthma might lead to a clearer understanding of the part played by histamine in an attack of asthma.

THE PRESENT INVESTIGATION

This work is a study of the imidazoles present in the urine of healthy subjects and of patients admitted to hospital in status asthmaticus. In addition the effect of corticotrophin on these imidazoles is observed during the treatment of status asthmaticus. This is followed by a study of the metabolism of infused C^{14} labelled histamine in patients with bronchial asthma and controls. At the same time the effect of the infused C^{14} histamine on pulmonary ventilation is noted. Based on the results of these experiments and other published work a hypothesis regarding the relationship of histamine metabolism to bronchial asthma is suggested.

Considerable numbers of patients are admitted to hospital in this country with dyspnoea and wheezing. The need to study a relatively homogenous group of subjects if consistent results are to be obtained is apparent. All the asthmatic subjects included in the present investigation fulfilled the criterion already laid down in the definition of bronchial asthma. In addition the patient usually (1) gave a family history of bronchial asthma (2) had had

infantile eczema (which in some cases had persisted to adult life) and (3) had an eosinophilia of at least 700 cells/c.mm. before treatment. Brief clinical details of each patient are given in the text.

The investigations outlined above are presented in two parts.

Part I

1. A preliminary experiment to confirm the work of Schayer and his associates by injection of ring labelled C^{14} histamine into guinea pigs and the preparation of autoradiograms for the identification of the radioactive metabolites.

2. The investigation of the imidazole compounds present in the urine extracts of patients admitted in status asthmaticus and of healthy non asthmatic subjects.

   a) The identification of urocanic acid in the urine of non asthmatic patients by paper chromatography and ultra violet absorption spectra.

   b) The effect of corticotrophin on the urocanic acid excretion and the identification of other imidazole acids

present in the asthmatic subject when on corticotrophin.

c) The identification of 1-methyl, 4-imidazole acetic acid by paper chromatography and ultra violet absorption. Confirmation of the identification of 1-methyl, 4-imidazole acetic acid by infra red spectrophotometry of its crystallised form and gas chromatography of its methyl ester. This was necessary as non isotopic 1-methyl, 4-imidazole acetic acid had not previously been identified in human urine.

d) Examination of the urine extracts of patients admitted in status asthmaticus for evidence of overloading of the alternative pathway of histamine metabolism to the riboside of imidazole acetic acid.

e) The identification of histamine in the urine of some patients in status asthmaticus whilst on corticotrophin.

Part II

a) The metabolism of C^{14} labelled histamine dihydrochloride infusion in patients with bronchial asthma and control subjects.

b) The effect of infused C^{14} labelled histamine dihydrochloride on pulmonary ventilation in patients with bronchial asthma and control subjects.

PART I

1. Preliminary Investigation

The injection of ring labelled C^{14} histamine into guinea pigs and the preparation of autoradiograms from paper chromatograms of the urine extracts from the guinea pigs.

Materials and Methods

The C^{14} histamine was obtained from the Radiochemical Centre, Amersham, England. The labelled carbon is in the 2 position of the imidazole ring and the specific activity of the C^{14} histamine was 39.4 µc/mg. (See Figure 1 page 14)

Weights of guinea pigs.

1. 360 g.
2. 380 g.

Dose of C^{14} histamine 50 µg. per kilogram.

The animals were injected subcutaneously at 10.30 a.m. and the urine collected for 24 hours. The urines from the guinea pigs were acidified with 2N HCL and the total 24 hour output evaporated to dryness in 250 ml. round

bottomed flasks. Methanol (25 ml.) was added to each flask and the urine extracted in boiling methanol for 2 hours. After cooling the methanol extract was filtered and again evaporated to dryness and the residue dissolved in 1 ml. water. A one dimensional paper chromatogram was run on Whatman No. 4 paper spotting 5 ul. of the extract. The chromatogram was developed with Propanol-ammonia, Propanol: $0.2N/NH_4OH$, 3:1 (Pr.Am.) and after drying exposed to X-ray film for 3 weeks. The X-ray film was then processed.

Control papers were run in Propanol-ammonia (3:1) spotting 5 µg. imidazole acetic acid, 200 µg. 1-methyl, 4-imidazole acetic acid, 10 µg. histamine and 20 µg. 1-methyl histamine.

The imidazole acetic acid and histamine were located with the Pauly reagent (page 52). The 1-methyl, 4-imidazole acetic acid was located with diazotised p-nitroaniline (page 52) and the 1-methyl histamine located with ninhydrin reagent.

Results

The X-ray negative prepared from the paper chromatogram of each guinea pig urine extract showed three distinct peaks

Figure 4 and Table I.

TABLE I

Radioactive Peak No.	Rf of Radio Peaks	
	GP 1	GP 2
Peak I	0.21	0.21
Peak II	0.42	0.44
Peak III	0.69	0.69

The Rf of imidazoles run on control papers are shown in Figure 5 and Table II.

TABLE II

Rf of Control Imidazoles	Rf
Imidazole acetic acid	0.37
1-methyl, 4-imidazole acetic acid	0.43
Histamine	0.59
1-methyl histamine	0.69

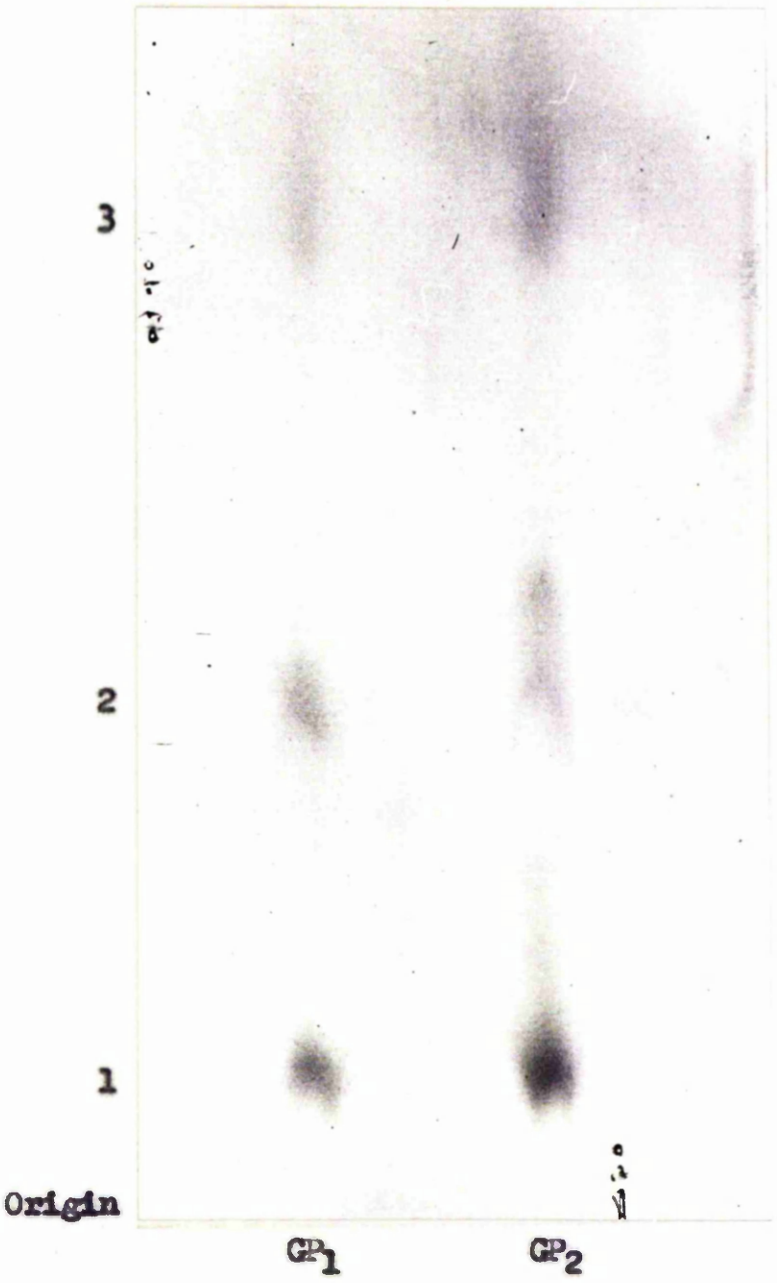


Figure 4. The autoradiograph of guinea pig urine extract run in Pr.Am. and exposed to X-ray film for 3 weeks. Three radioactive peaks can be identified. From the origin the first peak is the riboside of imidazole acetic acid, the second 1-methyl, 4-imidazole acetic acid, and the third 1-methyl histamine. The principal metabolite in the guinea pig is the riboside of imidazole acetic acid. The Rf's are shown in Table I.

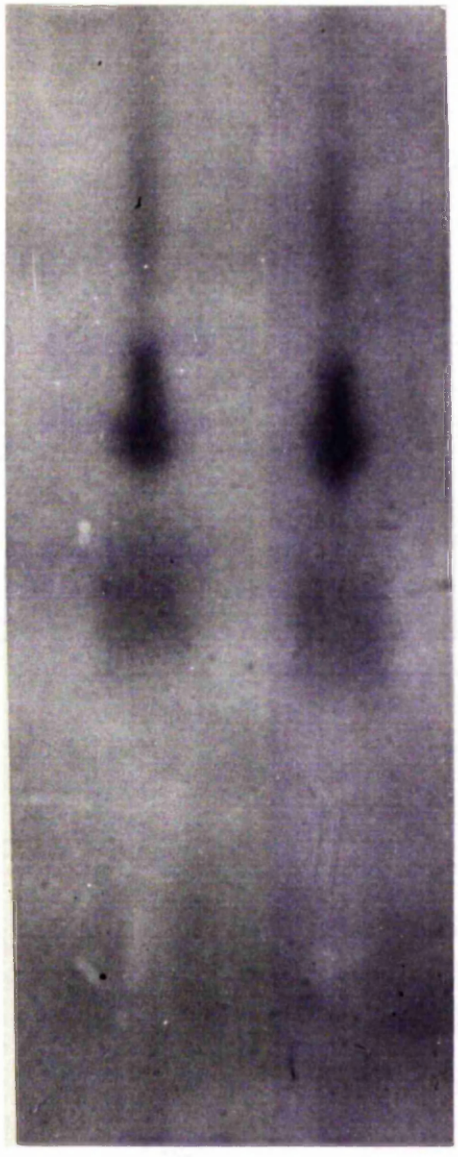


Figure 5 A one dimensional chromatogram of each GP urine extract with added non isotopic 1-methyl histamine, histamine, and 1-methyl, 4-imidazole acetic acid and run in Pr.Am. The Rf's are as shown in Table II.

Conclusions

The presence of three radioactive substances in paper chromatograms prepared from the urine extracts of guinea pigs after the injection of C^{14} histamine have been described. This observation confirms the findings of Karjala, Turnquest and Schayer (1956). The Rf of the second peak corresponds to that obtained for non isotopic 1-methyl, 4-imidazole acetic acid and the Rf of the third peak corresponds to the Rf obtained for 1-methyl histamine (1-methyl, 4-(β-amino ethyl) imidazole).

Schayer identified the first peak as 1-ribosyl-4-imidazole acetic acid and this can be seen to be the principal metabolite in the guinea pig (Figure 4), and runs slower than imidazole acetic acid on the paper chromatogram. In man the principal metabolite is the second peak which has been identified as 1-methyl, 4-imidazole acetic acid (Schayer and Karjala, 1956; Kerr, 1964).

As the aim of this work was to identify the metabolites of histamine in man and make observations on its metabolites in asthmatic subjects and during status asthmaticus no further work on the radioactive metabolites of C^{14} histamine in the guinea pig was carried out.

This preliminary experiment has also shown that the method of methanol extraction of the radioactive imidazoles followed by identification by paper chromatography was successful. It was therefore anticipated that development of this method with the addition of column chromatography using an ion exchange resin would enable non isotopic imidazole metabolites of histamine to be identified in human urine. Further in Part II the metabolism of C^{14} labelled histamine in man is reported using a method developed from the procedure used in this experiment.

The investigation of imidazole compounds in the urine of patients in status asthmaticus and control subjects

Materials and Methods

In 1952 Ames and Mitchell described the paper chromatography of some imidazoles using diazotised p-sulphanilic acid (i.e. Pauly reagent, Hunter, 1928) as a location reagent. They pointed out that imidazoles substituted at the 1-position in the ring did not give any reaction with diazotised p-sulphanilic acid and that this reagent was more sensitive than ninhydrin for locating histamine and l-histidine on paper chromatograms. Although it was realised that the principal metabolites of histamine were substituted in the 1-position in the ring all the paper chromatograms of urine extracts prepared from asthmatic and control subjects were stained with the Pauly reagent.

Reviewing the catabolism of physiological quantities of histamine in vivo, Schayer (1959) reported that there was no known isotopic procedure for the determination of the principal metabolite of histamine in man, 1-methyl, 4-imidazole acetic acid. On the other hand, Karjala and Turnquest (1955) had synthesised 1-methyl, 4-imidazole

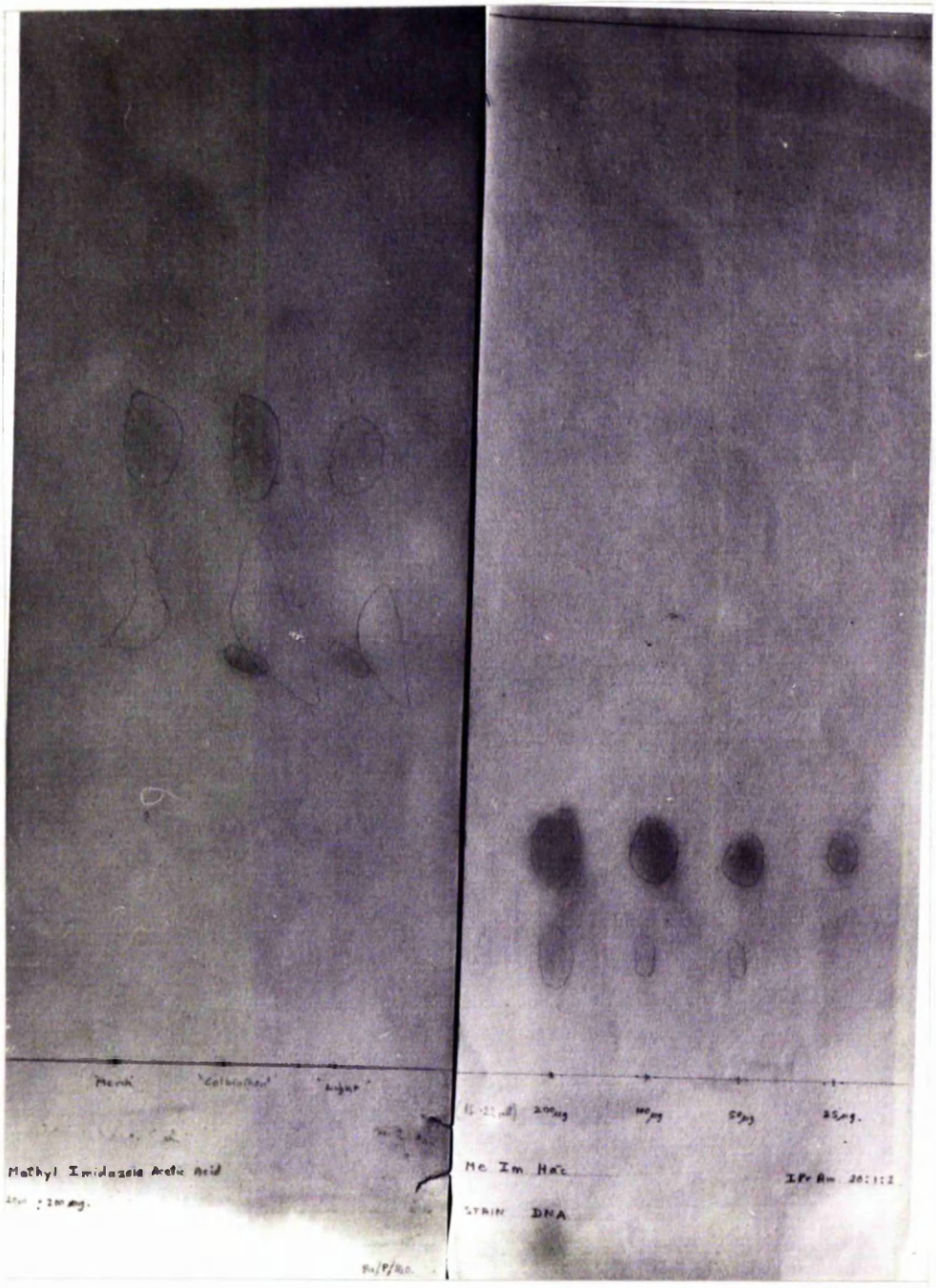
acetic acid and Cowgill (1955) had used iodine to locate imidazoles substituted in the 1-position. More recently McGreer, Robertson and McGreer (1961) had reported that 1-methyl, 4-imidazole acetic acid could be located in paper chromatograms with p-nitroaniline stain.

A gift of synthetic 1-methyl-4-imidazole acetic acid hydrochloride was received from Dr. R. W. Schayer of the Merck Institute, U.S.A. and was used to run one dimensional paper chromatograms in various solvents and test several locating reagents. At a later date 1-methyl, 4-imidazole acetic acid became available commercially, but on chromatography this material was not pure, there being at least two contaminants (Figure 6 A). For the critical experiments to identify 1-methyl, 4-imidazole acetic acid recovered from human urine the pure material supplied by Dr. Schayer was used as a reference compound. Although not in any way a specific stain, p-nitroaniline reagent proved most satisfactory to locate 1-methyl, 4-imidazole acetic acid, giving a red spot. At least 25 µg. of 1-methyl, 4-imidazole acetic acid was required to give a satisfactory spot (Figure 6 B).

Figure 6.

A. 1-methyl, 4-imidazole acetic acid from various commercial sources run in BuP and located with p-nitroaniline stain. The 1-methyl, 4-imidazole acetic acid is the fastest moving material. Rf = 0.53. The material supplied by the Merck Institute is the purest.

B. Various concentrations of 1-methyl, 4-imidazole acetic acid spotted and run in a one dimensional chromatogram in i-Pr.Am. The chromatogram is stained with p-nitroaniline and 25 µg. of 1-methyl, 4-imidazole acetic acid gives a satisfactory spot. Rf = 0.22.



A B

Iodine in ethyl alcohol (Cowgill, 1955) and iodine in carbon tetrachloride (Smith, 1960) proved poor location reagents and both were later found quite unsatisfactory when staining chromatograms prepared from urine extracts. Bromcresol green 0.05% in alcohol had also been used as a location reagent for 1-methyl, 4-imidazole acetic acid (Karjala and Turnquest, 1955) but this too proved quite unsatisfactory in test chromatograms.

In a search for metabolites of C^{14} labelled l-histidine Brown, Silva, McDonald, Synder and Keis (1960) described a method for the elution of urinary imidazoles from a Dowex I x 8 ion exchange resin column. Under the conditions described 1-methyl, 4-imidazole acetic acid should be eluted early from the column followed by imidazole acetic acid, the riboside of imidazole acetic acid and later still urocanic acid. The basic imidazoles - l-histidine, histamine and methyl histamine - were in the wash from this column. This formed the basis of the method used here to prepare the urine extracts from asthmatic subjects for paper chromatography. For the

identification of histamine, location by the Pauly stain on paper chromatograms run in several different solvent systems was considered sufficient. Where new compounds were located by paper chromatography additional chemical procedures for identification have been employed.

Clinical Material

The patients with asthma used in this study were admitted to hospital acutely ill in status asthmaticus. These patients fulfilled the definition of asthma given in the introduction. Each had a history of breathlessness dating from childhood and most had had atopic eczema and a family history of allergic disease. All had positive skin tests to inhalants such as house dust, grass pollens or moulds indicating the presence of reaginic antibodies and an eosinophilia of at least 700 cells/c.mm. Six patients and an equal number of control subjects were used for each phase of the investigations.

During the first 24 hours in hospital drug therapy was kept to a minimum but usually included a sedative such as soluble sodium phenobarbitone (gr. 3 I.M.) and simple antispasmodics such as ephedrine and adrenaline. A 24 hour collection of urine was made and following this each patient received corticotrophin 80 units I.M. in the next 24 hours and a second 24 hour urine collection was made. The two urine collections from each subject one before and one after corticotrophin therapy were prepared for chromatography. (Brief clinical details of each patient are

included in the Appendix, page 173.)

The control subjects were healthy young adults on a free diet and without a history of asthma or allergic disease. A 24 hour collection of urine was made from each and they were not given corticotrophin. In addition a 24 hour urine collection was made from two patients with eosinophilia but no history of bronchial asthma.

Preparation of Column

Approximately 50 g. of Dowex I x 8 ion exchange resin (100-200 mesh) was washed with distilled water and then mixed in a beaker with glacial acetic acid to form a slurry which was left overnight. Enough of the slurry was poured into a burette to give a 40 x 1 cm. column. The column was washed with 10% acetic acid followed by 20 ml. of a 50% solution of saturated sodium acetate and followed by further washings with 10% acetic acid till the wash from the column was free from chloride. Finally the column was eluted with distilled water till the wash from the column had a pH of 5 (Figure 7).

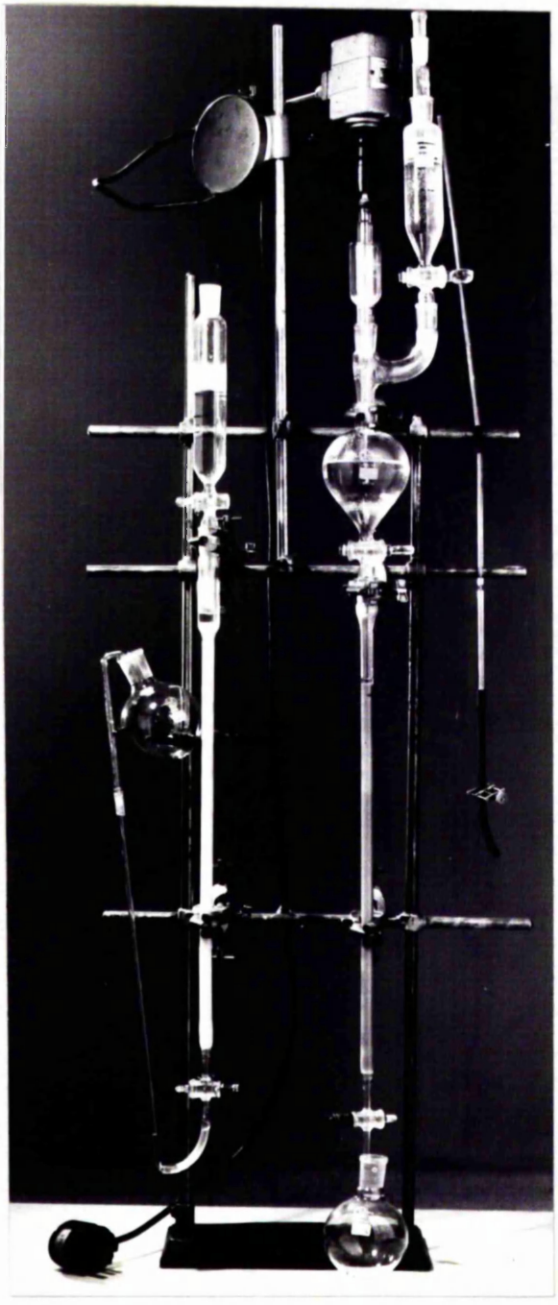


Figure 7. In the left hand column the ion exchange resin is being prepared. The column on the right contains the Dowex I x 8 resin in the acetate form and is prepared for the gradient elution.

Preparation of Urine

The urine collection from each subject was stored at -20°C until required. After filtration 1/3 total volume was evaporated to dryness in vacuo at 45°C. The residue was extracted in 100 ml. boiling methanol for 3 hours using a soxhlet apparatus again evaporated to dryness and the residue dissolved in 10 ml. water (Figure 8). This 10 ml. extract at pH 5 was added to the 40 x 1 cm. Dowex I x 8 acetate column. The column was washed with 40 ml. distilled water and a gradient elution of the column was then carried out. The first elution was with 0.75 N acetic acid dripping into a 100 ml. water reservoir and was collected in 5 aliquots; 1-5 ml.: 6-10 ml.: 11-15 ml.: 16-50 ml. and 51-100 ml. (Figure 7). Each aliquot was evaporated to dryness. The first three aliquots were taken up in 1 ml. water and the last two in 2 ml. water. The second elution was with 2N acetic acid dripping into a 100 ml. reservoir of water and was collected as one aliquot. After a preliminary ether extraction each aliquot was evaporated to dryness and taken up in 2 ml. water. The

final elution was with 25 ml. of 6 N acetic acid the eluant being evaporated to dryness and finally dissolved in 2 ml. water. The initial water wash from the column was evaporated to dryness and taken up in 4 ml. water. The eluants and wash were used to spot paper chromatograms and were stored at -20°C until required. In some early investigations the aliquots were pooled for paper chromatography.

Paper Chromatography

Ascending chromatograms were run on Whatman No. 4 paper in four solvents:-

1. Butanol/acetic acid glacial/water in the ratio 12:3:5 (BuA)
2. Butanol/Pyridine/water in the ratio 1:1:1 (BuP)
3. iso-propanol/ammonia 880/water in the ratio 20:1:2 (i-Pr.Am.)
4. Propanol -0.2N/NH_4OH in the ratio 3:1 (Pr.Am.)
5. iso-propanol/Pyridine/water in the ratio 3:2:1 (Pr.Py.)

Paper Chromatograms of Eluants from Column

Paper chromatograms were prepared spotting 40 µl. of

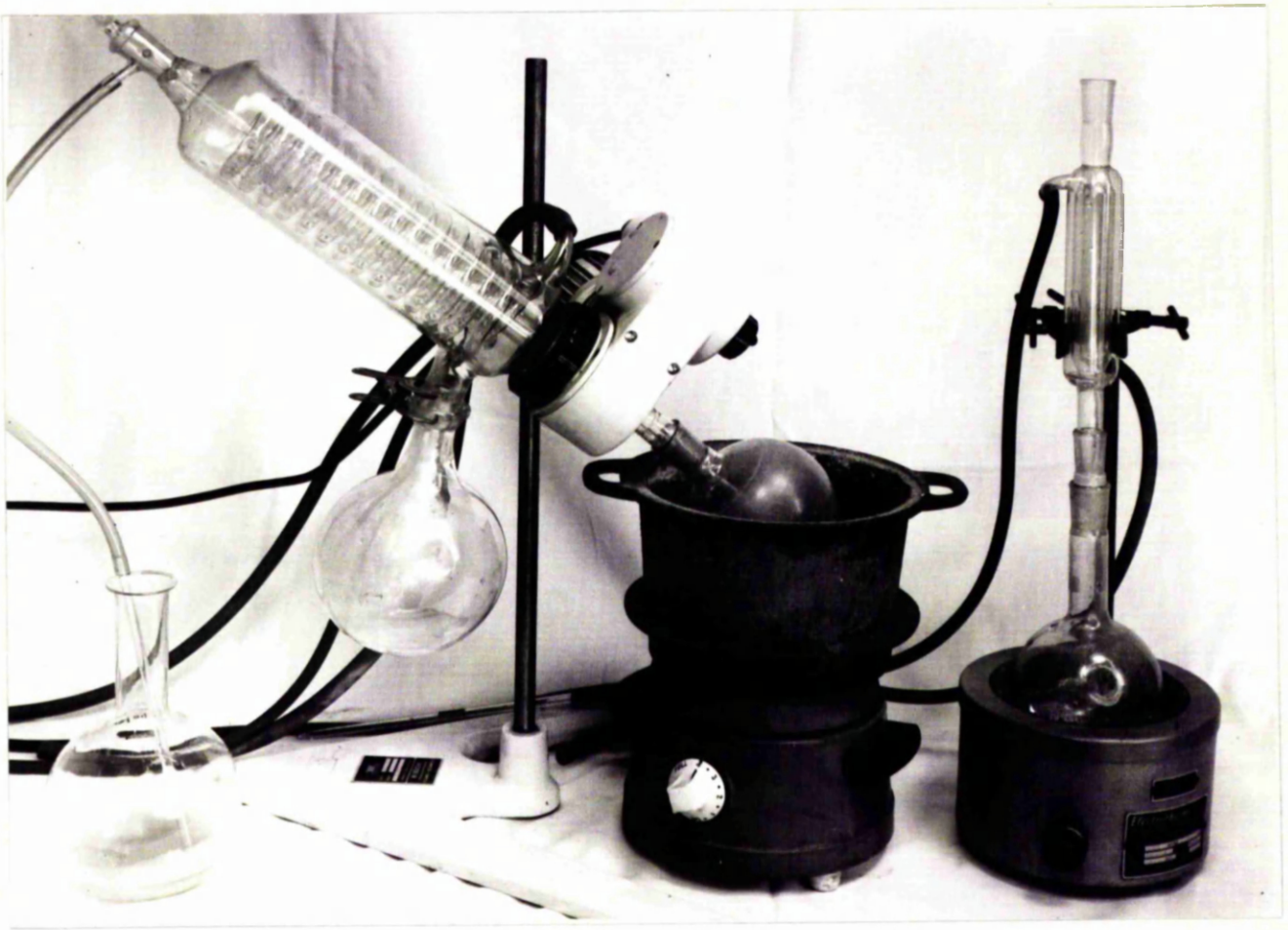


Figure 8. The rotary evaporator and soxhlet apparatus used in the preparation of the urine extracts for the ion exchange column.

each 1 or 2 ml. final preparation from the eluant of the column and the solvent flow was carried out overnight with solvents 1, 3, 4 and 5, a six hour solvent run being sufficient with solvent 2. Control papers were run spotting either 5 µg. urocanic acid in 20 µl. or 200 µg. in 20 µl. of 1-methyl, 4-imidazole acetic acid. In addition papers were prepared spotting 20 µl. of the unknown urine extract together with either 5 µl. urocanic acid or 10 µl. 1-methyl, 4-imidazole acetic acid applied as one spot.

Paper Chromatograms of Wash from Column

Using the 4 ml. final preparation from the wash of each column paper chromatograms were prepared spotting 40 µl. The solvent flow was carried out overnight with solvents 1 and 3; again a six hour solvent flow being sufficient with solvent 2. Control papers were prepared spotting 5 µl. (5 µg.) of l-histidine or histamine as required. Control papers were also run spotting 5 µl. histamine and 20 µl. wash applied as one spot.

Location Reagents

The paper chromatograms were dried and stained either

by dipping in diazotised sulphanilic acid stain (Smith, 1960) or for the location of 1-methyl, 4-imidazole acetic acid dipped in p-nitroaniline stain (Modification of Smith, 1960).

Sulphanilic Acid (Pauly) Reagent

A Sulphanilic acid, 9 g. in 90 ml. concentrated HCL made up to 900 ml. with water

B Sodium nitrate, 5 per cent w/v in water

C Sodium carbonate anhydrous, 10% in water

When required equal volumes of stock solutions of reagents A and B are mixed and cooled 4 or 5 minutes on ice. Two volumes of solution C is added carefully as the mixture effervesces vigorously. The dried chromatogram is then dipped rapidly through the solution and laid flat on a clean white paper.

Nitroaniline Reagent

A	p-nitroaniline 0.3% in 8% w/v HCL	50 ml.
B	Sodium nitrate 5% in water	3 ml.
C	Sodium carbonate anhydrous 10% w/v in water	50 ml.

When required solutions A and B are freshly prepared each time, cooled on ice and mixed. Solution C is added

slowly. The dried paper chromatogram is then dipped rapidly through the solution and laid flat on a clean white paper.

Identification by Ultra Violet Absorption Spectra

1. Urocanic Acid

Calculation of the amount of urocanic acid required to give a satisfactory optical density.

This is based on the Beer-Lambert law of light absorption which can be expressed as

$$\text{Optical density} = \varepsilon \text{ cl.}$$

Where ε is the absorption coefficient; c is the concentration and l is the path length. When c is expressed in gram moles per litre and l in cm. then ε becomes the molar extinction coefficient. OD = optical density and MW = molecular weight.

$$\text{Therefore } \varepsilon = \frac{\text{MW x OD}}{\text{c in mg/ml x cell path.}}$$

The molecular weight of urocanic acid is 174 and at pH 4 the absorption maximum is 277 mμ and ε = 18,800 (Organic Electronic Spectral Data Vol I and II) therefore for a 1 cm. light path and assumed OD = 0.6 at 277 mμ.

$$\varepsilon = 18{,}800 = \frac{174 \times 0.6}{c \times 1}$$

$$c = \frac{104}{18{,}800} \text{ mg/ml}$$

$$= \text{ca } 6 \text{ μg./ml.}$$

To allow for loss in chromatography and dilution after recovery 60 µg. urocanic acid was applied to the control paper chromatograms.

Two dimensional paper chromatograms were run in duplicate spotting 80 µl. of urine extract and two similar papers were spotted with 60 µg. urocanic acid. One urine extract and one urocanic acid paper was stained (Pauly reagent) and used to locate the unknown and urocanic acid respectively in the unstained papers. These areas were marked, cut out and eluted overnight with 90% Ethanol. The Ethanol was evaporated to dryness and the residue made up to 5 ml. with water. The ultra violet absorption spectra of the dissolved material from both papers was measured in a Unicam SP 500 spectrophotometer.

Identification by Ultra Violet Spectra

2. 1-methyl, 4-imidazole acetic acid

Calculation of the amount of 1-methyl, 4-imidazole acetic acid required to give a satisfactory optical density. The molecular coefficient for 1-methyl, 4-imidazole acetic acid was not known but was assumed to be log ε = ca 3.67. The molecular coefficient for N methyl imidazole is reported as log ε = 3.10 in ethanol absorption maxima = 270 mμ and for 4-methyl imidazole log ε = 3.67 in ethanol absorption maxima = 211 mμ. (Organic Electronic Spectral Data Vol. III and IV). The molecular weight of 1-methyl, 4-imidazole acetic acid hydrochloride = 176.5.

ε = log 3.67 = 4677: assuming OD = 0.60

$$\varepsilon = 4677 = \frac{176.5 \times 0.6}{c \times 1}$$

$$c = \frac{176.5 \times 0.6}{4677}$$

$$c = \text{ca } 22\ \mu\text{g./ml.}$$

To allow for loss in paper chromatography and dilution after recovery 200 μg. of 1-methyl, 4-imidazole acetic acid

was applied to the control chromatograms.

Two ascending chromatograms were run each with three spots side by side. Each spot in the first paper was 200 µg. of 1-methyl, 4-imidazole acetic acid and in the second paper each spot was 40 µl. of the first aliquot of the eluant from the Dowex I x 8 acetate column. This eluant was from the urine of a control subject containing the unknown substance as shown by paper chromatography. The papers were run in BuP overnight and each paper cut into 3 strips with one spot on each. The side strips were stained with p-nitroaniline stain and used to locate the unknown and 1-methyl, 4-imidazole acetic acid respectively on the unstained strips. These areas were marked, cut out and eluted with 90% ethanol together with a blank paper. The volumes were made up to 25 ml. the pH adjusted to 5 and the ultra violet absorption spectra of the material from both papers recorded against the blank eluant in a Unicam SP 500 spectrophotometer.

Identification of 1-methyl, 4-imidazole acetic acid extracted from urine by Infra-red spectrophotometry

After an overnight fast, a healthy control subject was given 60 mgm. histamine diphosphate by intragastric tube and a urine collection was made for 24 hours. One half of this 24 hour specimen was evaporated to dryness and prepared as before for addition to a Dowex I x 8 acetate column. Paper chromatography showed that large quantities of the suspected 1-methyl, 4-imidazole acetic acid was present in the first two aliquots of the gradient elution with 0.75 N acetic acid. This fraction was put in a Craig tube and on the addition of acetone, crystals were formed. The supernatant was removed by centrifugation and the crystals recrystalised from acetone-water. These crystals were dissolved in a few drops of water and a saturated ethanol solution of picric acid was added. Yellow feather like needles were obtained. The picrate was recrystallised from boiling water and on cooling long needle like crystals were obtained.

Since the urine extract thus prepared would not be in the hydrochloride form the synthetic 1-methyl, 4-imidazole acetic acid hydrochloride was desalted before preparing the

picrate. A Dowex I x 8 acetate column 30 x 0.75 cm. was prepared (see page 46) and 20 mgm. 1-methyl, 4-imidazole acetic acid hydrochloride in 2 ml. water was added to the column. The column was washed with 9 ml. distilled water. A gradient elution was set up, 100 ml. 0.75 N acetic acid dripping through a 200 ml. reservoir of distilled water. The fractions were collected in 1 ml. aliquots for 15 mls. and thereafter 15-60 and 61-100 mls. The column was monitored by paper chromatograms and the bulk of the 1-methyl, 4-imidazole acetic acid came out in the first 15 ml. This fraction was evaporated to dryness and dissolved in a small quantity of ethanol; diethyl ether was added till a turbidity appeared. The turbidity was cleared by adding ethanol and crystallisation allowed to take place. This was followed by recrystallisation from acetone - water and the picrate was formed as above.

The infra-red spectra of the unknown picrate and the picrate of 1-methyl, 4-imidazole acetic acid were obtained in KCl discs (about 0.5 mgm. of picrate crystals to 25 mgm. KCl). The spectra were determined using a Perkin-Elmer Infra-red spectrophotometer (Model 237).

Identification of imidazole acetic acid and 1-ribosyl imidazole acetic acid

Both these compounds are eluted in the 0.75 N eluate from the Dowex I x 8 acetate column. Imidazole acetic acid is easily located in paper chromatograms by the Pauly stain but acid hydrolysis of the eluant is required to split off the riboside from 1-ribosyl imidazole acetic acid before it can be located on paper chromatograms with the Pauly stain.

A small quantity of the 0.75 N acetic acid eluant (up to 0.5 ml.) was sealed in a 2 ml. ampoule with an equal volume of 2 N HCl. The ampoules were placed in a sand bath and autoclaved at 145-150°C for 5 hours. Paper chromatograms were prepared with 200 µl. spots of the autoclaved eluants and control papers were run spotting 20 µl. of imidazole acetic acid and also of the eluants before hydrolysis. The paper chromatograms were run in i-Pr.Am. and BuA and stained with the Pauly stain. The ribose was located with analine phosphate stain (Hough, Jones and Wodman, 1950).

Results

Imidazoles in the Eluant from the Dowex I x 8 Column

Identification of Urocanic Acid

Paper chromatograms were prepared from the urine extracts of six patients admitted in status asthmaticus, and were stained by dipping in the Pauly stain. The chromatograms prepared from the 0.75 N acetic acid eluant showed a spot which stained brick red with this location reagent (Figure 9). The maximum recovery was in the chromatograms prepared from the first half of the elution with 0.75 N acetic acid.

The Rf of this spot for patient J.N. in BuA = 0.60 in BuP = 0.53 and in i-Pr.Am. = 0.13 and identical Rf's were obtained from urocanic acid run on control papers in the same solvents (Figure 10). Table 3 shows the Rf of this spot for each patient in the three solvents together with values for urocanic acid run in control papers at the same time. In addition, when two dimensional chromatograms were run with the unknown substance in the urinary extract and the urocanic acid applied to the same origin on the paper only one Pauly positive spot appeared (Figure 11).

The ultraviolet absorption of the unknown compound was identical with that of urocanic acid, the absorption maximum being 263 mμ (Figure 12).

In the six normal control subjects no urocanic acid could be identified in similarly prepared chromatograms (Figure 13). During the second 24 hours in hospital the six patients in status asthmaticus received 80 units of corticotrophin and a further 24 hour urine collection was made. Paper chromatograms were prepared from the extracts of these urine collections and no urocanic acid was identified (Figure 14), (Kerr, 1963).

Paper chromatograms showing the presence of urocanic acid in the six asthmatic subjects are in the appendix page 149 to 156, (Figures 32-37).

Based on the Beer-Lambert equation the 24 hour output of urocanic acid in the urine of subject 1 (J.N.) was calculated to be 1.01 mgm. urocanic acid in 24 hours (see appendix page 170).

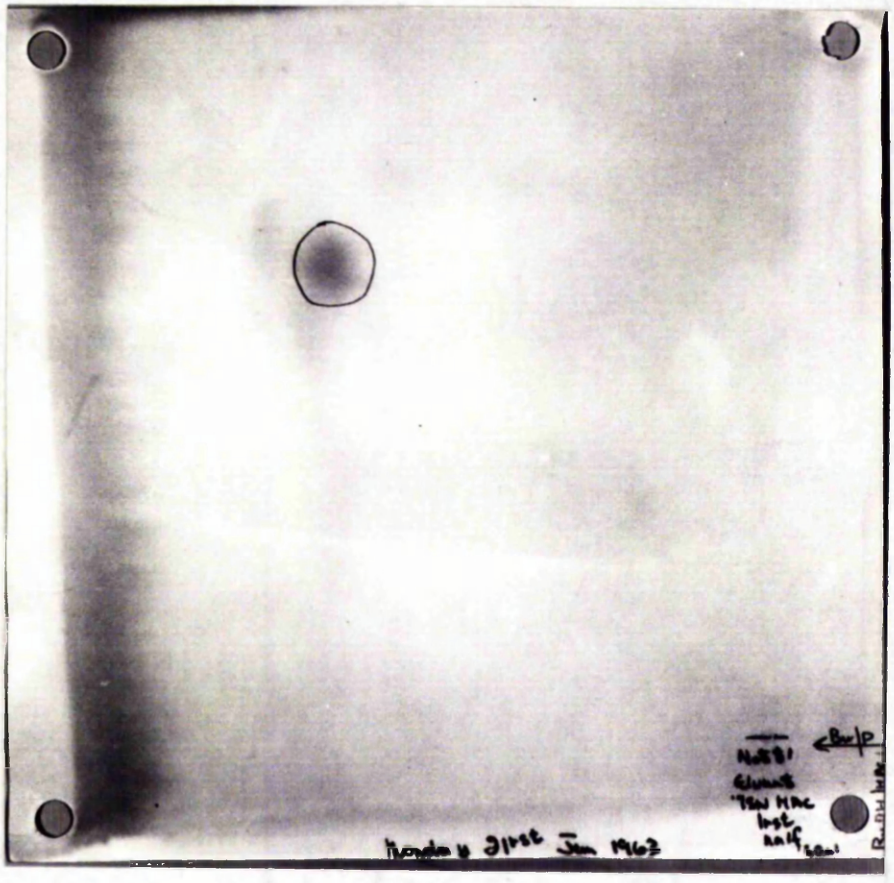


Figure 9. A two dimensional paper chromatogram run in Bu.A and Bu.P to show the brick red spot located by the Pauly stain in the urine extract of subject 1, (J.N.) admitted in status asthmaticus. The Rf for this spot in patient J.N. in Bu.P = 0.53 in Bu.A = 0.60

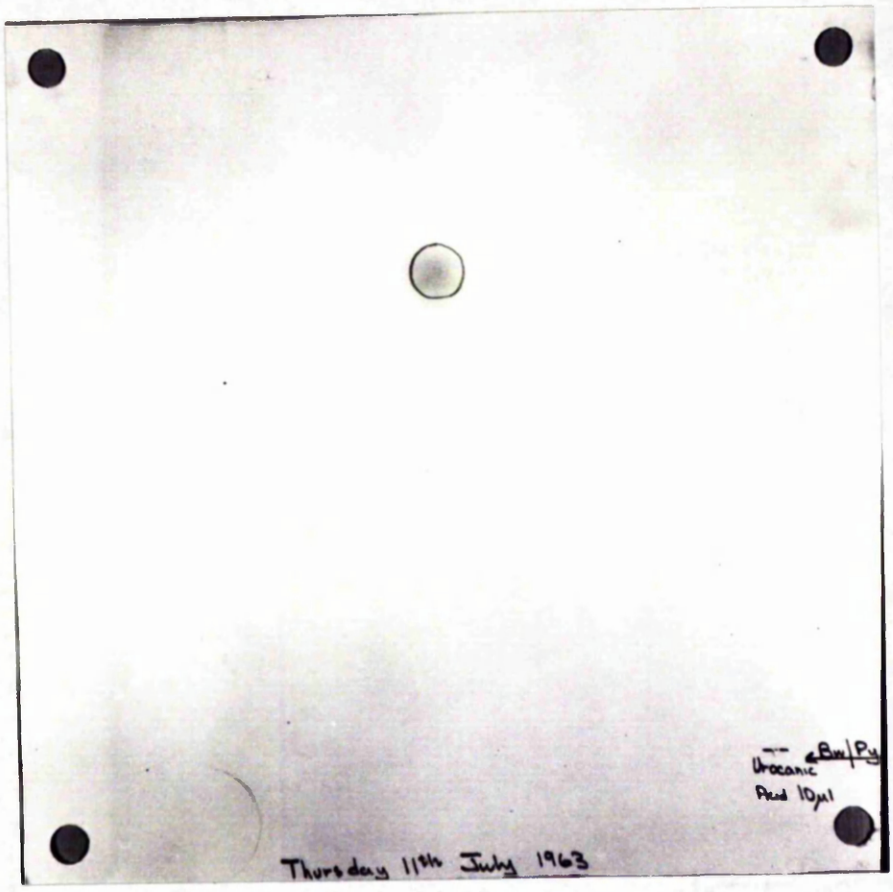


Figure 10. A two dimensional paper chromatogram run in Bu.A and Bu.P to show the brick red spot given by urocanic acid. The Rf of this spot in Bu.A = 0.60, in Bu.P = 0.53.

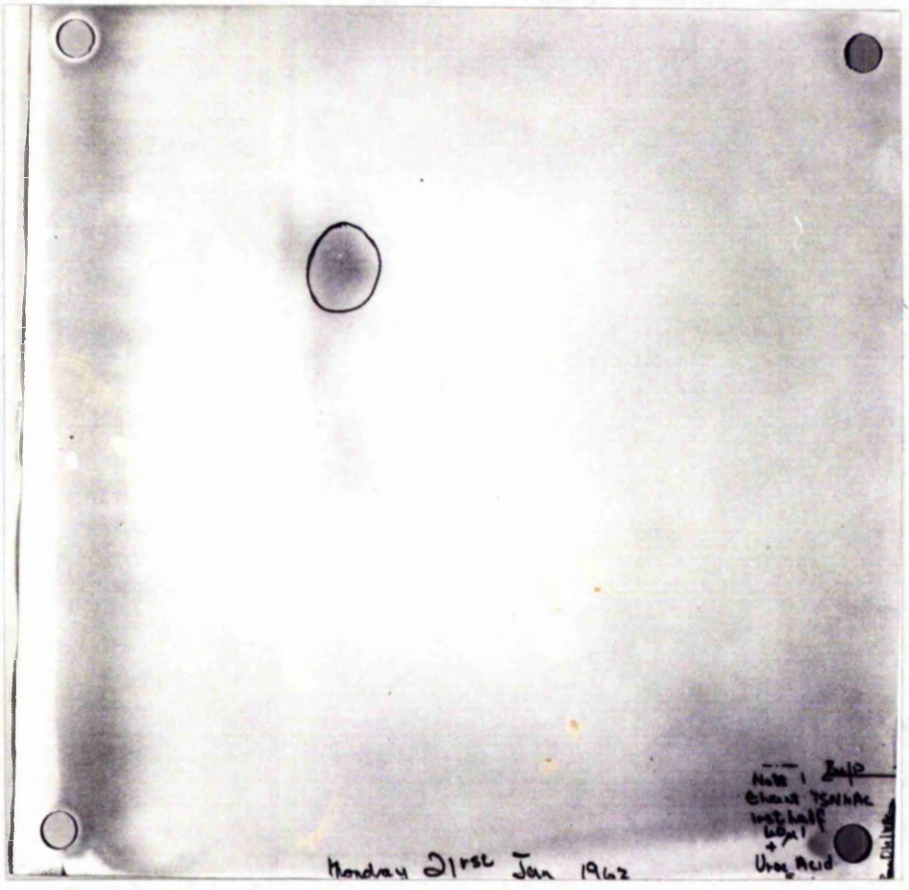
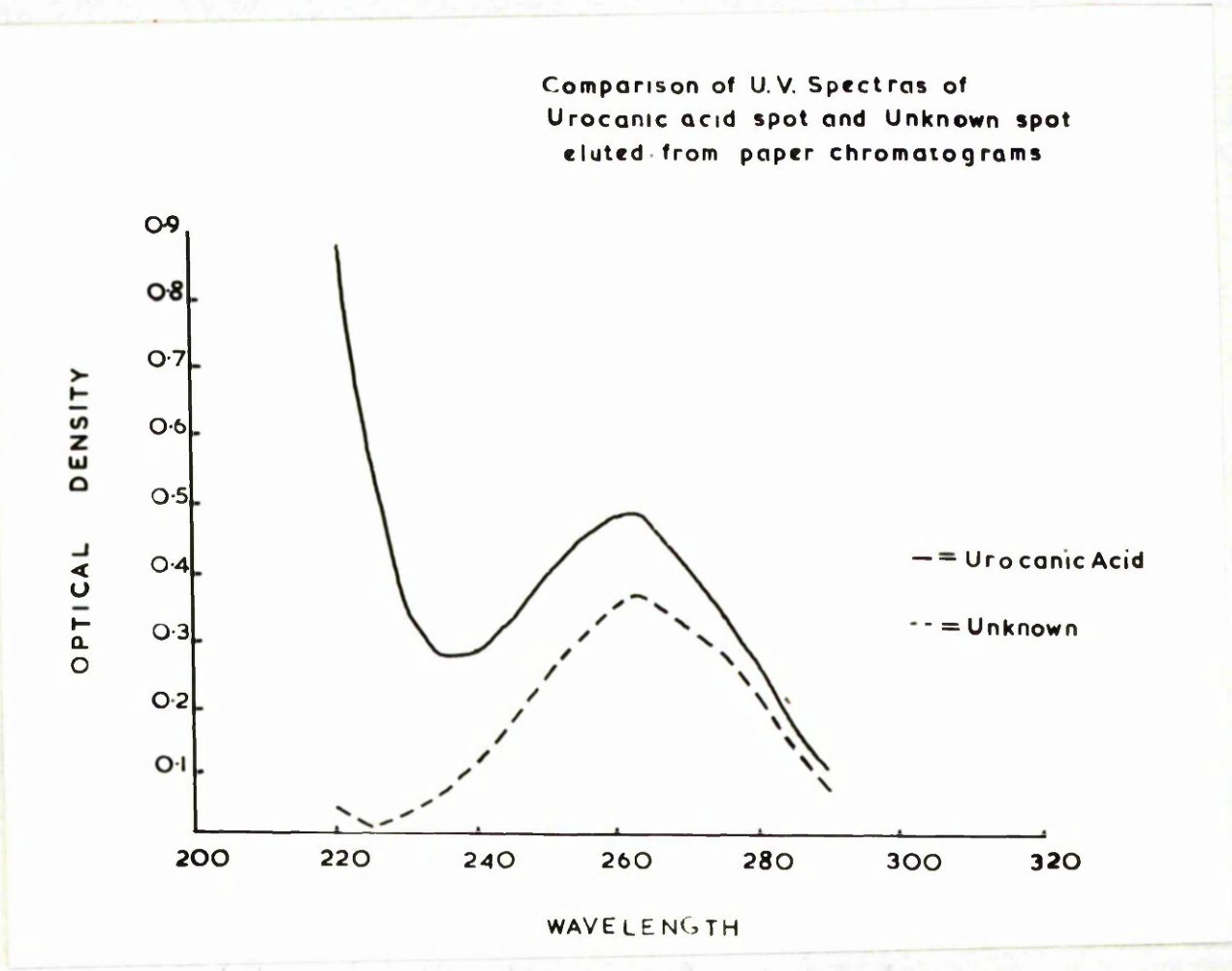


Figure 11. A two dimensional paper chromatogram run in Bu.A and Bu.P with the urine extract of subject 1 containing the unknown substance and urocanic acid applied to the same origin. The chromatogram is stained with the Pauly stain and only one brick red spot appears, i.e. the unknown substance and urocanic acid run as one spot.

TABLE III

The Rf's of the unknown Pauly positive spot in paper chromatograms of the eluants (0.75 N acetic acid) prepared from the urine of six patients in status asthmaticus and compared to the Rf's of urocanic acid run at the same time in control papers.

	Solvent		
Subject	BuA	BuP	i-Pr.Am
J.N.	0.60	0.53	0.13
Urocanic acid	0.60	0.53	0.13
I.P.	0.55	0.63	0.13
Urocanic acid	0.55	0.64	0.11
M.G.	0.57	0.54	0.13
Urocanic acid	0.57	0.54	0.13
D.T.	0.65	0.56	0.12
Urocanic acid	0.65	0.56	0.11
E. McF	0.69	0.62	0.13
Urocanic acid	0.69	0.62	0.12
H.T.	0.58	0.55	0.13
Urocanic acid	0.58	0.55	0.12



Wavelength (mμ)

Figure 12. Comparison of the ultra violet spectra of urocanic acid and the unknown spot eluted from paper chromatograms. The absorption maxima of the urocanic acid and unknown were identical at 263 mμ.

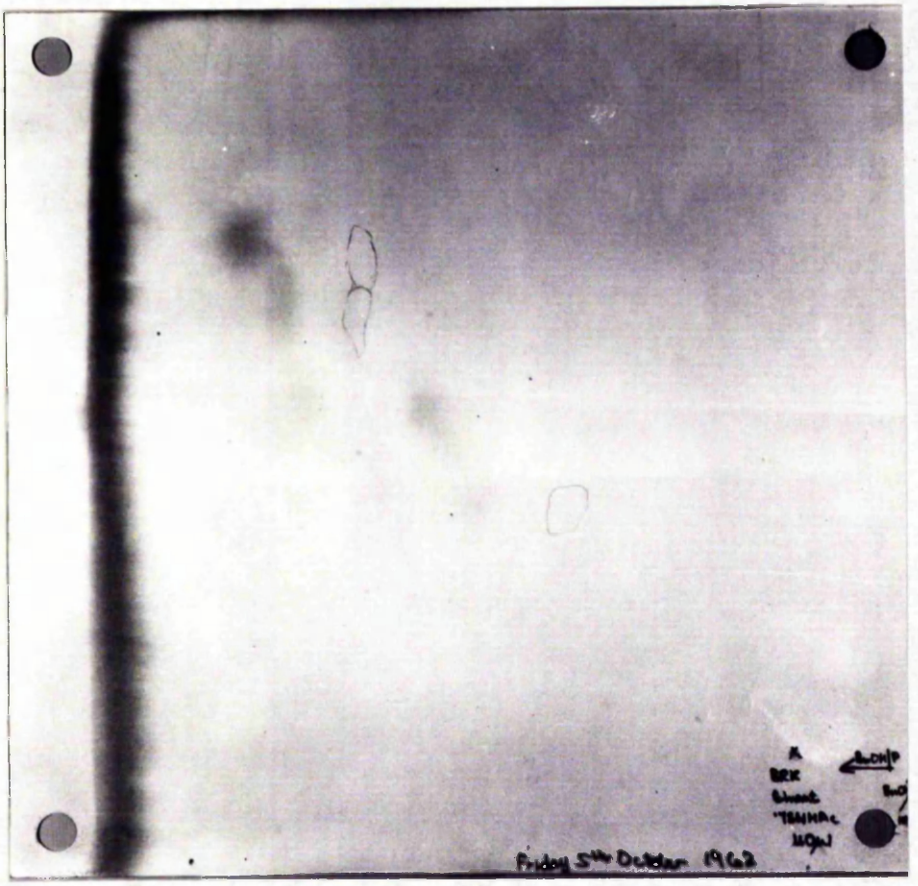


Figure 13. A two dimensional paper chromatogram run in Bu.A and Bu.P. The urine extract from a control subject was applied at the origin and after development the chromatogram was stained with the Pauly stain. There is no evidence of urocanic acid.

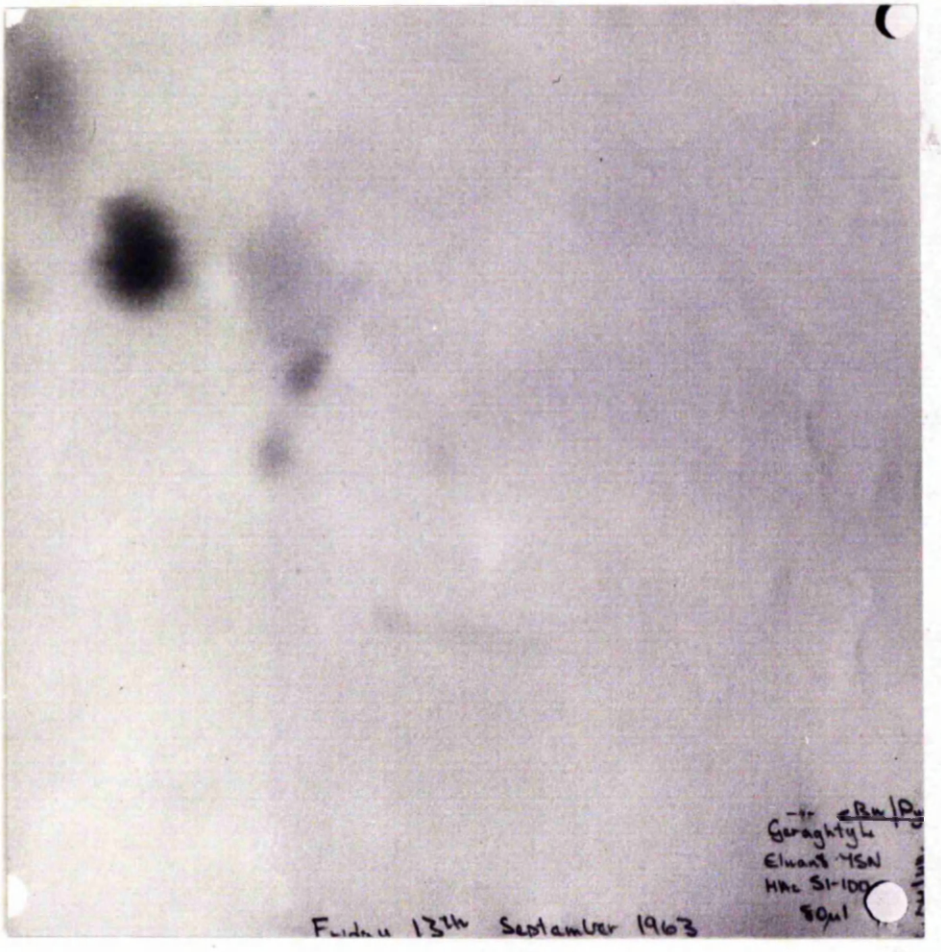


Figure 14. A two dimensional paper chromatogram run in BuA and BuP to show the absence of urocanic acid in the urine extract prepared after the patient (subject 3) had received 80 units of corticotrophin.

The paper chromatogram showing the presence of urocanic acid in this asthmatic patient before corticotrophin therapy is shown in Figure 34, page 152.

Identification of 1-methyl, 4-imidazole acetic acid

The paper chromatograms prepared from the first 5 ml. aliquot of eluant (0.75 N acetic acid elution) of the six control subjects and the two patients with eosinophilia but no history of asthma, showed a red spot when stained with p-nitroaniline location reagent (Figure 15 and 16). The Rf of this spot for control subject S.V. in BuA = 0.33, in BuP = 0.53 and in i-Pr.Am. = 0.25. Identical staining and Rf's were obtained with 1-methyl, 4-imidazole acetic acid run in control papers in the same solvents (Figure 17).

Table 4 shows the Rf's of this spot for each control subject and in the two patients with eosinophilia, together with the Rf's for 1-methyl, 4-imidazole acetic acid run in control papers.

In addition when paper chromatograms were run with the urinary extract and 1-methyl, 4-imidazole acetic acid applied as one spot on the paper, the unknown and 1-methyl, 4-imidazole acetic acid ran as one p-nitroaniline staining spot (Figure 40 (3)). Only one control subject did not appear to have

TABLE IV

The Rf's of the unknown spot located with p-nitro-aniline stain on the paper chromatograms of the eluants (0.75 N acetic acid) prepared from the urine of six control subjects and compared to the Rf's of 1-methyl, 4-imidazole acetic acid run in control papers.

	Solvent		
Subject	BuA	BuP	i-Pr.Am
M.W.	.31	.50	.24
Standard	.33	.50	.25
T.B.	.33	.54	.27
Standard	.32	.53	.27
S.V.	.33	.53	.25
Standard	.30	.53	.25
R.R.	.38	.53	.27
Standard	.38	.53	.27
D.M.	.35	.50	.25
Standard	.35	.50	.25
B.K.	Tr.	.51	Tr.
Standard	.33	.57	.29
Patients with Eosinophilia			
S.U.	.33	.53	.25
I.L.	.34	.50	.25

Standard = 1-methyl, 4-imidazole acetic acid.

Tr. = trace

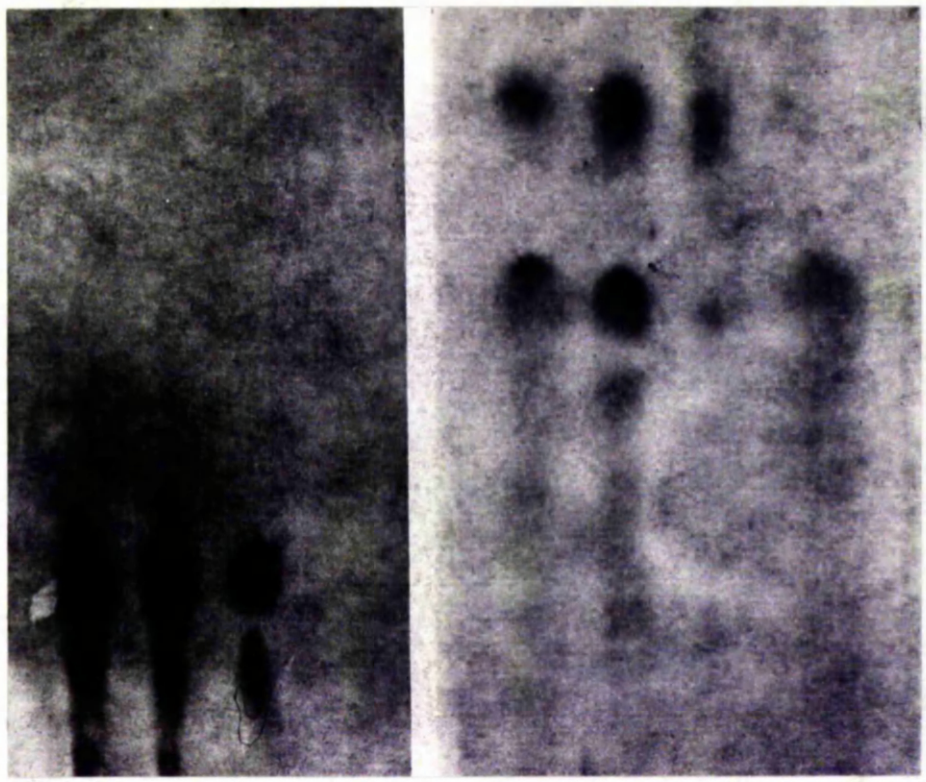
any of the unknown substance in the 24 hour urine collection. The two patients with eosinophilia (but no asthma) appeared to have at least twice the output of 1-methyl, 4-imidazole acetic acid as compared to the control subjects (Figure 16).

The ultra violet absorption of the unknown compound was identical with that of 1-methyl, 4-imidazole acetic acid, the absorption maximum being 211 mμ. (Figure 18).

The infra red spectrophotometry of the picrate of the unknown substance in the urine of a control subject and the picrate derivative of synthetic 1-methyl, 4-imidazole acetic acid were similar and are shown in Figure 19.

Gas chromatography of the authentic methyl ester of 1-methyl, 4-imidazole acetate and the methyl ester of the unknown urinary material was carried out by Dr. Charles Brooks of the Chemistry Department. The retention time of the authentic N-methyl, imidazole acetate was 7.6 min. and the retention time of the unknown methyl ester was 7.5 min. (Figure 20).

In the six patients admitted in status asthmaticus no 1-methyl, 4-imidazole acetic acid could be identified in the first 24 hour urine collection. Paper chromatograms were



1-methyl, 4-imidazole acetic acid

1 2 3 4 5 6

Figure 15. Two one dimensional paper chromatograms developed in i-Pr.Am. and stained with p-nitroaniline reagent.

1 and 2 are eluants from the urine extracts of a control subject and 1-methyl, 4-imidazole acetic acid is present in both fractions. Rf = .24.

3 is a control chromatogram of synthetic 1-methyl, 4-imidazole acetic acid. Rf = .24.

4, 5 and 6 are eluants from the urine extracts of asthmatic subject 5 before treatment. No 1-methyl, 4-imidazole acetic acid can be identified in these fractions.

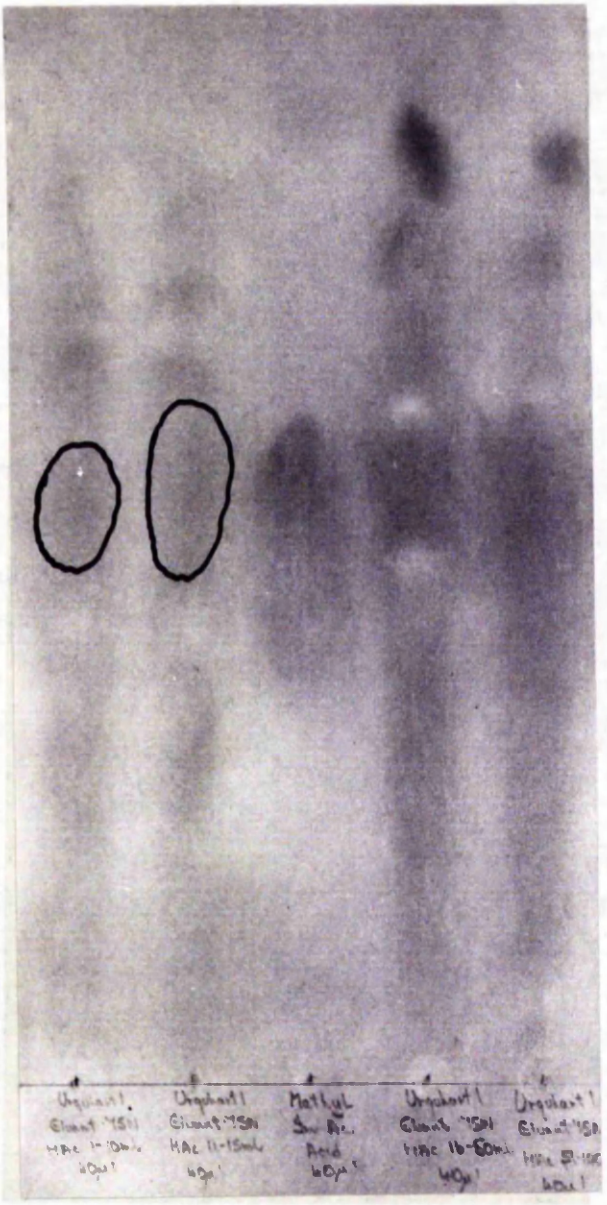


Figure 16. A one dimensional chromatogram spotted with the eluants from the urine extracts of control subject Urquhart (with eosinophilia) run in BuP and located with p-nitroaniline reagent. Considerable quantities of 1-methyl, 4-imidazole acetic acid are present in the 4 fractions shown. Rf = .53.

Figure 17. A two dimensional paper chromatogram run in Pr.Am. and BuP. 1-methyl, 4-imidazole acetic acid was spotted at the origin and after development the chromatogram was stained with p-nitroaniline reagent to locate the 1-methyl, 4-imidazole acetic acid. The Rf in BuA = 0.35 and in Pr.Am. = 0.25.

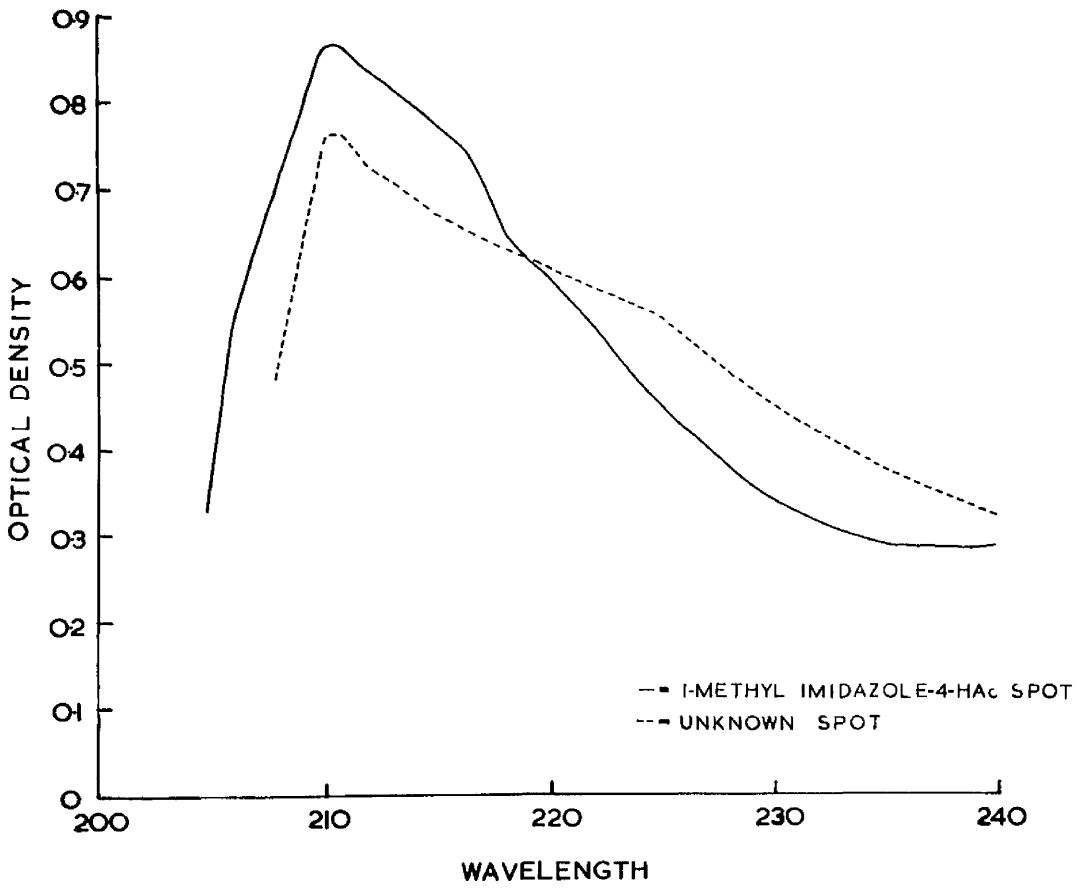


Figure 16. Comparison of the ultra violet spectra of 1-methyl, 4-imidazole acetic acid and the unknown spot eluted from paper chromatogram of a non-asthmatic control subject. The absorption maximum of the unknown compound is identical with that of 1-methyl, 4-imidazole acetic acid being 211 mμ.

prepared in an identical way from the second 24 hour urine collection whilst the patient was having corticotrophin therapy. In these chromatograms 1-methyl, 4-imidazole acetic acid could be identified (Figures 21 and 22). The paper chromatograms of the 6 asthmatic patients before and after corticotrophin are in the Appendix, page 158 to 169, (Figure 38 to 43).

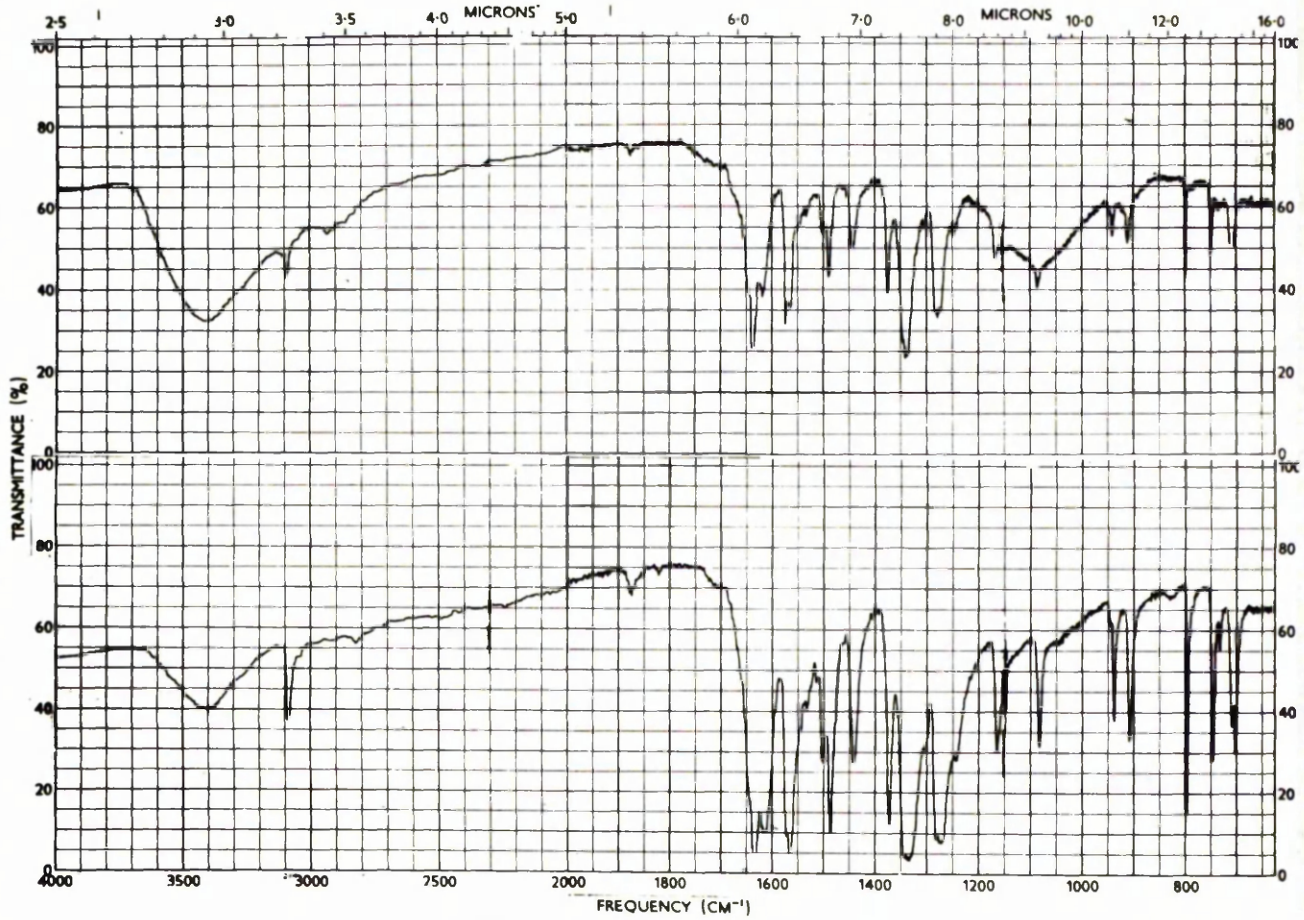


Figure 19. The infra-red spectrophotometry of the picrate of the unknown substance in the urine of a control subject (A) and the picrate derivative of synthetic 1-methyl, 4-imidazole acetic acid (B). Both have identical peaks.

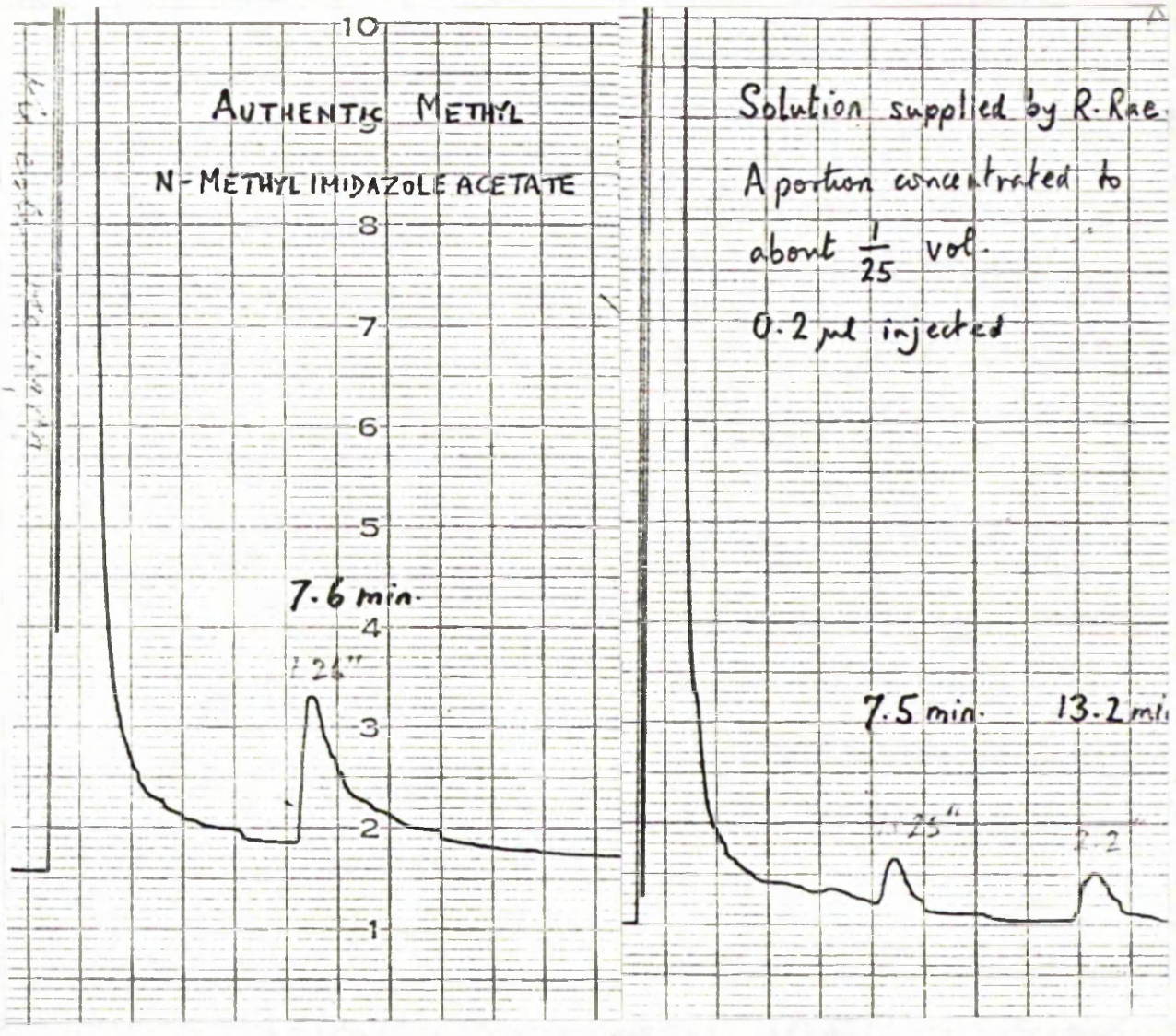


Figure 20. The gas chromatograph of authentic N-methyl imidazole acetate and the N-methyl ester of the unknown urinary material.

The retention time of the authentic N-methyl imidazole acetate is 7.6 minutes and of the unknown methyl ester 7.5 minutes. (Carried out by Dr. Brooks)

Figure 21. Asthmatic subject 9.

Two one dimensional paper chromatograms of this patient before and after corticotrophin therapy. The chromatograms were run in i-Pr.Am and stained with p-nitroaniline reagent. 1, 2 and 3 are eluant fractions of the urine extract before treatment and show no evidence of 1-methyl, 4-imidazole acetic acid. 5, 6 and 7 are eluant fractions of the urine extract after corticotrophin treatment. In 6 and 7 1-methyl, 4-imidazole acetic acid is present. 4 and 8 are control spots with 1-methyl, 4-imidazole acetic acid.

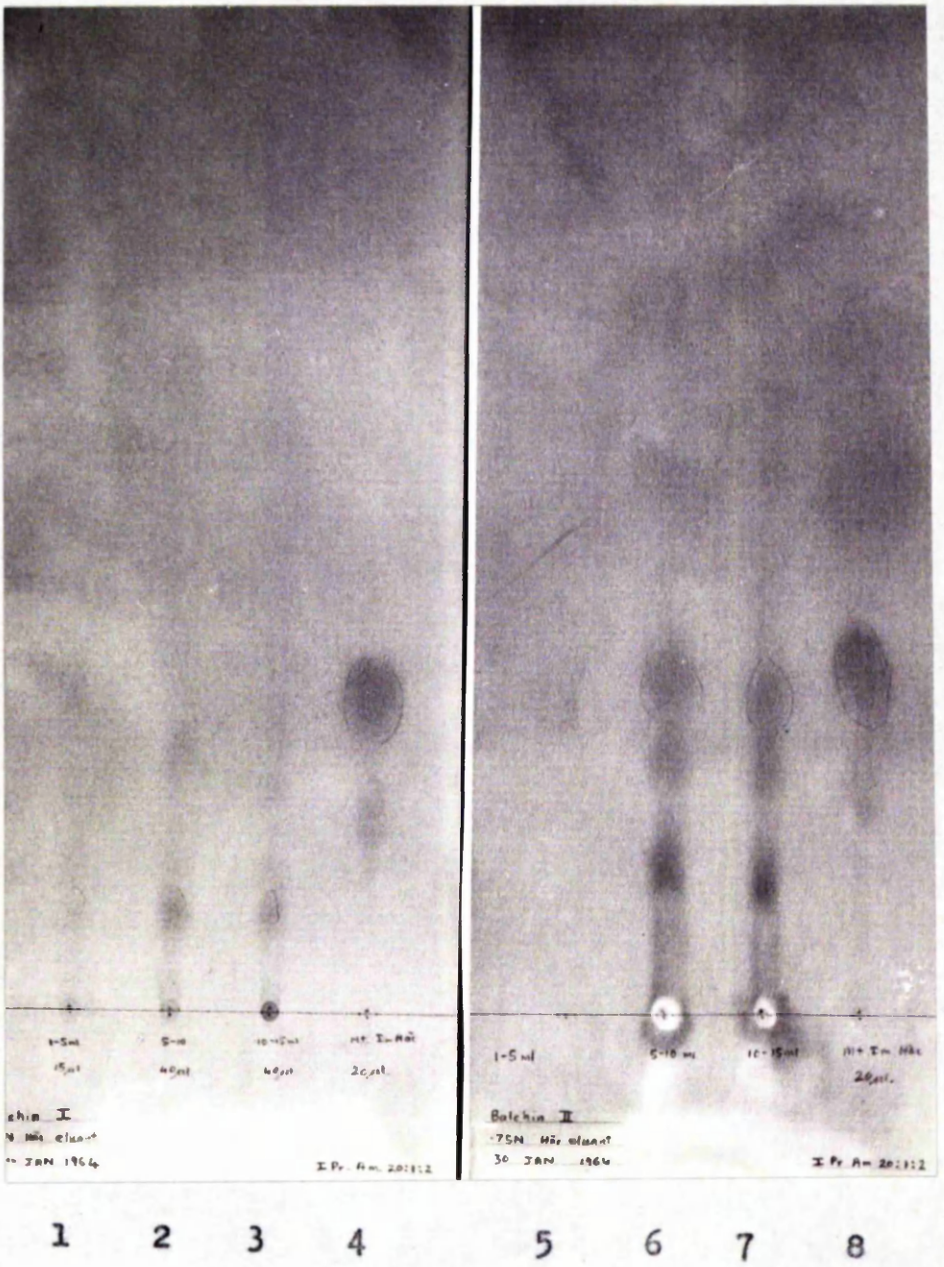


Figure 21. Asthmatic subject 9.

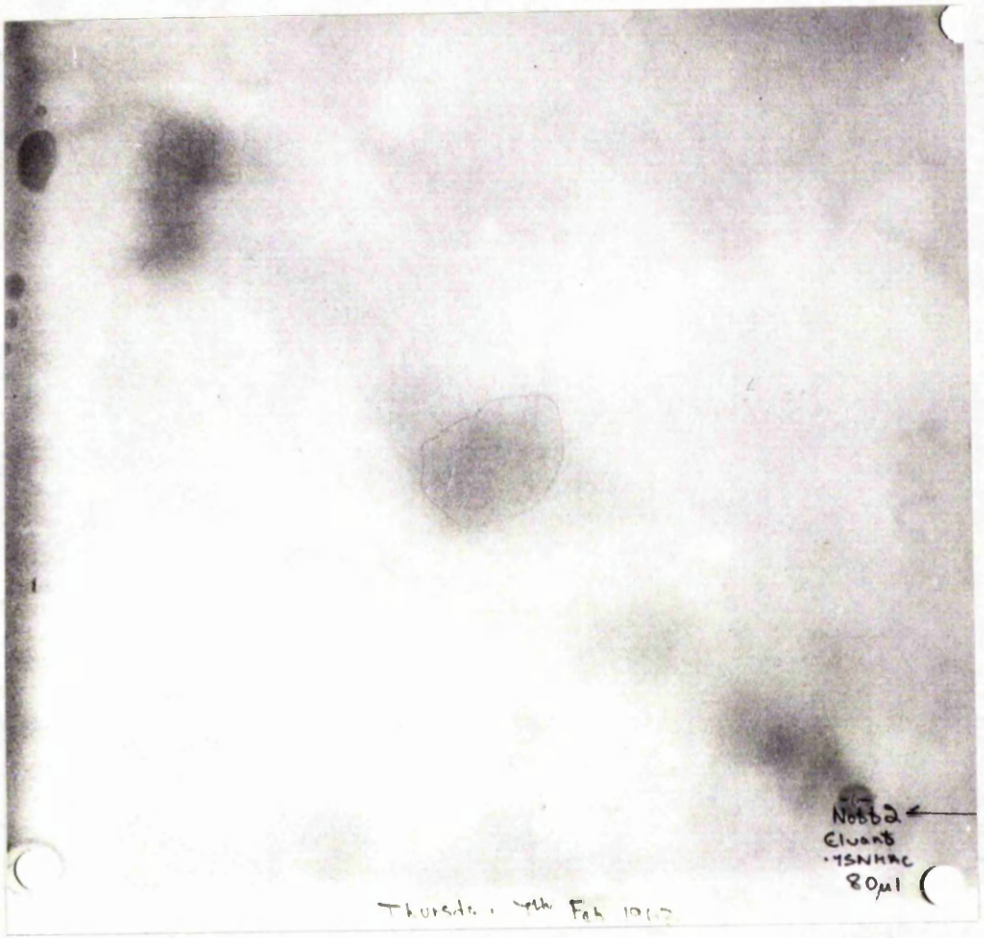


Figure 22. Asthmatic subject 1.

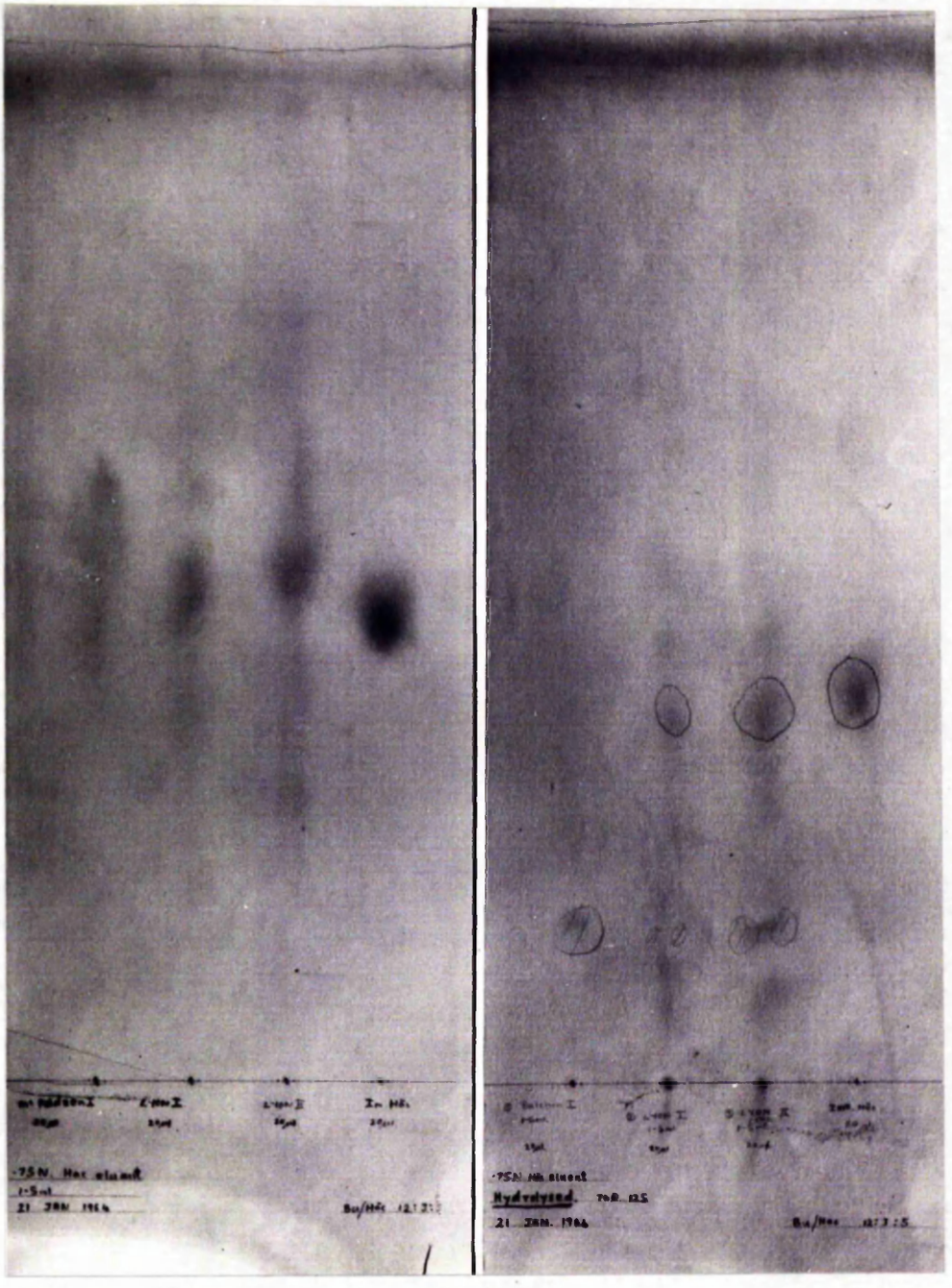
A two dimensional paper chromatogram run in BuA and BuP spotting the eluant prepared from the second urine collection after corticotrophin. After development the chromatogram is stained with p-nitroaniline reagent to locate 1-methyl, 4-imidazole acetic acid which is marked with a circle. Pre-treatment paper chromatograms of eluant from urine extract is shown in Figure 38 A.

Identification of imidazole acetic acid

1-ribosyl imidazole acetic acid

The paper chromatogram prepared from the 0.75 N acetic acid eluants of the Dowex I x 8 before and after acid hydrolysis and stained with the Pauly stain did not show the presence of imidazole acetic acid in any of the asthmatic patients. The control subjects were the same with one exception. This was patient Lyon who was admitted to hospital with a megaloblastic anaemia of pregnancy due to folic acid deficiency. The eluant of the urine sample from this patient after acid hydrolysis revealed a red spot with the Pauly stain. The Rf's of this unknown material and the Rf's of imidazole acetic acid were identical; in BuA = 0.35 and i-Pr.Am. = 0.20 (Figure 23 and 24 A). Further, the eluants of the hydrolysed sample when run in BuA and located with aniline phosphate stain showed evidence of a carbohydrate spot with the same Rf as authentic ribose, i.e. Rf = 0.37 (Figure 24 B).

Figure 23. Two one dimensional paper chromatograms run in BuA and stained with the sulphanilic acid stain. Chromatograms 2 and 3 show eluants of subject Lyon before hydrolysis. There is no evidence of imidazole acetic acid. Chromatograms 6 and 7 show eluants of same subject after hydrolysis. A Pauly positive spot corresponding to imidazole acetic acid is present. Rf = 0.35; 4 and 8 are control spots with authentic imidazole acetic acid.



1 2 3 4 5 6 7 8

Figure 23.

Figure 24. Two one dimensional paper chromatograms to show the eluants of urine extract from subject Lyon after hydrolysis. Paper chromatogram A is run in i-Pr.Am and located with Pauly stain. A Pauly positive spot due to imidazole acetic acid can be identified; Rf = 0.20. Chromatogram B is run in BuA and located with aniline phosphate reagent. A spot with the same staining and Rf as ribose is identified; Rf = 0.37.

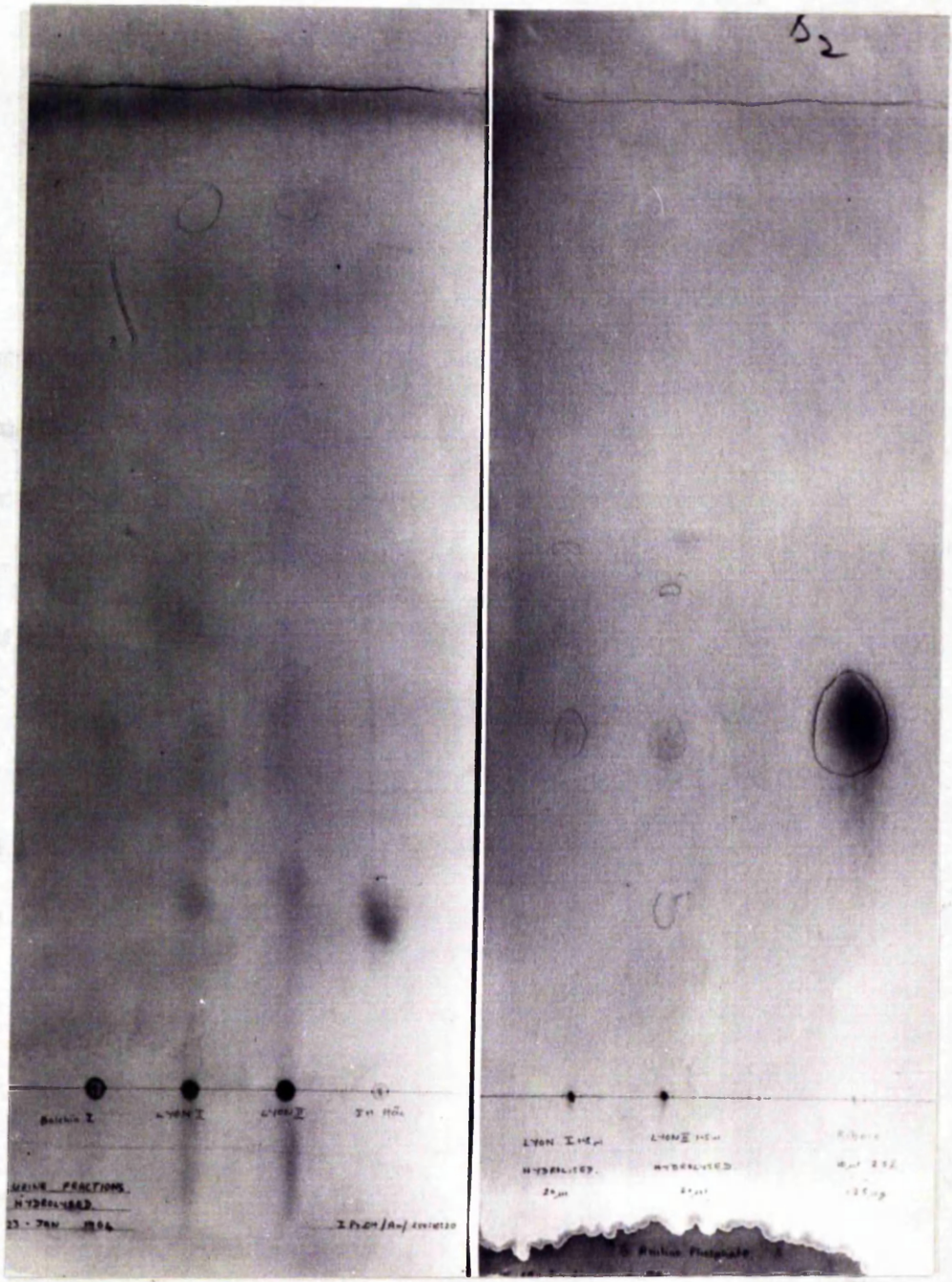


Figure 24.

Imidazoles in the Wash from the Dowex I x 8 Column

Identification of Histamine

Paper chromatograms prepared from the wash from the Dowex I x 8 acetate column in the six patients admitted in status asthmaticus (first 24 hour urine collection) were also stained with the Pauly stain and compared to the corresponding chromatograms in the six control subjects. No differences could be identified. The only Pauly positive spot being due to l-histidine (Figure 25, chromatogram 1 and 3).

In the asthmatic subjects paper chromatograms were prepared from the wash from the Dowex I x 8 acetate column of the second 24 hour urine collection made during corticotrophin therapy and stained with the Pauly stain. In three of these patients a second Pauly spot could be identified in addition to the one due to l-histidine (Figure 25 and 26).

The Rf of this spot corresponded to that of histamine (Table 5). Further when two dimensional chromatograms were run with the unknown substance in the column wash and

histamine applied to the same spot on the paper only one Pauly positive spot appeared in addition to the spot due to l-histidine (Figure 27).

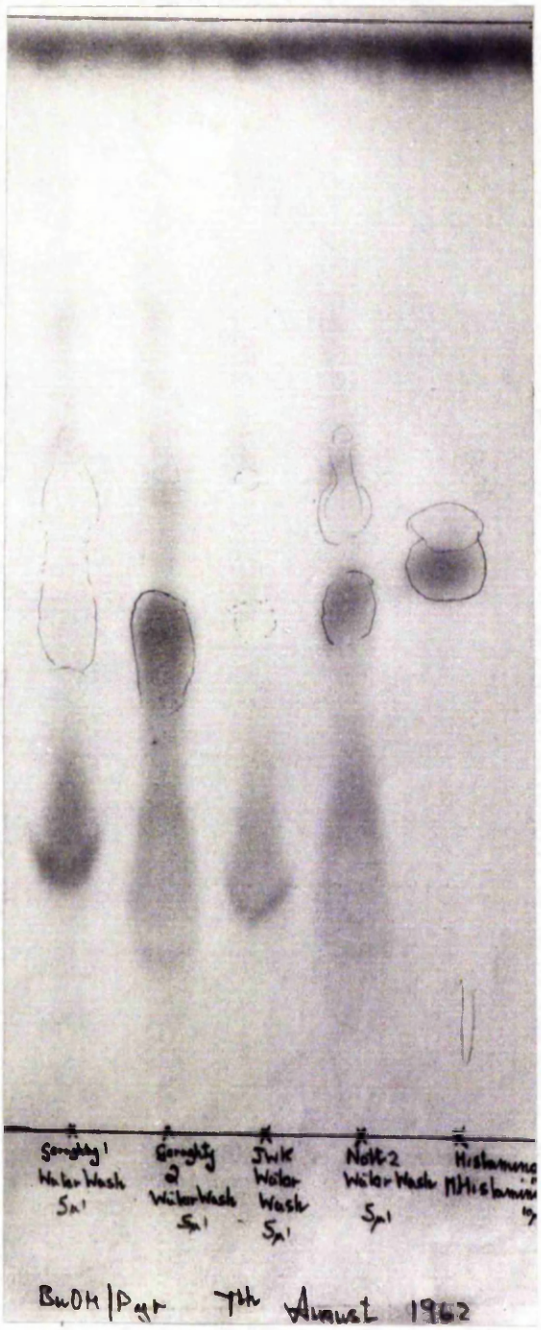
TABLE 5

The Rf's of the second Pauly positive spot in the wash from the Dowex I x 8 column of three asthmatic patients after 80 units of A.C.T.H. Gel compared to a histamine standard.

	Solvent	
Subject	i-Pr.Am	BuP
J.N.	.38	.47
M.G.	.38	.48 (.50)
H.T.	.37	.50
Histamine	.38	.50 (.50)

( ) from a two dimensional paper chromatogram

Figure 25. A one dimensional paper chromatogram run in BuP and stained with the sulphanilic acid stain. 1 and 3 are the water washes of an asthmatic (subject 3) and control subject respectively. The one Pauly positive spot in each is l-histidine. After 80 units of corticotrophin the water washes 2 and 4 of asthmatic subjects (3 and 1) have an additional Pauly positive spot due to histamine. Spot 5 is a control with histamine and l-methyl histamine.



histamine

l-histidine

Origin

1 2 3 4 5

Figure 25.

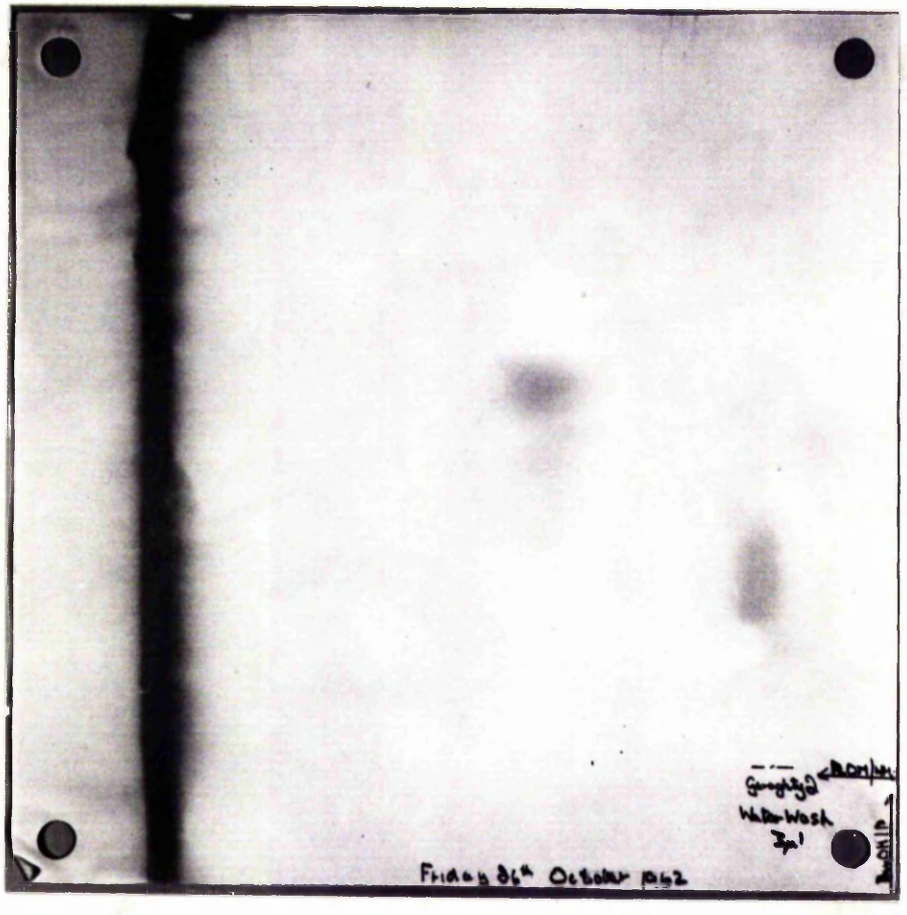


Figure 26. A two dimensional chromatogram run in BuP and BuA and stained with p sulphanilic acid reagent. The chromatogram shows the water wash of subject 3 prepared from the urine collection in the 24 hours after 80 units of corticotrophin. Two positive spots are present due to l-histidine and histamine. The Rf's of histamine are i-Pr.Am = .38; BuP = .50.

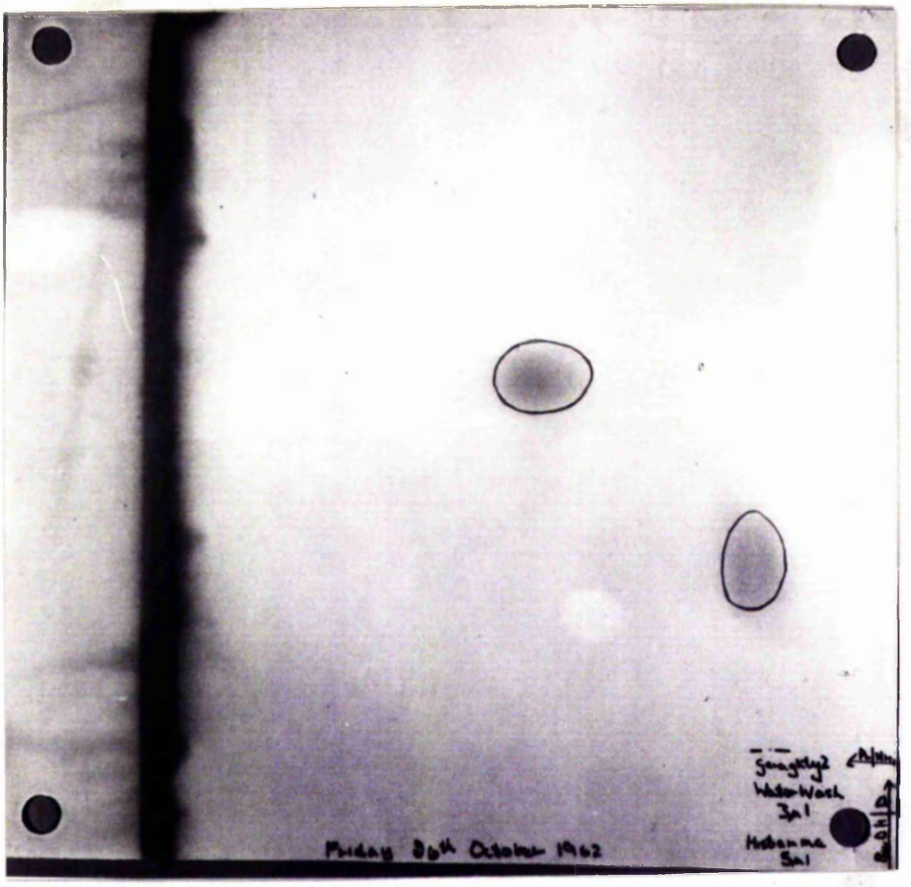


Figure 27. A two dimensional chromatogram run in BuA and BuP and stained with the sulphanilic acid reagent. The origin is spotted with the water wash of the second 24 hour urine collection from subject 3 together with 5 µg. of histamine. After development two Pauly positive spots are present due to l-histidine and histamine. The second Pauly positive spot (see Figure 25) coincides with the added histamine. The Rf's in i-Pr.Am = .37 and BuP = .50.

Summary of Results in Part I

In each of the six patients admitted in status asthmaticus the first 24 hour urine collection has been shown to contain the imidazole, urocanic acid. Histamine and the histamine metabolite 1-methyl, 4-imidazole acetic acid were not identified. During the second 24 hours whilst the patients were receiving corticotrophin the urocanic acid disappeared from the urine and the histamine metabolite 1-methyl, 4-imidazole acetic acid was identified. In addition histamine was identified by paper chromatography in 3 of these patients.

The urine specimens from control subjects did not contain urocanic acid, and histamine was not identified by the methods used. The histamine metabolite 1-methyl, 4-imidazole acetic acid was identified in all but one of the control subjects. The 1-methyl, 4-imidazole acetic acid was recovered from one subject and the identification confirmed by UV spectra, infra red spectrophotometry and gas chromatography.

Discussion

In the preliminary investigation the three isotopic metabolites of C^{14} histamine were clearly identified in the autoradiograms prepared from the urine of the guinea pig after an injection of isotopic histamine. This confirmed the work of R. W. Schayer and his associates (1959). Injected histamine is catabolised by two pathways. The first is by ring methylation to form 1-methyl histamine and 1-methyl, 4-imidazole acetic acid and in the second the amine group is oxidised to form imidazole acetic acid which is largely excreted as a conjugate with ribose (Figure 28). Clearly the first objective was to see if either of these two metabolites, 1-methyl, 4-imidazole acetic acid or the riboside of imidazole acetic acid could be identified in the urine of patients admitted in status asthmaticus.

The finding of a Pauly positive substance which was not extracted by ether from the acidified eluants prepared from the urine of patients admitted in severe status asthmaticus excluded a phenolic compound and suggested an imidazole such as imidazole acetic acid. Paper chromatography

in various solvents using synthetic imidazole acetic acid and urocanic acid as control substances soon ruled out the former. Further, the unknown compound was recovered from paper chromatograms and the UV spectra of the recovered material gave the very characteristic absorption spectra of urocanic acid. Urocanic acid was identified in the urine of all six patients admitted in status asthmaticus and rapidly disappeared from the urine when these patients were treated with corticotrophin gel (Kerr, 1963).

Although urocanic acid has not previously been reported in the urine of patients in status asthmaticus it has been shown to be an important catabolite of l-histidine in various species (Mehler and Tabor, 1953) and has been identified as a constituent of human sweat (Zenisek and Kral, 1953). More recently urocanic acid has been identified in the urine of patients with liver disease (McIsaac and Page, 1961; Merritt et al., 1962) and Kwashiorkor (Whitehead and Arnstein, 1961). Urocanic acid is not normally found in the urine in any measurable quantity and none was found in the control subjects by the method used here. Bennett and Chanarin (1961) reported the appearance of urocanic

acid in the urine after a 15 g. oral load of l-histidine in normal subjects and in patients with megaloblastic anaemia.

There are several known pathways for man to metabolise l-histidine; it may be incorporated into the body protein synthesis, decarboxylated and bound as histamine, metabolised via urocanic acid to glutamic acid or excreted in the urine as l-histidine (Figure 28). When a physiological quantity of C^{14} ring labelled l-histidine is given to man, only a small part of the radioactivity is recovered in the urine, suggesting that the bulk is incorporated in the body (Brown et al., 1960). When massive doses of l-histidine (15 g.) are given by mouth to normal people (Bennett and Chanarin, 1961) or to recovered Kwashiorkor patients (Whitehead, 1962) quantities of urocanic acid (8-10 mgm./12 hr.) appear in the urine. The amount of urocanic acid identified in the urine of subjects in status asthmaticus is small. In patient J. N., subject 1, not allowing for urocanic acid lost in the Dowex column the 12 hour out-put was 1.01 mgm. (page 170). Retrospective dietary surveys indicate that these asthmatic subjects were not consuming a diet rich in l-histidine

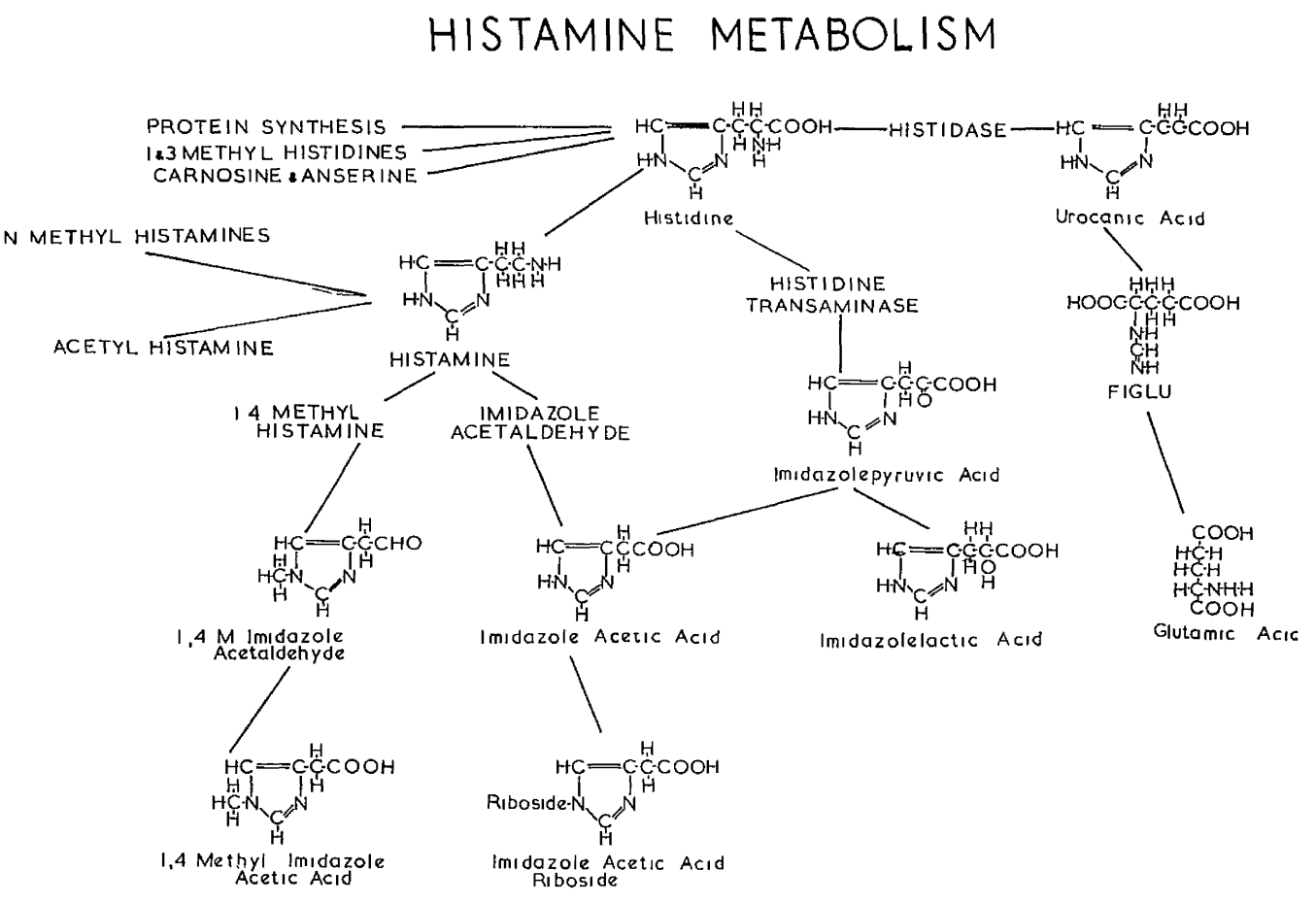


Figure 28. The metabolic pathways for l-histidine are shown. Histamine is formed by the decarboxylation of l-histidine. Note the metabolic pathway from l-histidine to imidazole acetic acid (riboside) does not necessarily require the formation of histamine.

before admission. One explanation could be that these asthmatic patients were subject to a physiological l-histidine overload which could be brought about by a block in one of the pathways of l-histidine metabolism. This has already been observed in patients with a folic acid deficiency where formamino glutamic acid and urocanic acid appear in the urine (Bennett and Chanarin, 1961). One of our controls, a patient with a megaloblastic anaemia of pregnancy, was shown to have the riboside of imidazole acetic acid in her urine (Figure 23). This is a demonstration of an alternative pathway for l-histidine metabolism being overloaded when the metabolic route via urocanic acid to glutamic acid is blocked due to folic acid deficiency (Figure 28). Similarly, a block in the pathway from l-histidine via histamine to l-methyl, 4-imidazole acetic acid and the riboside of imidazole acetic acid (Figure 28) could lead to overloading of the alternative metabolic route via urocanic acid to glutamic acid. In support of this view is the observation that within 48 hours of corticotrophin therapy in the six asthmatic patients, urocanic acid had disappeared from the urine.

The histamine metabolite l-methyl, 4-imidazole acetic

acid was identified in the urine extract of control subjects. The identification was confirmed by histamine loading of a healthy subject so that large quantities of the metabolite were present in the urine. The unknown substance was extracted from the urine in crystalline form and compared to synthetic 1-methyl, 4-imidazole acetic acid using paper chromatography, UV absorption spectra, infra red spectrophotometry and gas chromatography to confirm the identification. With one exception the 1-methyl, 4-imidazole acetic acid was identified in the urine of all the control subjects and was also present in considerable quantities in the urine of the two patients admitted with eosinophilia but no history of asthma. This suggests that these two patients were having an increased rate of histamine metabolism and contrasts with the failure to identify significant quantities of 1-methyl, 4-imidazole acetic acid in the urine of patients admitted in status asthmaticus.

Ring methylation is reported to be specific for histamine, no other imidazole serving as a substrate (Brown, Tomchick and Axelrod, 1959) and suggests that failure to identify 1-methyl, 4-imidazole acetic acid in the urine of patients

in status asthmaticus is further evidence of a disturbance of histamine metabolism in this condition. A deficiency in the enzyme system required for methylation should lead to overloading of the alternative pathway to imidazole acetic acid and the riboside of imidazole acetic acid. Or in status asthmaticus histamine may not be made available for methylation or oxidation. The failure to identify imidazole acetic acid in the hydrolysed urines of these asthmatic subjects favours the latter possibility. The identification of both imidazole acetic acid and ribose in the hydrolysed urine from the patient admitted with a megaloblastic anaemia of pregnancy already described confirms that the method was satisfactory; but it should be remembered there are alternative pathways for the formation of imidazole acetic acid and its conjugate with ribose which do not involve histamine. Nevertheless, if histamine is not being made available for catabolism in status asthmaticus both metabolic pathways should be affected. This would appear to be the case from the results reported here, as in asthmatic subjects there was no evidence of overloading the alternative metabolic pathway to the riboside of imidazole acetic acid.

The observation of increased quantities of histamine in the urine of patients in an attack of asthma when given corticotrophin or corticosterone therapy has already been reported by several authors using a sensitive bio-assay method to measure histamine in the urine (Grob, 1952). In 1951 Rose et al., reported that some cases of asthma treated with corticotrophin showed considerable increase in the urinary output of histamine in the 24 hours after the onset of treatment. In two cases the output of histamine was 200 mgm. in 24 hours. This high output of histamine was not observed in cases of rheumatoid arthritis given the same treatment with corticotrophin. Mitchell, Logan and Peters (1954) have also shown that cortisone causes a rise in the histamine concentration in the urine of asthmatic subjects which is greater than that found in control subjects on cortisone. These authors observed that the amount of histamine released was proportional to the severity of the asthmatic attack. More recently, Telford and West (1963) have reported that the newer synthetic corticosteroids act as powerful histamine liberators in some species.

In three of the six asthmatic subjects studied here

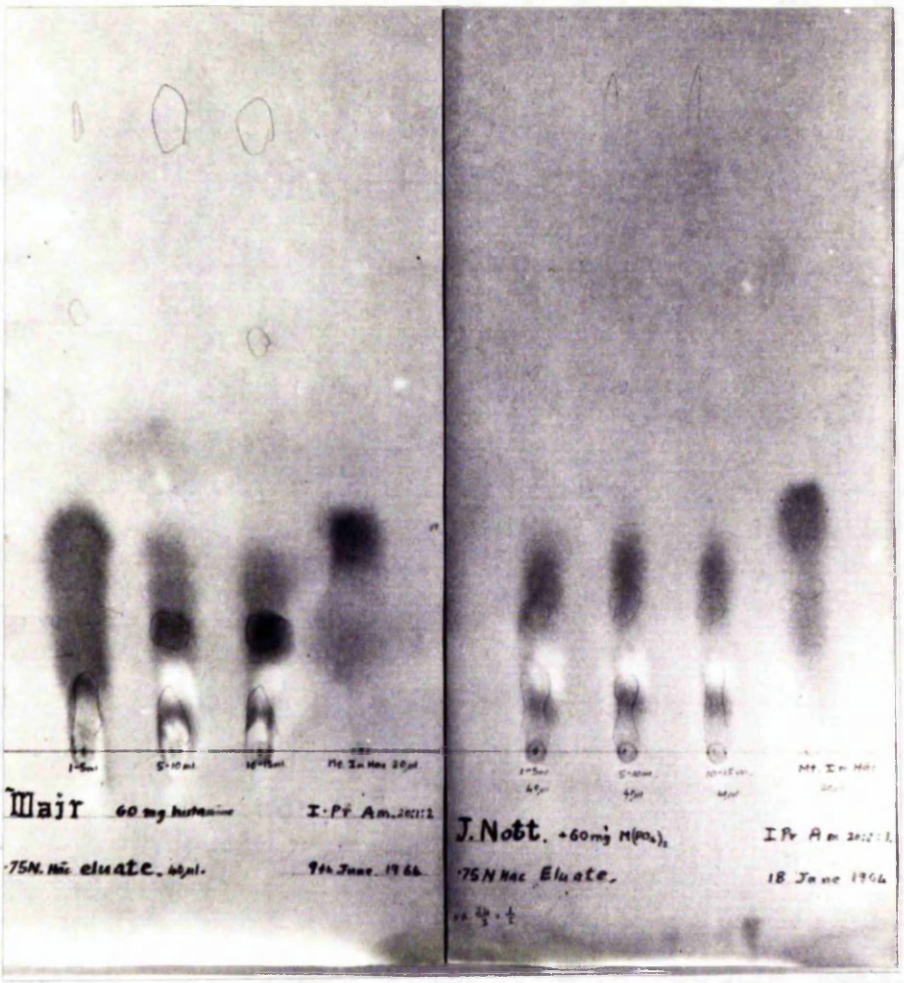
histamine was identified in the urine whilst these patients were on corticotrophin and 1-methyl, 4-imidazole acetic acid appeared in the urine of all six. It appears that not only is histamine not being made available for metabolism in an attack of asthma but also that it is accumulating in the tissues and can be released by corticotrophin.

At this point it is relevant to consider some recent work on the metabolism of amino acids. Although Garrod (1923) suggested that inborn errors of metabolism would be associated with a specific enzyme deficiency this has not always been proven. For example, in certain inborn errors of amino acid metabolism the basic defect appears to be in the amino acid transport system. There is now good evidence that the amino acid transport systems in the kidneys and intestine are similar. In Cystinuria there is defective tubular resorption of cystine, arginine, ornithine and lysine and in the intestine defective absorption of lysine and ornithine (Dent and Rose, 1951 and Milne, Asatoor and Loughbridge, 1961). A similar situation exists for the neutral amino acids in Hartnup disease (Baron, Dent, Harris, Hart and Jepson, 1956 and Milne,

Crawford, Lewis and Loughbridge, 1959). If the defect in asthma was in the transport of amines, we would expect to find a poor absorption of histamine together with an increased urinary excretion of histamine or its metabolites due to a failure of tubular resorption.

In 1939, Myhrman and Tomenius reported that the histamine content of the stool of some asthmatic subjects (5 out of 9 examined) was many times that found in normal or patients with urticaria or ulcerative colitis. On culture the bacterial flora in the stools was similar to that from normal subjects (Myhrman, 1943). On the other hand the low output of histamine in the urine during an asthmatic attack, (Mitchell, Logan and Peters, 1954) and the failure to find histamine metabolites in the urine during an asthmatic attack does not suggest a defect of tubular resorption. One asthmatic subject given 60 mg. oral histamine certainly did not show the massive increase in 1-methyl, 4-imidazole acetic acid which occurred with the control (Figure 29). Nevertheless it is unlikely that there is an abnormal histamine transport of this pattern in patients with bronchial asthma.

Figure 29. Two one dimensional paper chromatograms run in i-Pr.Am and located with p-nitroaniline reagent. Both subjects were given 60 mg. orally of histamine diphosphate. The eluants of the urine extract prepared from a 12 hour urine collection are shown. There is a high output of 1-methyl, 4-imidazole acetic acid 1, 2 and 3 in the non asthmatic control. In asthmatic subject 1 the eluants 5, 6 and 7 contain 1-methyl, 4-imidazole acetic acid but in much lesser quantity than in the control subject. 4 and 8 are control spots with 20 µg. 1-methyl, 4-imidazole acetic acid.



1 2 3 4 5 6 7 8

Figure 29.

The findings reported here together with much of the published work revieved supports the hypothesis that in severe asthma increased quantities of histamine are being retained in the body and not made available for metabolism. The high blood levels of histamine occasionally found in asthmatic subjects have been shown by Rose (1941) to be erratic and do not necessarily contradict the above hypothesis. Further, both Rose et al. (1950) and Schild (Personal Communication) have been able to estimate the amount of histamine in the lung tissue of a subject with bronchial asthma and they report levels four times that found in controls.

Most of the histamine in the body is found in the mast cells and in recent years the biosynthetic pathways for histamine in tissue cultures of tumour mast cells has been extensively studied by Green and Day (1963). Day and Green (1962 a & b) have shown that tissue cultures of tumour mast cells will concentrate l-histidine against a gradient and form histamine. These cells will also take up exogenous pre-formed histamine. The endogenous C^{14} histamine has a half life of 27 hours but the half life

of exogenous C^{14} histamine was not measurable, as after an initial loss of isotope no further loss occurred (Green and Day, 1961; Day and Green, 1962 (c)).

One explanation of these findings is that endogenous and exogenous histamine are held in separate pools in these mast cells, the exogenous histamine not being associated with an effective mechanism for elimination. When endogenous histamine levels are low exogenous histamine is transferred to the endogenous pool and can be eliminated for catabolism (Green and Fuzano, 1962).

These observations suggested that further information about the metabolism of histamine in the asthmatic subject would be obtained by studying the metabolism of C^{14} histamine in asthmatic and normal subjects.

Observations on the serum metabolites of C^{14} histamine would provide evidence about the exogenous pool of histamine and possibly indirect evidence as to the state of the endogenous histamine pool.

These studies are reported in Part II.

PART II

The metabolism of C^{14} histamine in patients with asthma and control subjects

In 1960 Beall and Van Arsdel reported on the plasma and urinary radioactivity after an intravenous injection of C^{14} histamine in normal individuals and patients with various diseases including 3 patients with asthma. The asthmatic patients developed mild wheezing but were otherwise symptom free. These authors reported no difference between the asthmatic subjects and controls either as regards the rate of decline of plasma radioactivity or in the rate of urinary excretion of radioactivity. The pattern of urinary metabolites was the same in both groups and they considered the major histamine metabolite in man to be imidazole acetic acid and its conjugate with ribose. These two substances accounted for 77 per cent of the radioactivity eluted from paper chromatograms and 1-methyl, 4-imidazole acetic acid only accounted for 20 per cent of the elutable radioactivity.

More recently Holander, Lindell, Nilsson and Westling (1962) have reported on the catabolism of C^{14} ring labelled histamine in patients with asthma and other allergic diseases.

These workers identified the histamine metabolites by a rigorous purification of the urinary metabolites using an isotope dilution technique. They reported 1-methyl, 4-imidazole acetic acid as the principal metabolite which accounted for 40-70 per cent of the recovered radioactivity. Imidazole acetic acid and its conjugate with ribose accounted for 20-38 per cent of the recovered radioactivity. The total radioactivity recovered in the urine in 12 hours (50-64%) was lower in the asthmatic subject as compared to controls (82 per cent recovery) both when the asthmatic patient was acutely ill with asthma and when free from symptoms. The differences in the total radioactivity recovered were not considered significant as the figures for the control subjects were quoted from an earlier publication by the same authors.

In these two papers on the metabolism of C^{14} histamine there is disagreement both as to the total recovery of radioactivity in asthmatic subjects as compared to controls and also in the identification of the principal radioactive metabolite in the urine after an injection of C^{14} histamine. Further, no analysis of the serum metabolites of C^{14} histamine

was carried out in the first few minutes just after the C^{14} histamine was administered. This would appear to be the critical period as Curry (1946) reported the maximum fall in vital capacity in asthmatic subjects after intravenous histamine was between five and twenty minutes.

The present investigation was carried out in view of the differing results already reported following the injection of C^{14} histamine. In addition by observing the metabolism of C^{14} histamine in the serum in the few minutes after completion of the infusion it was hoped results would be obtained which might help to elucidate the metabolic basis for the well known hypersensitivity of the asthmatic subject to histamine. On account of the possibility of severe respiratory distress with histamine in asthmatic subjects C^{14} histamine was administered to these patients in a period when they were relatively free from wheezing.

Permission was obtained from the M.R.C. Isotope Advisory Panel for the use of up to 3 μc. of C^{14} histamine in a suitable group of six adult asthmatic patients and six non asthmatic subjects.

Material and Methods

Clinical

Each asthmatic patient was admitted to hospital on the day prior to the test; and each patient fulfilled the definition of asthma given in the introduction. Brief clinical details of each patient are given in the Appendix (page 173). Each patient remained on a normal diet and on the morning of the test a urine sample was collected and the bladder emptied. A 10 ml. sample of venous blood was collected and an intravenous infusion set up with 200 ml. saline containing 3 μc. ring labelled C^{14} histamine di-hydrochloride (equivalent to 25 μg. histamine base) the infusion being given over a period of 15-20 minutes. Timed from the end of the infusion 10 ml. blood samples were taken from the other arm at 0, 5, 10, 20, 40, and 60 minutes and at 2, 6, 12, and 24 hours. All blood samples were collected using disposable syringes and needles. The blood was allowed to clot, centrifuged and the serum collected and stored at -20^{o}C till required. The total urine voided in the 24 hours after the IV infusion was collected in aliquots representing 0-1 hr., 1-6 hr., 6-12 hr.,

and 12-24 hr. and, after measuring the volumes, each collection was stored at -20°C until required.

The vital capacity (VC) and forced expiratory volume in one second (FEV_1) were recorded for each subject before the infusion of C^{14} histamine and again following the collection of each blood sample.

Material

Histamine (2-ring C^{14}) di-hydrochloride
Specific activity 116 µc./mg. M.W. = 184
Radiochemical purity 98% was obtained from the Radiochemical Centre, Amersham.

0.1 mc C^{14} histamine di-hydrochloride was made up in 100 ml. saline and divided into 20 ampoules each containing 5 uc. C^{14} histamine. The ampoules were sterilised by heat in an autoclave. For use 3 ml. from each ampoule (= 3 µc. C^{14} histamine di-HCl) was added to 200 ml. sterile normal saline and administered by IV infusion.

Measurement of Recovered Radioactivity in Urine

In preliminary experiments to establish the method it was apparent that our internal standard C^{14} histamine di-hydrochloride was being quenched by saline. To correct for this and calculate the total possible recovery of

radioactivity in the urine 0.1 ml. C^{14} toluene was added to a duplicate sample in every case. In addition, internal standard solutions containing C^{14} toluene in Bray's liquid scintillation solution and C^{14} toluene together with C^{14} histamine in Bray's liquid scintillation solutions were prepared and counted.

The recovered radioactivity was measured by liquid scintillation counting carried out in special 15 ml. vials obtained from the Packard Instrument Company. Using a blank urine and C^{14} toluene standard counting was carried out at different gain settings in all three windows of the Model 3003 Packard TriCarb Spectrometer. There was little difference in the three windows and the optimum gain setting was ten.

The liquid scintillation counting was carried out using Bray's liquid scintillation solution (Bray, 1960).

Naphthalene	60 gms.
PPO	4 gms.
POPOP	0.2 gms.
Methanol	100 mls.
Ethylene glycol	20 mls.

Made up to 1 litre with dioxan

PPO = 2, 5-diphenyloxazole
POPOP = 1, 4-di-2 (5-phenyloxazole)

Preparation of urine samples for liquid scintillation counting

To count the radioactivity in a 24 hour urine collection the following samples were prepared in 15 ml. vials together with the necessary blanks and standards and refrigerated before counting.

1. Blank vial
2. 0.5 ml. blank urine + 1 drop 4N HCl (in duplicate)
3. 0.5 ml. blank urine + 1 drop 4N HCl + 0.1 µl. C^{14} toluene
4. 0.5 ml. 0-1 hr. urine + 1 drop 4N HCl (in duplicate)
5. 0.5 ml. 0-1 hr. urine + 1 drop 4N HCl + 0.1 µl. C^{14} toluene
6. 0.5 ml. 1-6 hr. urine + 1 drop 4N HCl (in duplicate)
7. 0.5 ml. 1-6 hr. urine + 1 drop 4N HCl + 0.1 µl. C^{14} toluene
8. 0.5 ml. 6-12 hr. urine + 1 drop 4N HCl (in duplicate)
9. 0.5 ml. 6-12 hr. urine + 1 drop 4N HCl + 0.1 µl. C^{14} toluene
10. 0.5 ml. 12-24 hr. urine + 1 drop 4N HCl (in duplicate)
11. 0.5 ml. 12-24 hr. urine + 1 drop 4N HCl + 0.1 µl. C^{14} toluene
12. 0.1 µl. C^{14} toluene (in duplicate)
13. 0.1 µl. C^{14} toluene + 0.1 µl. C^{14} histamine
14. 0.1 µl. C^{14} histamine
15. 0.1 µl. C^{14} toluene + 0.1 µl. C^{14} histamine

Each vial was made up to 15 ml. with Bray's liquid scintillation solution and placed in a refrigerated Packard TriCarb liquid scintillation spectrometer (Model 3003) and permitted to adapt to darkness and cold for 30 minutes before counting for 1 minute with the gain setting at 10.

Measurement of Radioactivity in Serum Samples

The problem in counting radioactive serum is to solubilize the proteins so that a solution in scintillation fluid can be obtained with the minimum amount of quenching of the radioactivity. The method used here has been described by Chen (1958). Serum is dissolved in hydroxide of hyamine, acidified to overcome photo luminescence and once the serum is completely in solution scintillation fluid is added.

Preparation of serum samples for liquid scintillation counting

To count the radioactivity in the serum samples collected during each experiment, the following samples were prepared in 15 ml. vials together with the necessary blanks

and standards, and refrigerated before counting.

1. Blank vial
2. 0.1 ml. (0.1 μc) C^{14} toluene (in duplicate)
3. 0.1 ml. C^{14} toluene + 0.1 ml. C^{14} histamine (in duplicate)
4. 0.4 ml. serum before infusion of C^{14} histamine (in duplicate)
5. 0.4 ml. serum before infusion + 0.1 ml. C^{14} toluene
6. 0.4 ml. serum after infusion of C^{14} histamine (in duplicate)
7. 0.4 ml. serum after infusion of C^{14} histamine + 0.1 ml. C^{14} toluene

Similar vials were prepared with 0.4 ml. serum samples taken at 5, 10, 20, 40, 60, 120, 360, 720 minutes and 24 hours after completion of the infusion of C^{14} histamine. To each vial containing 0.4 ml. serum was added 1 ml. hydroxide of hyamine-10X (obtained from Packard Instrument Company) and 1 drop of 4N HCl. The mixture was shaken till clear when Bray's scintillation solution was added to give a total volume of 15 ml. in the vial. The vial was shaken

for 1 minute and refrigerated in darkness for 30 minutes before counting each sample for 10 minutes with the gain setting at 10. The standards and blanks were handled in the same way except hydroxide of hyamine-10X was not added.

Paper Chromatography of Serum Samples

The serum samples collected at 0, 5, 10 and 20 minutes after the infusion were prepared for chromatography as follows. A 2 ml. sample of serum in 4 x 0.5 ml. aliquots was precipitated with 2.5 ml. methanol. After centrifugation the supernatant was decanted into a test tube. The precipitate was washed with methanol and the supernatant added to the test tubes containing the first extracts. The extracts were evaporated to dryness under vacuo and the residue dissolved in 0.5 ml. water. Five 0.05 ml. spots of the extract were applied to Whatman No. 4 chromatography paper together with 0.01 ml. of a non-isotopic standard. The standards used were histamine dihydrochloride; 1-methyl histamine; 1-methyl, 4-imidazole acetic acid and imidazole acetic acid. The chromatogram was run overnight by ascending chromatography in iso-propanol: ammonia (3:1) and then thoroughly dried. The chromatogram from one spot was cut off and stained with either sulphanilic acid reagent or diazotised p-nitroaniline reagent to locate the non isotopic standards. The remaining four chromatograms were required to give adequate counts. The paper was divided into 1 cm.

horizontal strips extending from the origin of the four spots to the solvent front (Figure 30 A). Each strip was folded and placed in a counting vial containing Bray's liquid scintillation mixture so that the orientation of all the strips was uniform. The vials were then refrigerated until counted for ten minutes. The total radioactivity on the paper chromatogram was calculated. The number of counts on each 1 cm. strip was expressed as a percentage of the total radioactivity on the chromatogram and was plotted against its Rf value (Figure 30 B). The percentage radioactivity on all 1 cm. strips between the base points of each peak were summed to give the radioactivity due to that peak.

Paper Chromatography of Urine Samples

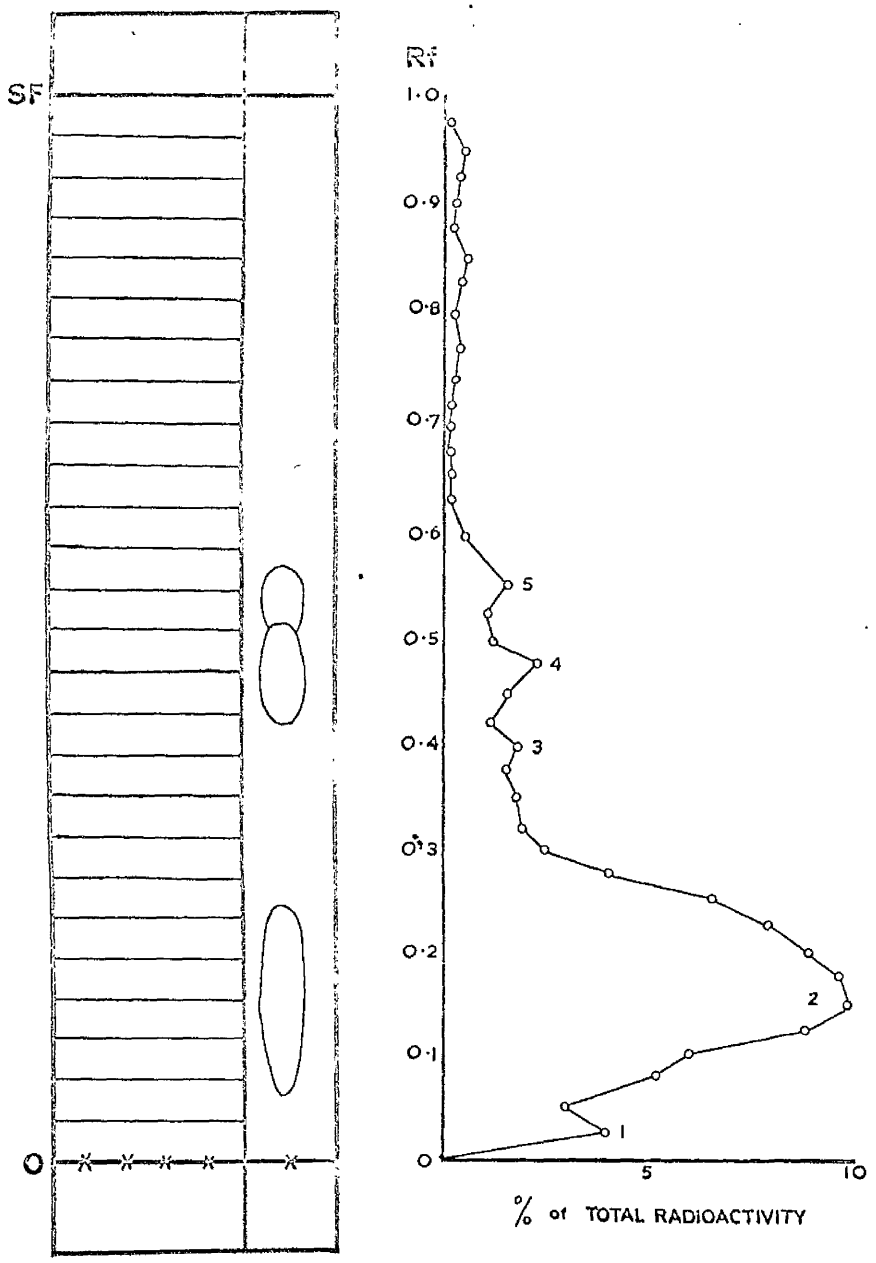
Paper chromatograms were prepared from the urine collected in the first hour. A second aliquot was refluxed with 8 ml. methanol and filtered. The filtrate was evaporated to dryness and re-dissolved in 1 ml. water. Two 0.04 ml. spots of this extract were spotted on Whatman No. 4 chromatography paper; to one spot were added non isotopic standards of histamine dihydrochloride and imidazole acetic acid. The

chromatogram was run as described above and after drying divided in two. The portion containing the non isotopic standards was stained with sulphanilic acid reagent. The remaining portion was divided into 1 cm. strips and prepared for counting as already described and counted for ten minutes.

Figure 30.

A. This shows a paper chromatogram with five radioactive spots applied to the origin (O) together with non-isotopic standards. The chromatogram is run overnight and one spot is cut off as shown and stained to locate the non isotopic standards. The standards shown from the origin are 1-methyl, 4-imidazole acetic acid, histamine, and 1-methyl histamine. The paper with the remaining four chromatograms is divided into 1 cm. strips extending from the origin to the solvent front (SF) and the radioactivity on each strip is counted.

B. This shows the radioactivity on each 1 cm. strip expressed as a percentage of the total radioactivity and plotted against its Rf value. Peak 1, riboside of imidazole acetic acid; Peak 2, 1-methyl, 4-imidazole acetic acid; Peak 3, unidentified; Peak 4, histamine; Peak 5, 1-methyl histamine.


SF
O
Rf
1·0
0·9
0·8
0·7
0·6
0·5
0·4
0·3
0·2
0·1
0
5
4
3
2
1
5
10
% of TOTAL RADIOACTIVITY

A B

Figure 30.

Pulmonary ventilation after infusion of C^{14} histamine

The Garthur vitalograph was used to measure the vital capacity (VC) and forced expiratory volume in one second (FEV_1). Base line readings had been obtained before the infusion of the isotope and thereafter readings were taken following the collection of each blood sample.

RESULTS

Vital capacity and forced expiratory volume.

The percentage change in vital capacity and FEV_1 from the baseline readings is shown in Table 6 and Figure 31. During the first hour of the experiment there is a marked fall in vital capacity and FEV_1 in all the asthmatic subjects, which is not seen in the controls. Following the infusion of C^{14} histamine, the changes in vital capacity and FEV_1 show that the asthmatic patients form a distinct population from the control subjects. For 11 degrees of freedom, t is significant at 0.02 level for VC at 10 minutes and t is significant at the 0.01 level at 20, 40 and 60 minutes for VC and FEV_1 (see appendix page 188).

Radioactivity in serum

The radioactivity in serum is expressed in counts per millilitre per minute, and the results are shown in Table 7. There is no statistically significant difference in the radioactivity present in the serum of the two groups of subjects at any period during the first 24 hours. It should be noted that the decline in radioactivity runs parallel in both groups, with the mean counts in the asthmatic patients slightly lower than those found in the

TABLE 6

Percentage change in Vital Capacity and Forced Expiratory Volume, 1 second, after intravenous infusion of 3 μc of C^{14} histamine dihydrochloride. The values shown are the means from six subjects in each group.

Time after Infusion	Asthmatic Patients				Control Subjects			
	V.C.	S.D.	FEV_1	S.D.	V.C.	S.D.	FEV_1	S.D.
0 mins.	-5.8	5.9	-7.7	5.0	-3.43	2.5	-2.6	4.3
5 mins.	-17.0	8.0	-16.6	11.2	-1.43	6.1	-1.1	9.03
10 mins.	-15.2	11.1	-15.5	11.6	+3.14	9.1	+5.3	6.8
20 mins.	-23.2	11.5	-24.8	15.5	+2.28	17.0	+1.6	5.3
40 mins.	-24.4	17.7	-29.2	15.8	+4.0	11.1	+1.3	9.2
60 mins.	-16.8	10.6	-22.8	14.2	+0.0	4.97	-1.3	9.4
2 hrs.	-14.3	13.9	-21.3	14.2	+5.6	10.3	+5.4	12.1
6 hrs.	-7.0	9.3	-9.8	7.5	+8.0	8.8	+6.2	12.7
12 hrs.	0.0	1	-10.5	2.5	19.0	2.0	+11.0	11.7
24 hrs.	-8.3	14.3	-15.0	22.5	+13.0	9.4	+14.0	8.7

(S.D. = Standard Deviation)

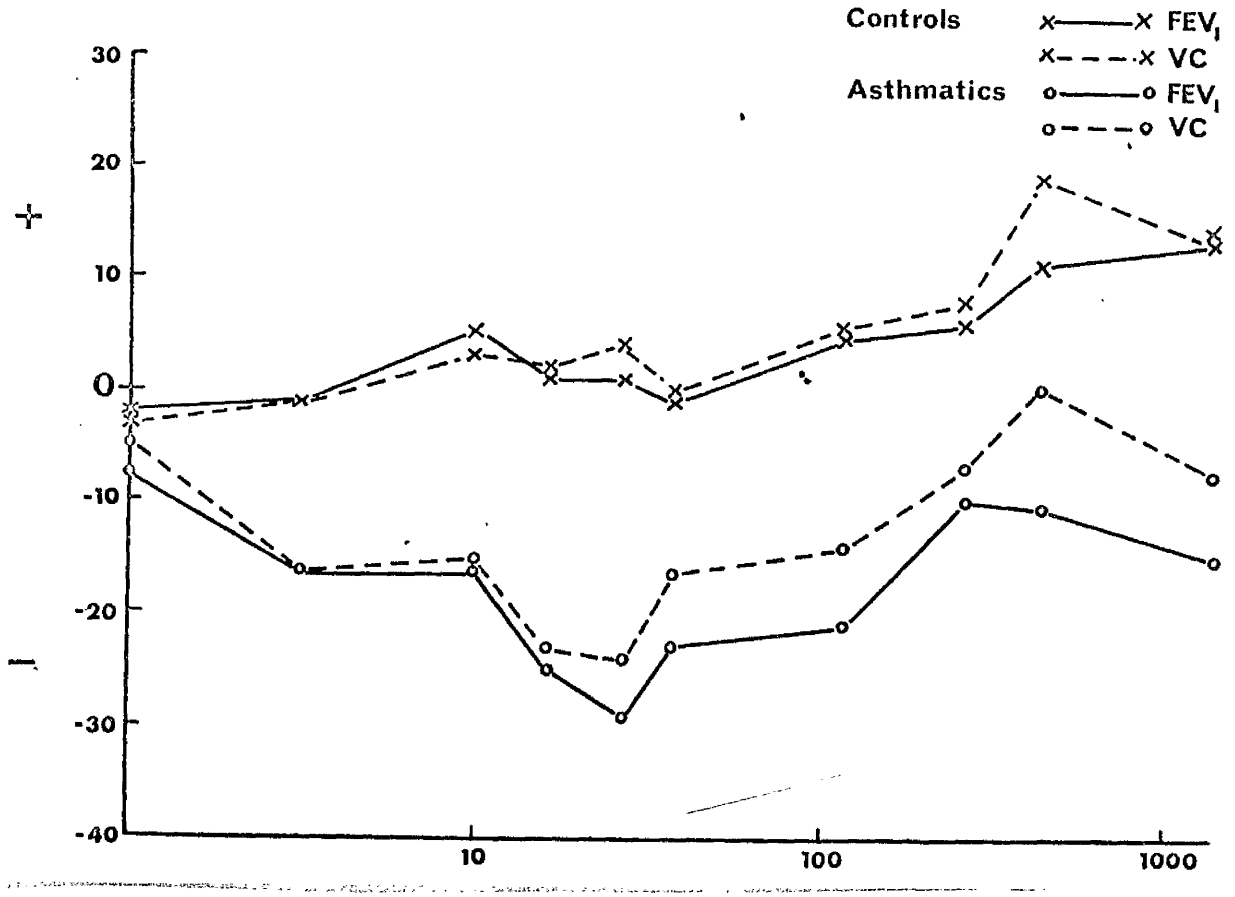


Figure 31. This shows the percentage change in vital capacity and FEV_1 from baseline readings in asthmatic and control subjects following the infusion of 3 µc. of C^{14} histamine (25 µg. histamine dihydrochloride).

The values are the means from six subjects in each group. The difference in the VC and FEV_1 second is statistically significant, see appendix page 186.

TABLE 7

Radioactivity in the serum expressed in counts per ml. per minute after intravenous infusion of 3 μc C^{14} histamine dihydrochloride. The values shown are the means from six subjects in each group.

Serum Aliquot	Asthmatic Patients		Control Subjects	
	Radio-activity	S.D.	Radio-activity	S.D.
0 mins.	121	19	107	13
5 mins.	106	14	108	32
10 mins.	101	13	107	28
20 mins.	100	12	107	25
40 mins.	90	11	99	25
60 mins.	78	11	83	25
2 hrs.	66	14	75	23
6 hrs.	35	6	46	23
12 hrs.	25	8	29	17
24 hrs.	18	6	16	16

controls.

The radiograms prepared from the serum extracts showed five distinct peaks which were identified as Peak 1, the riboside of imidazole acetic acid; Peak 2, 1-methyl, 4-imidazole acetic acid; Peak 3, unidentified; Peak 4, histamine; and Peak 5, 1-methyl histamine (Figure 30 B). Table 8 shows the amount of radioactivity accounted for by each peak in the serum samples obtained at 0, 5, 10 and 20 minutes after the infusion of 3 μc of C^{14} histamine. By the end of the infusion, the principal metabolite present in the serum of both groups is 1-methyl, 4-imidazole acetic acid, which accounts for 74.7 per cent of the radioactivity in the asthmatic subjects and 52.8 per cent in the controls. This difference is not statistically significant, ($P>0.05$) and by 5 minutes the amount of radioactivity due to 1-methyl, 4-imidazole acetic acid was the same in both groups. Histamine accounted for 6.8 per cent of the serum radioactivity by the end of the infusion in the asthmatic subjects and for 23.3 per cent of the radioactivity in the controls. Again this difference is not statistically significant and by 5 mimutes the amount of radioactivity due to histamine is similar in both groups ($P>0.05$). The remaining peaks and the residual radioactivity showed no difference between the two groups.

TABLE 8

The distribution of radioactivity in the serum chromatograms after intravenous infusion of 3 μc C^{14} histamine dihydrochloride. The radioactivity of each peak is expressed as a percentage of the total radioactivity on the chromatogram. The values shown are the means from six subjects in each group.

Peak	Rf	Group	Time in Minutes after Infusion 0		5		10		20	
			%	S.D.	%	S.D.	%	S.D.	%	S.D.
1. Riboside of Imidazole Acetic Acid	0.025	Asthmatics	9.8	2.2	0.4	4.0	9.2	5.0	9.0	1.7
		Controls	7.0	3.9	9.2	2.5	5.9	3.5	6.5	2.4
2. 1-methyl, 4-Imidazole Acetic Acid	0.15	Asthmatics	74.7	5.4	71.3	7.2	75.1	10.8	75.6	6.6
		Controls	52.8	21.3	67.4	11.0	75.6	4.9	80.2	9.0
3. Unidentified	0.40	Asthmatics	5.6	3.3	6.1	3.8	6.5	4.0	5.4	1.7
		Controls	6.7	4.5	7.7	2.4	5.9	3.1	4.6	2.4
4. Histamine	0.48	Asthmatics	6.8	1.6	6.8	5.6	3.6	2.5	2.3	0.9
		Controls	23.3	21.6	11.7	9.8	6.0	2.2	3.6	2.2
5. 1-methyl Histamine	0.55	Asthmatics	4.1	2.9	2.8	3.3	3.7	2.7	3.1	3.2
		Controls	4.3	3.1	5.0	3.3	4.3	4.2	2.5	1.9
Miscellaneous		Asthmatics	4.6	2.1	7.6	6.1	6.6	2.0	8.0	4.4
		Controls	9.5	3.6	8.0	7.4	2.9	1.9	6.1	4.2
Total Radioactivity		Asthmatics	103.6		109.0		100.6		103.5	
		Controls	105.6		104.0		104.7		103.4	

Radioactivity in the urine

Radioactivity in the urine.ctivity in the urine is expressed as a percentage of the total radioactivity administered and is shown in Table 9. The total radioactivity administered is recovered in 24 hours and there is no significant difference in the amount of radioactivity recovered in each group at 0 to 1 hour, 2 to 6 hours, and 13 to 24 hours. The urine collected during the first hour accounts for 32.9 per cent of the radioactivity in the asthmatic subjects and 25.9 per cent in the controls; the difference is not significant ($P > 0.05$).

The radiograms prepared from the urine collected in the first hour showed three distinct peaks, identified as the riboside of imidazole acetic acid, 1-methyl, 4-imidazole acetic acid, and histamine. In addition, a fourth, unidentified peak was present in eight patients, five of them being asthmatic subjects. Table 10 shows the amount of radioactivity accounted for by each peak in the asthmatic and control subjects. No significant difference was found between the groups ($P > 0.05$).

TABLE 9

Recovery of Radioactivity in the urine expressed as a percentage of the total radioactivity infused. The values shown are the means from six subjects in each group.

Time	Asthmatic Patients		Control Subjects	
	Percentage Radioactivity	Standard Deviation	Percentage Radioactivity	Standard Deviation
0-1 hrs.	32.9	7.4	25.9	3.9
2-6 hrs.	38.3	5.2	40.1	6.1
7-12 hrs.	15.3	3.2	19.9	4.7
13-24 hrs.	13.9	4.3	15.3	2.6
Total	100.4		101.3	

TABLE 10

The distribution of radioactivity in the chromatogram prepared from the urine extract after the infusion of 3 μc C^{14} histamine dihydrochloride. The radioactivity of each peak is expressed as a percentage of the total radioactivity on the chromatogram. The values shown are the means from six subjects in each group.

		Asthmatic		Control	
Peak	Rf	%	Standard Deviation	%	Standard Deviation
Riboside of Imidazole Acetic Acid	0.056	7.8	4.9	16.6	8.3
1-methyl, 4-Imidazole Acetic Acid	0.16	58.1	15.8	54.9	9.7
Histamine	0.55	24.5	6.0	19.1	6.9
Unidentified	0.78	8.1	7.7	3.4	6.6
Miscellaneous Radioactivity		3.0		8.2	
Total Radioactivity		101.7		102.2	

Discussion

Weiss, Robb and Blumgart (1929) were the first to report on the fall in pulmonary ventilation in asthmatic subjects following the infusion of histamine. This observation was confirmed by the work of Curry (1947) and Bouhuys et al. (1960). The fall in VC and FEV_1 is again clearly seen following the infusion of isotopic histamine (Figure 31). The quantity of isotopic histamine administered was equivalent to 25 µg. of base and gave rise to a 24.4 per cent fall in VC and a 29.2 per cent fall in FEV_1 in the asthmatic subjects at 40 minutes. This contrasts with the lack of change in VC and FEV_1 (+ 4% and + 1.3% respectively) in the control subjects and is statistically significant (Appendix, page 186). Asthmatic subjects appear to form a distinct population with a marked hypersensitivity to intravenous histamine, the basis of which remains unexplained. This sensitivity should not be confused with the non specific reaction of the bronchioles of patients with chronic bronchitis or emphysema to numerous substances administered by inhalation. The asthmatic subject remains hypersensitive to histamine even in quiescent periods

when the patient is free from asthmatic attacks (Bouhuys et al., 1960). The intravenous infusion of histamine effects respiration in asthmatic subjects in about two minutes and the effect begins to wear off after forty minutes. Although ventilation in normal subjects can be reduced by histamine it required at least fifty times the amount of histamine usually administered to asthmatic subjects, and the dose administered here had no measurable effect on the ventilation of normal subjects.

In searching for an explanation of the increased sensitivity of these subjects to histamine it is worth considering the possible mechanisms for terminating the biological active life of injected or released histamine.

1. Metabolism of histamine by methylation or oxidation.
2. Inactivation by storage, for example in mast cells.
3. A carrier mechanism whereby histamine is inactivated by being bound to protein (histaminopexy).
4. A binding process at the receptor sites.

1. The results reported here together with those of Helander

and co-workers (1962) and Beall and Van Arsdel (1960) do not suggest that the hypersensitivity to histamine is due to a defect in the metabolic pathway for the metabolism of exogenous histamine. In both asthmatic and control subjects over 95 per cent of the administered radioactivity is recovered in the urine in 24 hours. Paper chromatograms prepared from the serum samples collected during the first hour and the first urine collection after administration of the isotope show no significant difference in the radioactive peaks in the asthmatic or control subjects. The principal metabolite is C^{14} ring labelled 1-methyl, 4-imidazole acetic acid with lesser quantities of C^{14} ring labelled riboside of imidazole acetic acid and C^{14} 1-methyl histamine (Dowell, Kerr and Park, 1966). The asthmatic subject metabolises exogenous histamine in the circulation normally and presumably endogenous histamine once released and in the circulation would be handled in a similar fashion.

2. The observations on asthmatic subjects with exogenous C^{14} histamine suggest a more rapid breakdown of the histamine

as compared to control subjects although this did not reach statistically significant levels. It has been shown by Lilja, Lindell and Saldeen (1960) that C^{14} l-histidine when incubated with normal lung forms C^{14} histamine but no C^{14} histamine metabolites. On the other hand the same lung when incubated with C^{14} histamine forms C^{14} methyl histamine and C^{14} 1-methyl, 4-imidazole acetic acid. This shows that the metabolism of exogenous histamine does not reflect the metabolism of endogenous histamine. The latter when formed is not available for metabolism but rather bound in storage sites. The rapid recovery of the total administered radioactivity does not suggest that exogenous histamine is being taken up and stored for later release. Indeed the asthmatic subject seems particularly efficient in metabolising exogenous histamine.

Tissue cultures with tumour mast cells have shown two pools for histamine. One pool is formed from exogenous and the other from endogenous histamine (Furano, 1962, Green, Furano and Carlini, 1962). The endogenous pool has a fairly rapid turnover and only when the

endogenous level of histamine is low are sites available for the binding of exogenous histamine. The exogenous pool of histamine is separate and not associated with an efficient elimination mechanism (Green and Fuxano, 1962). The observations reported in Part I show that endogenous histamine is being retained in the body in an attack of asthma and not made available for metabolism. The storage pools for histamine are therefore full and the rapid metabolism of C^{14} labelled exogenous histamine supports the experimental observations of Green and his associates. It is unlikely that increased stores of histamine and failure of elimination alone accounts for the histamine hypersensitivity of asthmatic subjects, although this could lead to an increased number of receptor sites available for histamine on smooth muscle and hence the hypersensitivity.

3. Parrot and Laborde (1953) have used the term histaminopexy to denote the immediate fall in the biological activity of histamine when mixed with dialysed human serum and tested on isolated guinea pig ileum. The same authors (1964)

suggested that histamine was not enzymatically destroyed by normal human serum but bound to a gamma globulin. This work has always been controversial and was not confirmed by Kaplan and Davis (1953) or Beall (1964), or Kertcher and Frankland (1965). In the studies reported here within 10 minutes of the infusion of C^{14} histamine 75 per cent of the radioactivity in the serum samples was accounted for by 1-methyl, 4-imidazole acetic acid. Less than 6 per cent of the radioactivity on the paper chromatograms was not identified as histamine or its known metabolites. It is unlikely that failure of histaminopexy is a factor in the sensitivity of asthmatic subjects to histamine.

4. The most likely explanation for the histamine hypersensitivity of the asthmatic subject would be an increased avidity of receptor sites or an increased number of receptor sites for histamine in the bronchial smooth muscle. The fall in free histamine in the urine in an attack of asthma (Mitchell, Logan and Peters, 1953) and the disappearance of the histamine metabolite 1-methyl, 4-imidazole

acetic acid from the urine in status asthmaticus (Kerr, 1964) is suggestive that endogenous histamine is being retained in the body in active asthma. Further, the excessive release of histamine and its metabolites identified in the urine of asthmatic subjects given steroid or corticotrophin therapy as compared to controls (Rose et al., 1951, Mitchell et al., 1953, Kerr, 1964) supports the conclusion that the asthmatic subject retains increased quantities of histamine in the body. As already noted, Rose et al. and Schild have had the opportunity to measure the concentration of histamine in biopsy material from the lung of an asthmatic subject and both report this to be at least 4 times that in normal subjects. Can this failure of histamine turnover in asthmatic subjects explain the known hypersensitivity of these subjects to histamine? It is worthwhile considering the supersensitivity of skeletal muscle which occurs after degeneration of the motor fibre; here the acetycholine sensitive area (receptor) which is normally restricted to the end plate region extends until it covers the whole muscle membrane (Axelson and

Thesleff, 1954; Miledi, 1960; Thesleff, 1960) and the pharmacological blocking of the pathway to the end plate, e.g. by botulinum toxin can also produce this type of supersensitivity of skeletal muscle (Thesleff, 1960). Smooth muscle has no obvious end plate, the receptors are probably uniformly spread over the cell membrane but could be visualised like skeletal muscle to be increased in number and thus lead to histamine hypersensitivity.

The neurogenic control of smooth muscle is part of the sympathetic nervous system with two types of antagonistic receptors alpha and beta (Ahlquist, 1948). A disturbance of the adrenergic control of smooth muscle might lead to smooth muscle supersensitivity similar to that described for skeletal muscle. Schayer (1956) has shown that the amount of histamine formed from C^{14} l-histidine in mast cells can be greatly enhanced in animals if they are given a small injection of epinephrine. In 1953 Bain and Meier showed that the normal biphasic physiological responses of the cat to histamine are dependant on the adrenal medulla and that a simultaneous infusion of

trace amounts of adrenaline or noradrenaline fully restored the physiological responses to histamine in adrenalectomised animals. In certain species histamine supersensitivity can be induced following the injection of a killed vaccine prepared from bordetella pertussis (Parfentjev and Goodline, 1948, Kind, 1958, Munoz, 1964). Whilst investigating histamine metabolism in animals sensitised to histamine with bordetella pertussis vaccine Fishel, Szentivanyi and Talmage (1962) reported that a similar degree of supersensitivity to histamine could be produced in certain species of animals by the beta adrenergic receptor blocking drug dichloroisoproterenol (DCI). This observation has been confirmed (Perioni and Levine, 1967) and it has been shown that beta adrenergic receptor blocking drugs intensify and prolong the anaphylactic response (Collier and James, 1967).

The above observations suggest that in man the hypersensitivity to histamine in the asthmatic subject could be based on a disturbed function of the adrenergic system. Bouhuys et al. (1960) reporting on the effects

of histamine on pulmonary ventilation in man noted that in two asthmatic subjects whilst on the ganglion blocker, hexamethonium bromide, much larger quantities of histamine were required to cause the same change in the Lung Clearance Index than they had observed in the original observations. These authors rather vaguely suggested a neurogenic mechanism of reflex or other nature as the basis for the histamine supersensitivity.

With the introduction of the beta adrenergic receptor blocking drug propranolol for clinical use there have been several reports confirming the first observations by McNeill (in Dundee) that beta adrenergic blockade can cause a fall in ventilation in asthmatic subjects (McNeill, 1964; Meier, Lydtin, Zolliner, 1966; McNeill and Ingram, 1966; Sly, Heimlich, Busser and Strick, 1967).

The relationship between hay fever and asthma is poorly understood other than the well known similarity in antibody response and the frequent occurrence of allergic asthma and hay fever together. What factors make the hay fever subject develop seasonal asthma in addition to his hay fever? Ouellette and Reed (1967) have shown that a pharmacologically

induced beta adrenergic receptor blockade can shift the response of ragweed hay fever subjects on exposure to antigen towards more severe airways obstruction and asthma. These subjects had never previously experienced asthma in association with their hay fever. Cookson and Reed (1963) observed that infusion of isoproterenol caused a greater decrease in diastolic blood pressure and a greater depression of the height of the diastolic pulse waves in normal than in asthmatic subjects reflecting a greater decrease in peripheral vascular resistance in the normal subjects. The authors interpreted these results as showing that vasodilation mediated by beta adrenergic receptors seemed partially blocked in asthmatic individuals.

In an attempt to produce histamine sensitivity in non allergic adults Zaid and Beall (1966) administered the beta adrenergic receptor blocking drug propranolol but were unable to alter histamine sensitivity and therefore concluded that beta blockade was not the cause of bronchial asthma in man. On the other hand McNeill and Ingram (1966) have shown that beta adrenergic receptor blockade does increase airways resistance in normal subjects measured by the sensitive body

plethysmograph where no change can be measured in the FEV_1 second. In addition, beta adrenergic blockade has produced an attack of asthma in a subject who had been free from asthma for many years (Bewsher, 1967).

Following the observation that beta receptor blocking drugs produce histamine hypersensitivity in animals most investigations in man have concentrated on the beta receptors of bronchial smooth muscle which on stimulation cause relaxation and little attention has been given to the possibility that asthmatic subjects have active alpha receptors giving rise to bronchial constriction. Fisher et al. (1962) reported that the alpha receptor blocking drug phenoxybenzamine could prevent the lethal effects of histamine in B. pertussis sensitised mice. In addition it has been shown that phentolamine (an alpha receptor blocking drug) exerts a prophylactic action in experimental asthma in the guinea pig produced either by histamine aerosol or by ovalbumin aerosol in previously sensitised animals (Wiskiewska, 1964). These reports have led us to investigate the effect of alpha receptor blocking drugs on the fall in ventilation observed in asthmatic subjects after an infusion of histamine.

Preliminary observations have shown that phentolamine 5 mgm. I.V. inhibits the fall in VC and FEV_1 produced by 50 µg. histamine dihydrochloride. The results are shown in Tables 11 and 12 (page 190 and 192) and on statistical analysis there is a highly significant inhibition in the fall in VC and FEV_1 by phentolamine at 5, 10, and 20 minutes. Phenoxybenzamine, a long acting alpha adrenergic receptor blocking drug, has a similar effect and this investigation is continuing.

The tension developed by contractile smooth muscle is dependant on the ionized calcium concentration in the environment of the contractile protein of the myofibrils (Filo, Bohr and Ruegg, 1965). It has been suggested that alpha receptor activity normally causes an increase in ionized calcium in the environment of the myofibrils and an increase of tension, whereas beta receptor activity causes a sequestration of ionized calcium and a relaxation of the myofibrils (Bohr, 1966). The activity of histamine on smooth muscle in an organ bath is known to be dependant on the concentration of ionized calcium in the Tyrode solution (Daniel, 1964). Therefore it can be postulated that beta receptor blocking drugs by increasing the ionized calcium

of the smooth muscle myofibrils increases the histamine response and alpha receptor blocking drugs by lowering the ionized calcium inhibits the histamine response of bronchial smooth muscle. In contrast the potent beta receptor stimulating drug isoprenaline rapidly overcomes the fall in ventilation produced by histamine in asthmatic subjects. That is, the histamine response of smooth muscle is dependant on the concentration of ionized calcium at the myofibrils and this is controlled by the adrenergic receptors.

Accepting a disturbed function of the alpha and beta receptors of bronchial smooth muscle as the basis of histamine hypersensitivity in the asthmatic subject, could this explain the retention of endogenous histamine in these same subjects in an attack of asthma? Histamine supersensitivity may lead to a "feed back" phenomenon whereby the asthmatic subject, to protect himself, slows down the elimination of histamine from storage sites. This in turn leads to a vicious circle as more receptor sites become available for histamine, the situation being further aggravated if the patient is allergic and liable to a sudden release of histamine on exposure to the appropriate antigen. Steroids used in the relief of

a severe asthmatic attack take some hours to produce relief. This could be due to the time required to release and metabolise histamine in the presence of the histamine supersensitive state.

The hypothesis that histamine supersensitivity in the asthmatic subject is due to an inbalance between the alpha and beta adrenergic receptors remains a promising one and explains many of the experimental and clinical observations about bronchial asthma. For example this hypothesis provides a common basis for infective, allergic or psychological asthma. In each a disturbance of the adrenergic balance of bronchial smooth muscle leads to histamine supersensitivity.

Although in this thesis attention has been focused on histamine it is realised that the other biologically active amines known to be liberated in anaphylaxis can give rise to bronchial reactions in asthmatic subjects and may play a part in bronchial asthma.

The relationship of the action of biologically active amines on smooth muscle to the sympathetic control of smooth muscle provides ample opportunity for further clinical investigation and holds the promise of a better understanding of the mechanisms responsible for bronchial asthma.

APPENDIX

Paper chromatograms from asthmatic subjects to show urocanic acid

Figures 32 to 37 are paper chromatograms prepared from the eluants of the first 24 hours collection of urine from each asthmatic subject on admission to hospital in status asthmaticus.

Urocanic acid was identified in each paper chromatogram.

Note: Imidazoles are located on paper chromatograms either with the Pauly stain or p-nitroaniline and appear as red or reddish-purple spots respectively; black and white photography does not bring out the true quality of the chromatograms.

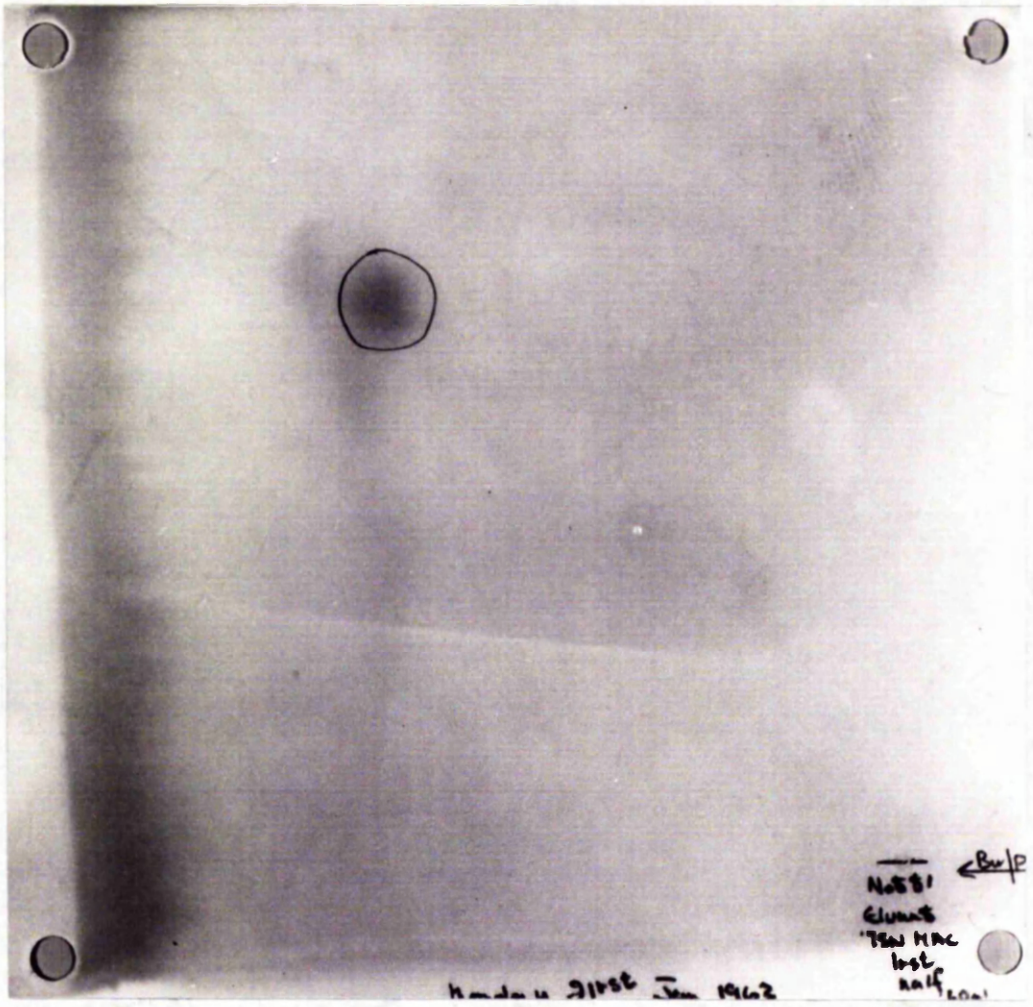


Figure 32. Asthmatic subject 1. Urocanic acid.

A repeat of Figure 9. A two dimensional chromatogram run in BuA and BuP. After development the chromatogram is stained with Pauly stain to locate urocanic acid.

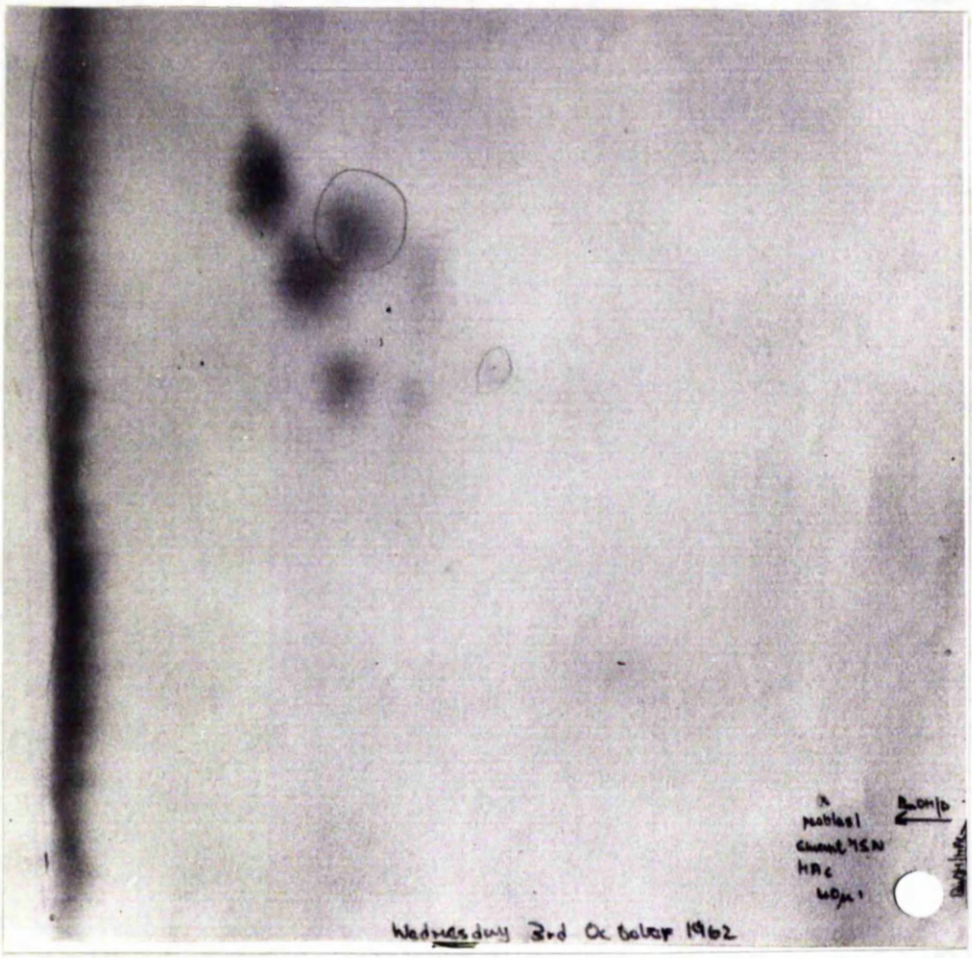


Figure 33. Asthmatic subject 2. Urocanic acid.

A two dimensional chromatogram run in BuA and BuP. After development the chromatogram is stained with Pauly stain to locate urocanic acid.

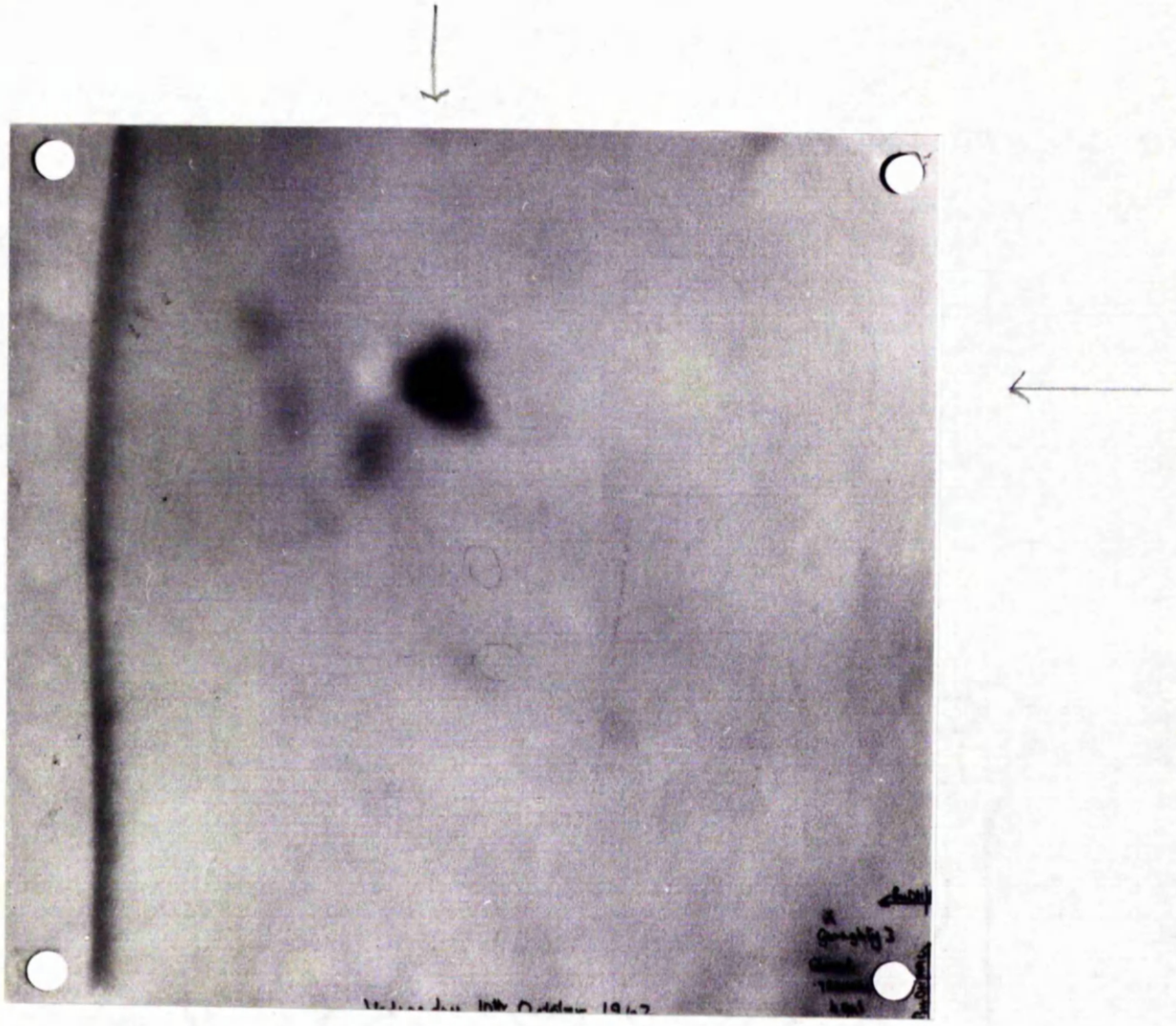


Figure 34. Asthmatic subject 3. Urocanic acid.

A two dimensional chromatogram run in BuA and BuP. After development the chromatogram is stained with Pauly stain to locate urocanic acid.

Figure 35. Asthmatic subject 4. Urocanic acid.
A two dimensional chromatogram run in BuA and BuP. After development the chromatogram is stained with Pauly stain to locate urocanic acid.

Figure 36. Asthmatic subject 5. Urocanic acid.
A two dimensional chromatogram run in BuA and BuP. After development the chromatogram is stained with Pauly stain to locate urocanic acid.

Figure 37. Asthmatic subject 6. Urocanic acid.

A one dimensional chromatogram developed in i-Pr.Am and located with the Pauly stain to show the presence of urocanic acid.

1. Urine extract plus urocanic acid
2. Urine extract 50-100 ml. eluant, urocanic acid present
3. Urine extract 15-50 ml. eluant, no urocanic acid present
4. Urocanic acid control

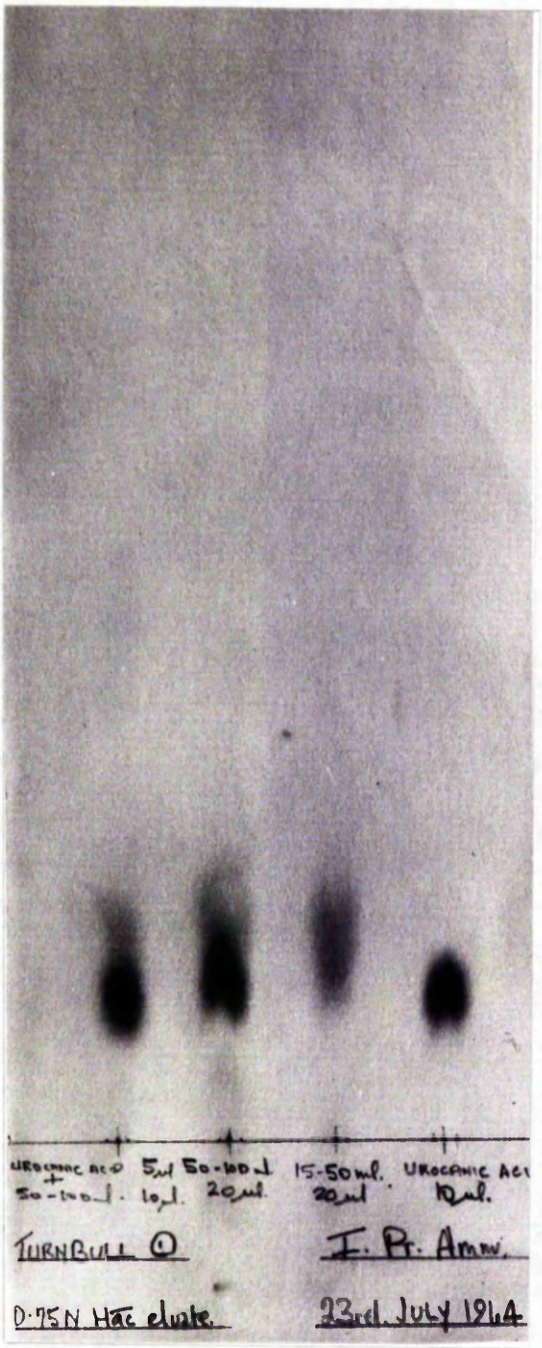

Urocanic Acid + 50-100 µl. 10 µl.
5 µl 50-100 µl 20 µl.
15-50 µl. 20 µl
Urocanic Acid 10 µl.
TURNBULL ①
I. Pt. Amm.
0·75 N Hac elute.
23rd. July 1964
1
2
3
4

Figure 37

Paper chromatograms from asthmatic subjects after A.C.T.H. to show 1-methyl, 4-imidazole acetic acid

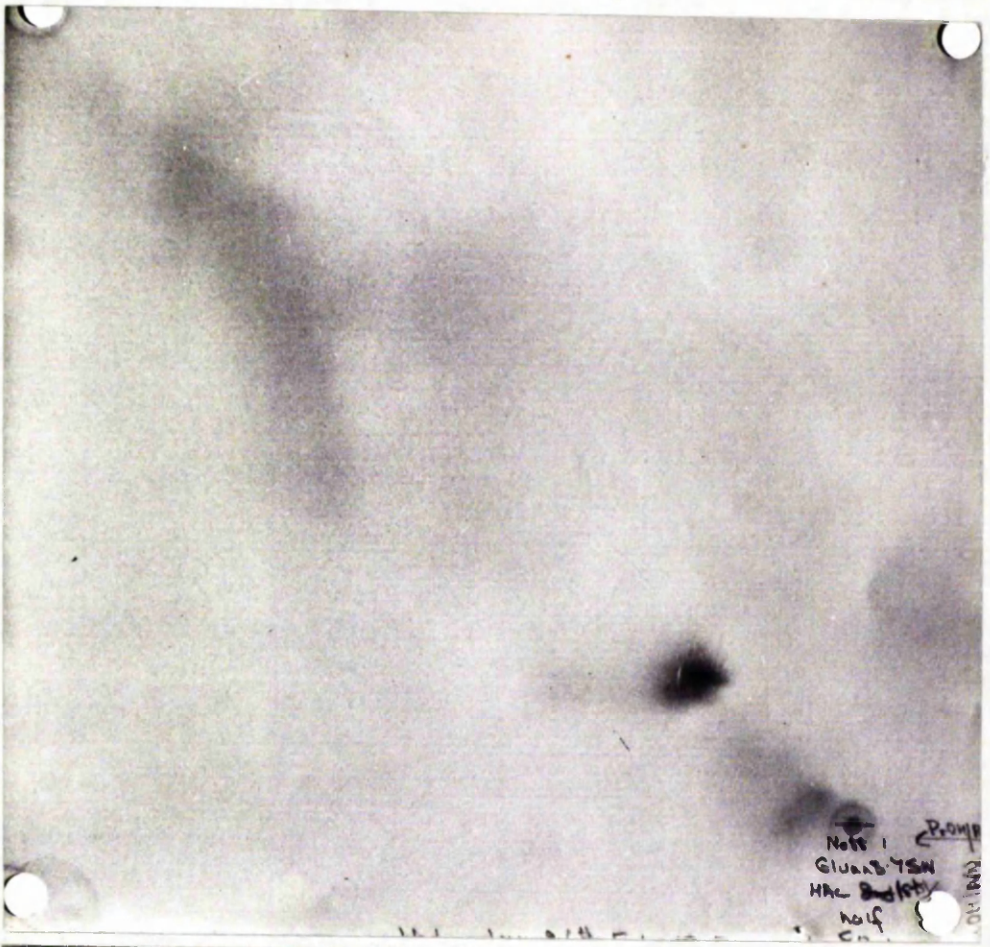
Figures 38 to 43 are paper chromatograms prepared from the eluants of the first and second collection of urine from six asthmatic subjects. In each Figure chromatogram A is from the first urine collection and shows no evidence of 1-methyl, 4-imidazole acetic acid. Chromatogram B is prepared from the second urine collection whilst the patient is receiving corticotrophin and 1-methyl, 4-imidazole acetic acid can be identified.

Figure 38. Asthmatic subject 1.

The paper chromatograms are run in BuA and Pr.Py and stained with p-nitroaniline reagent. A shows the eluant prepared from the first urine collection. There is no evidence of 1-methyl, 4-imidazole acetic acid. B shows the eluant prepared from the urine extract after corticotrophin therapy and 1-methyl, 4-imidazole acetic acid is present.

Figure 38.

A


Nov 1
Gluan B·YSN
HAc

B

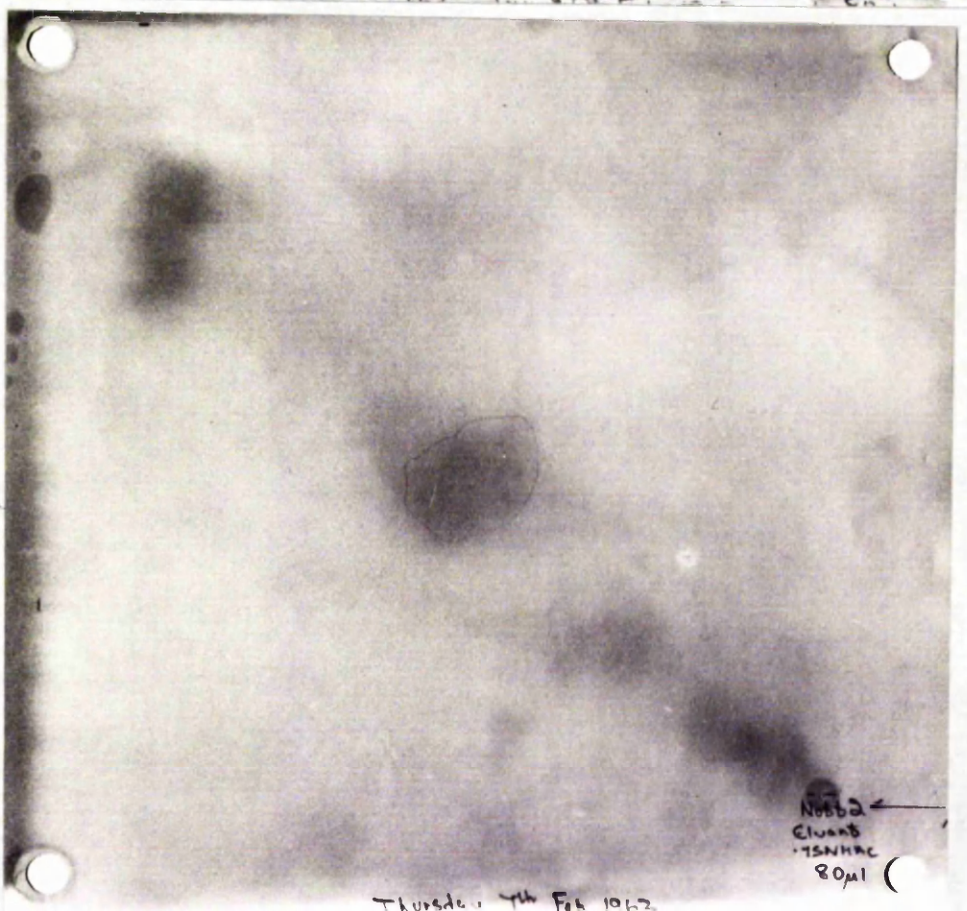

Nov 2
Gluan
·YSNHAc
80µl
Thursday 7th Feb 1963

Figure 39. Asthmatic subject 3.

Two paper chromatograms are run in BuA and Pr.Py and stained with p-nitroaniline reagent. A shows the eluant prepared from the first urine collection. There is no evidence of 1-methyl, 4-imidazole acetic acid. B shows the eluant prepared from the urine extract after corticotrophin therapy and 1-methyl, 4-imidazole acetic acid is present.

Figure 39.

A


Geraghty
Cluard 7SN
HAC
80M!

B


Geraghty 3C
Cluard
7SNHAC
80M!
Thursday 4th Feb 1963

Figure 40. Asthmatic subject 5.

Two one dimensional chromatograms run in i-Pr.Am and stained with p-nitroaniline stain.

There is no evidence of 1-methyl, 4-imidazole acetic acid in chromatograms 1 and 2. In chromatograms 5, 6 and 7 the eluants from the urine extract after corticotrophin small quantities of 1-methyl, 4-imidazole acetic acid are present. In chromatogram 3 the first eluant fraction after treatment is spotted together with 1-methyl, 4-imidazole acetic acid and the known and unknown run as one spot. 4 and 8 are control spots with 1-methyl, 4-imidazole acetic acid.

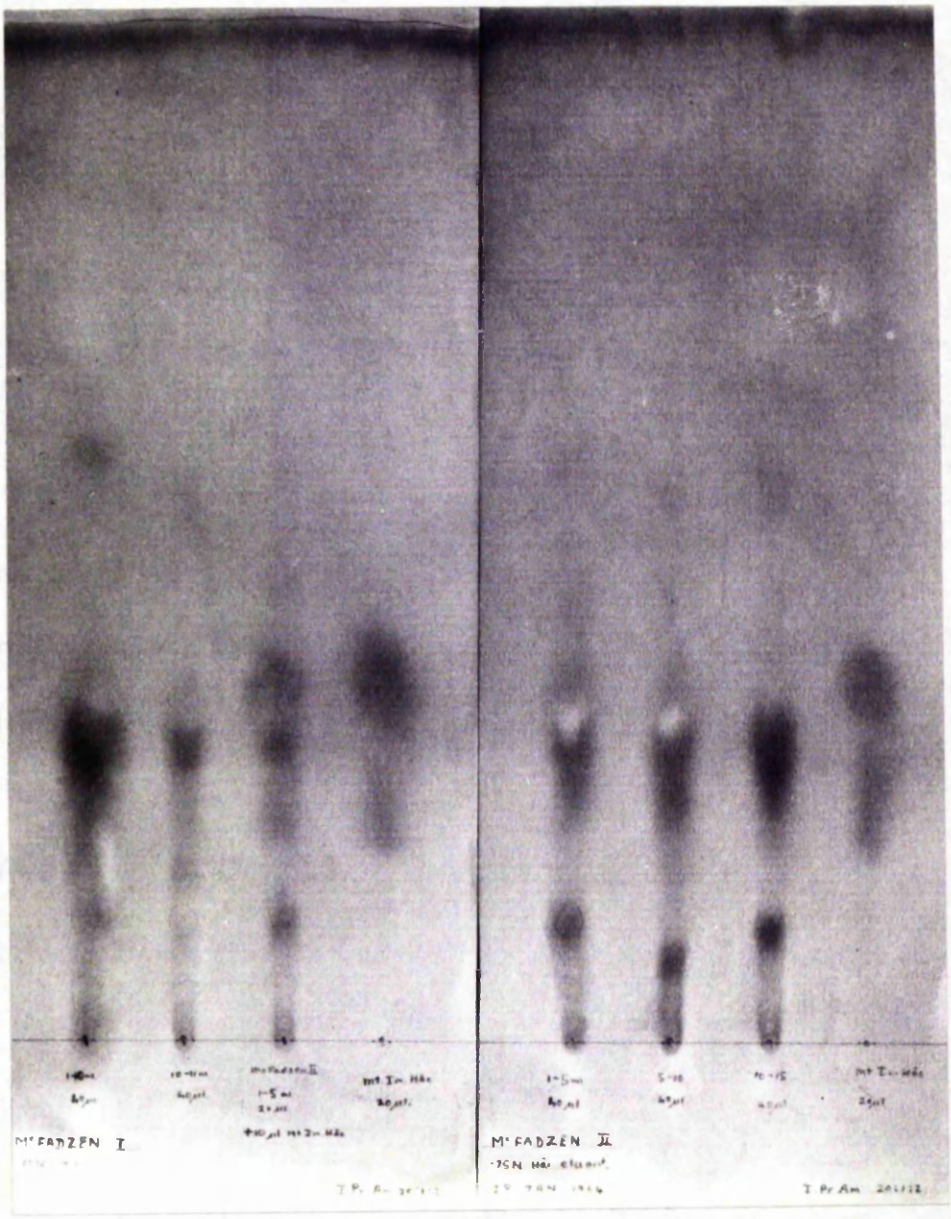


Figure 40. Asthmatic subject 5.

Figure 41. Asthmatic subject 7.

The paper chromatograms are run in BuA and Pr.Py and stained with a p-nitroaniline reagent. A shows the eluant prepared from the first urine collection. There is no evidence of 1-methyl, 4-imidazole acetic acid. B shows the eluant prepared from the urine extract after corticotrophin therapy and 1-methyl, 4-imidazole acetic acid is present.

Figure 41.

A


D. Young 1
Eluant 7SN
HAc Ist part
80μl

B

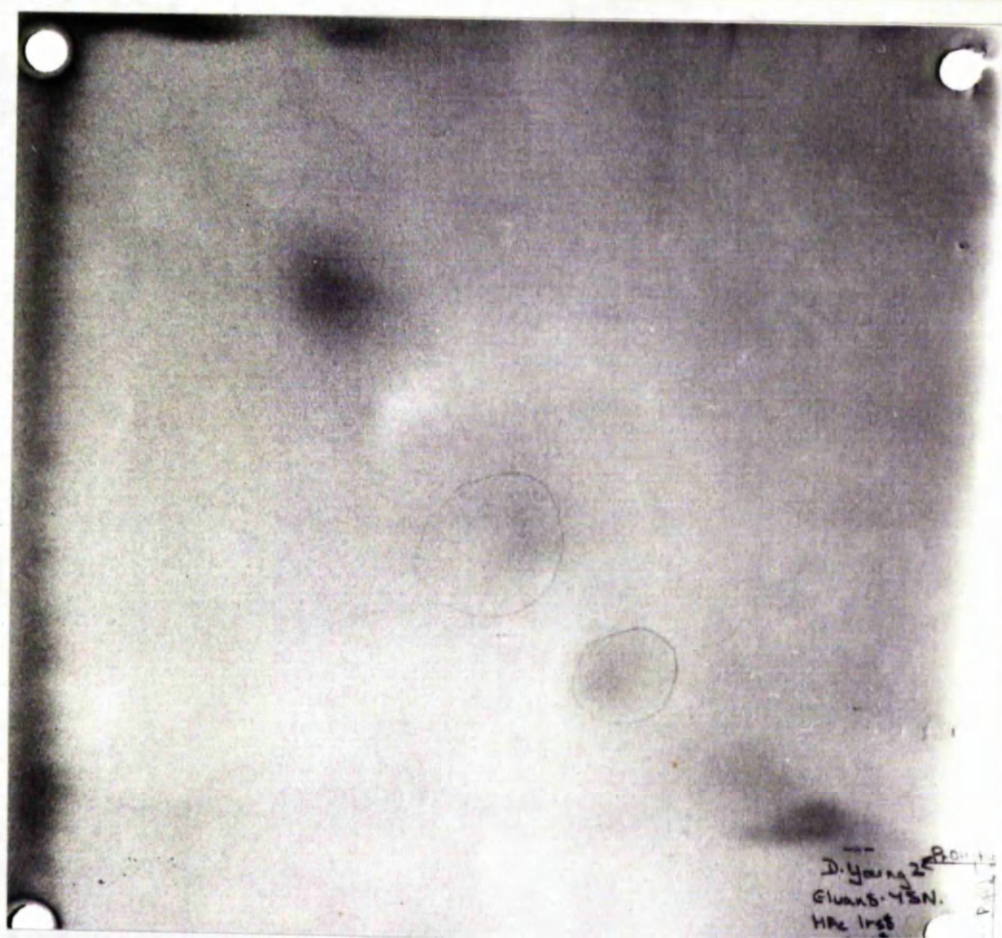
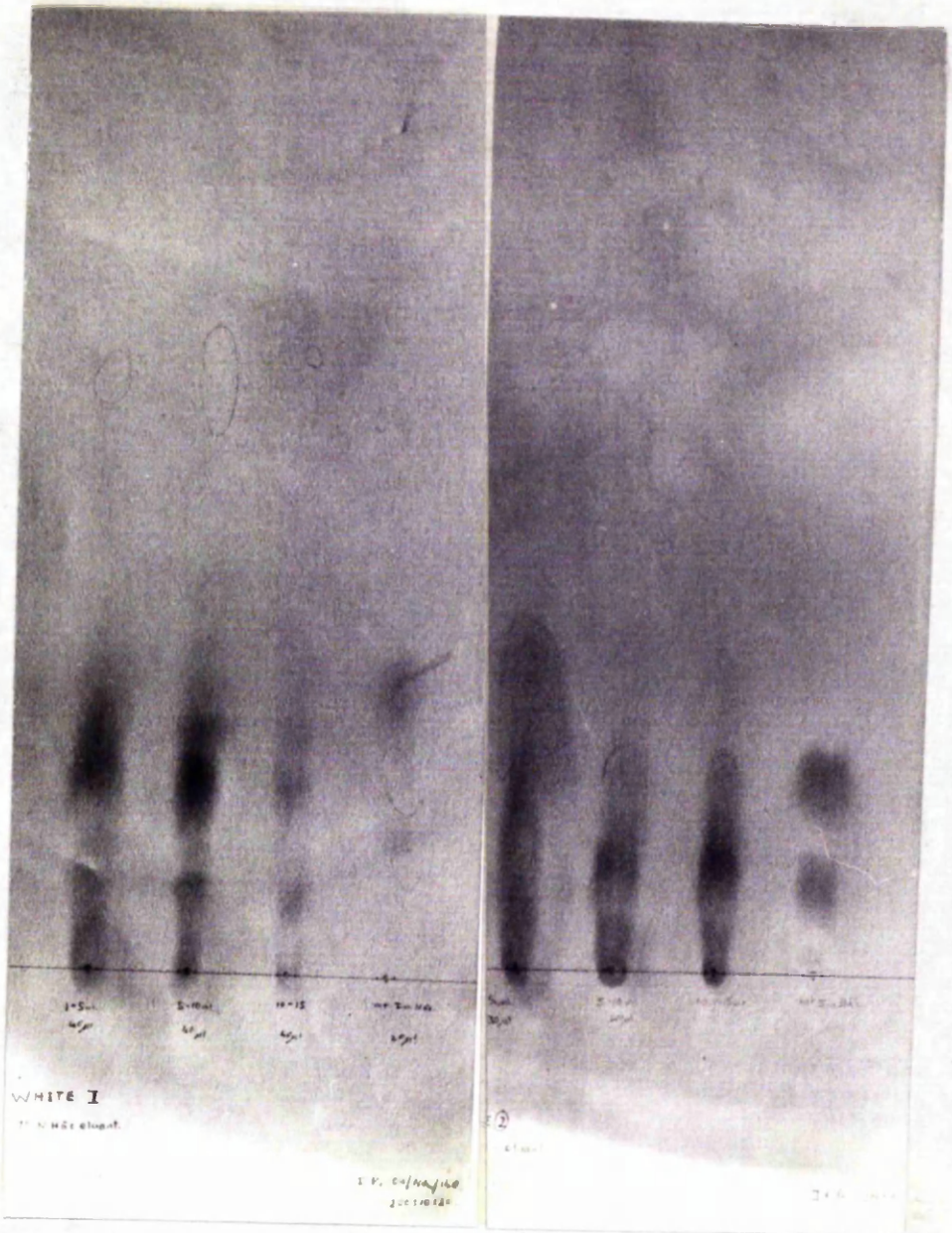

D. Young 2
Eluant 4SN.
HAc Ist

Figure 42. Asthmatic subject 8.

Two one dimensional chromatograms run in i-Pr.Am and located with p-nitroaniline reagent.

A. The first three eluant fractions show no evidence of 1-methyl, 4-imidazole acetic acid.

B. The eluant fractions prepared after corticotrophin therapy. The first, second and third fractions contain 1-methyl, 4-imidazole acetic acid.



A B

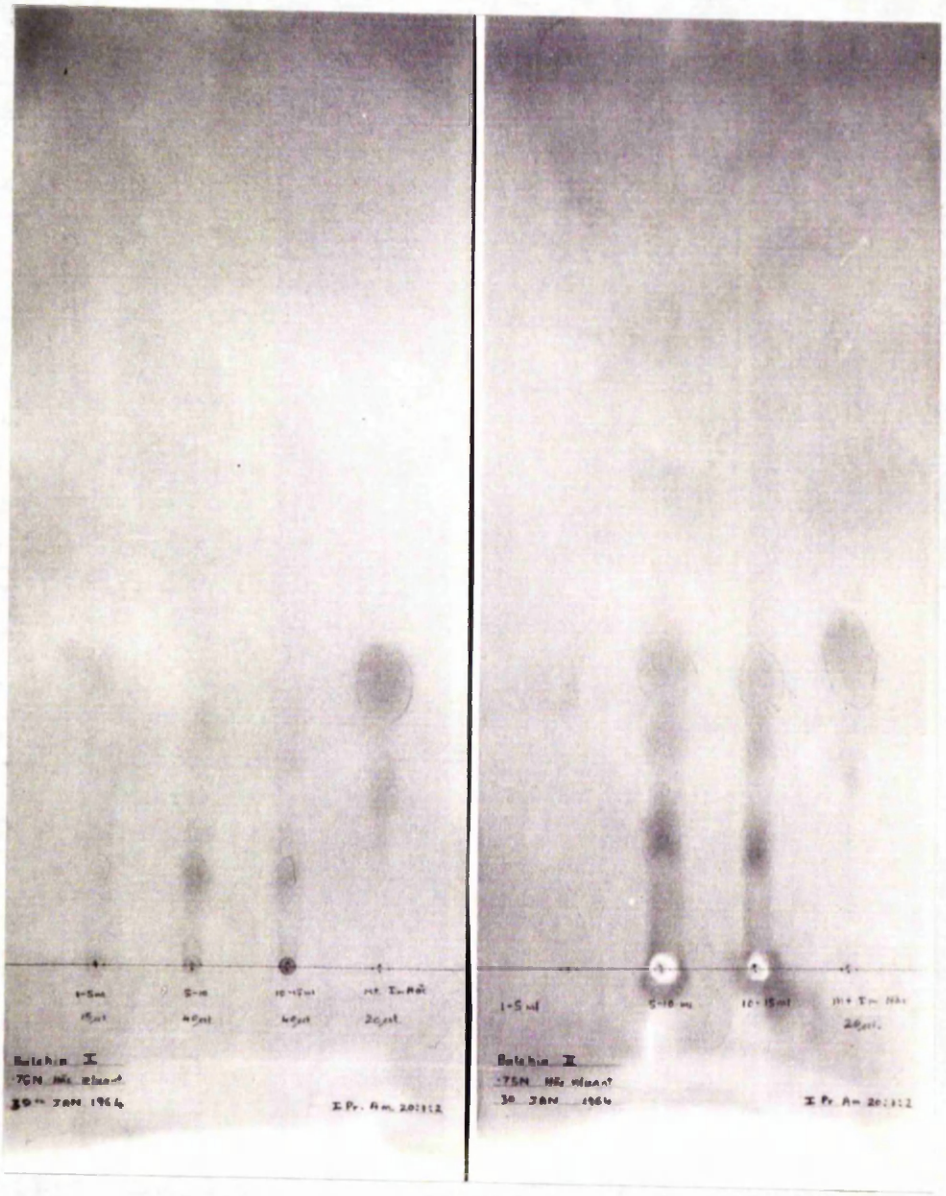
Figure 42. Asthmatic subject 8.

Figure 43. Asthmatic subject 9.

Two one dimensional chromatograms run in i-Pr.Am and located with p-nitroaniline reagent.

A. The first three eluant fractions show no evidence of 1-methyl, 4-imidazole acetic acid.

B. The eluant fractions prepared after corticotrophin therapy. The second and third fractions contain 1-methyl, 4-imidazole acetic acid.



A B

Figure 43. Asthmatic subject 9.

Measurement of recovery of Uroconic Acid

from Asthmatic Subject 1 (J.N.).

Measurement of recovery of urocanic acid from paper chromatogram of asthmatic subject 1 (J.N.).

1. The recovery from 60 ug. of urocanic acid spotted in on control chromatogram.

Eluted material diluted 1 in 10. OD = 475 mμ.

C = concentration in mg./ml. ε = 18,800

and MW = 174. OD = optical density in mu.

$$\varepsilon = \frac{MW \times OD}{C \times \text{cell path}}$$

$$\therefore 18,800 = \frac{174 \times 0.475}{C \times 1} \times 10$$

$$18,800 \times C = 174 \times 4.75$$

$$\therefore C = 0.04395 \text{ mg./ml.}$$

$$C \quad 44 \text{ μg./ml.}$$

$$\therefore \text{percentage recovery} = \frac{44}{60} \times 100$$

$$= 73\%$$

2. The urocanic acid recovered from asthmatic patient 1 was diluted 1 in 25. OD = 355 mμ.

$$\therefore 18,800 = \frac{174 \times 0.355}{C \times 1} \times 25$$

$$18,800 \times C = 174 \times 0.355 \times 25$$

$$\therefore C = 0.08213 \text{ mgm/ml.}$$

= 82 µg./ml.

82 µg./ml. represents 73% recovery

∴ 100% recovery = 112.3 µg./ml.

The volume of eluant from which chromatogram was spotted = 3 ml.

∴ Total urocanic acid in eluant = (112.3 x 3) µg.

= 336.9 µg.

This eluant represents a third of total urinary output.

∴ Total urocanic acid = 336.9 x 3

= 1010.7 µg.

Brief clinical details of asthmatic subjects used for the investigation of histamine metabolism in Part I and II.

Subject 1. J.N. (M) Birth 14.5.33. School Teacher.

This patient had infantile eczema and his asthma developed when he was one year old. Both conditions have been troublesome throughout his life. He is not aware of any family history of allergy.

He is a nasal carrier of coagulase positive staphylococci.

Blood: W.B.C. = 10,000/c.mm. Eosinophils 1070/c.mm.

Skin tests: Positive reactions to house dust and grass pollens.

Respiratory function: V.C. = 4.55 l. and has a variable FEV_1 sec. depending on the degree of airways obstruction.

The atopic eczema has responded well to measures to control the staphylococcal carrier state and topical steroids. Desensitisation to house dust and grass pollens has been of some benefit. He has been on small doses of prednisolone since 1955 although in the past 2 years he has only required intermittent steroid therapy.

Subject 2. I.P. (M) Birth 4.10.31. Journalist.

This patient developed asthma and atopic eczema when 5 years old. The asthma tends to be worse in the summer months and exposure to animal dander can precipitate wheezing attacks. Between 18 and 28 years he had very little breathlessness but since 1959 the seasonal asthma has been much more troublesome. Bronchial infections and emotional difficulties can precipitate attacks of wheezing.

He is a nasal carrier of coagulase positive staphylococci.

Blood: W.B.C. = 12,600/c.mm. Eosinophils 1330/c.mm.

Skin tests: Positive reactions to house dust, grass pollens and hormodendrum. No precipitins to aspergillus or Farmer's Lung antigens.

Respiratory function: Best V.C. = 3.4 l. and FEV_1 sec. variable depending on degree of airways obstruction.

He has been desensitised to house dust and grass pollens but still requires occasional courses of prednisolone.

Subject 3. M.G. (F) Birth 13.10.38. Chemist's Assistant. This patient has had infantile eczema and asthma since infancy. There have been acute exacerbations of both throughout her life. The home environment was poor and psychological factors together with bronchial infections aggravated her symptoms. There is a family history of asthma and eczema.

When first admitted to the Western Infirmary she had a right upper lobe staph. aureus pneumonia and was a nasal staphylococcal carrier.

Blood: W.B.C. = 9,900/c.mm. Eosinophils 840/c.mm.

Skin tests: Positive skin tests to house dust and grass pollens.

Respiratory function: V.C. = 2.05 l. FEV_1 sec. seldom more than 60 per cent of the vital capacity.

The atopic eczema cleared completely when the infective element was coped with. With a change of environment, the patient going into lodgings, and desensitisation to house dust and grass pollens the patient had much less trouble with her asthma.

Subject 4. D.T. (F) Birth 14.5.40. Medical Student.
This patient had infantile eczema which cleared when she was 5 years old but recurred when she was 17 years old. Bronchial asthma developed when she was 2 years old and has been troublesome throughout her life. The asthma was aggravated in the summer months and on exposure to cat. In 1961 she had a tuberculous synovitis of the left foot. There is a family history of asthma and eczema.
This patient was a nasal carrier of staphylococcus aureus and an inguinal lymph node biopsy showed a lipomelanic reticulosis due to the chronic infection complicating the atopic eczema.

Blood: W.B.C. = 10,400/c.mm. Eosinophils 2080/c.mm.

Skin tests: Showed immediate positive reactions to house dust, cat fur and fish extracts.

Respiratory function: V.C. = 2.9 l., FEV_1 sec. variable, the best reading being 1.72 l.

Once the infection and carrier state had been dealt with the skin lesion cleared with topical steroids and has not recurred. This patient's asthma has been less troublesome in recent years and she has graduated and completed her pre-registration posts.

Subject 5. E. McF. (F) Birth 8.3.37. Almoner/Housewife. This patient has had asthma since she was 18 months old. There is no history of atopic eczema. There is a family history of asthma and hay fever. Throughout her life she has had frequent wheezing attacks and at University had almost continuous low grade wheeze. More severe attacks are related to infections and are

associated with a purulent sputum. Exposure to house dust aggravates the wheezing.

Nasal swabs cultured staphylococcus aureus.

Blood: W.B.C. = 6,900/c.mm. Eosinophils 955/c.mm.

Skin tests: Positive to house dust.

Respiratory function: V.C. = 2.21 l. and the best FEV_1 recorded is 1.85 l.

Although this patient has bronchial asthma there is no doubt that there is also an irreversible element in the airways obstruction in this case.

Subject 6. H.T. (M) Birth 18.8.43. Barman.

This man has been liable to attacks of bronchial asthma since he was seven years old. There is no history of atopic eczema and he is not aware of a family history of allergy. He had 3 admissions to hospital with acute exacerbations of his asthma and on one occasion had a staphylococcal lobar pneumonia with abscess formation which responded well to antibiotic therapy. This patient is believed to have died from an acute asthmatic attack whilst on a cycling holiday.

Blood: W.B.C. = 12,000/c.mm. Eosinophils 1500/c.mm.

Skin tests: Showed him to be markedly sensitive to house dust and grass pollens.

Respiratory function: V.C. = 4.1 FEV_1 sec. 2.94 l.

Subject 7. D.Y. (F) Birth 13.2.41. Hairdresser.

This patient had infantile eczema which cleared when she was 2 years old. Her asthma began when she was 10 years old and although worse in the summer months she was never free from wheezing for more than a few weeks. There was little interference with schooling. There is a family history of asthma.

Blood: W.B.C. = 10,400/c.mm. Eosinophils 810/c.mm.

Skin tests: These gave immediate positive reactions to house dust, aspergillus. (No arthus reaction to aspergillus)

Respiratory function: V.C. = 2.75 l.; FEV_1 sec. variable and best recorded = 1.8 l.

X-ray of the chest shows a non specific apical fibrosis.

This patient responded well to a change of environment when she came to Glasgow and was free of serious wheezing for over a year. She has had more wheezing in recent months and has required short courses of steroid therapy.

Subject 8. M.W. (F) Birth 18.2.35. Housewife.

This patient had attacks of asthma but no atopic eczema or hay fever since she was 7 years old. After 15 years old she had only one attack of wheezing yearly until October 1961 when her asthma recurred and has remained persistent since then except when she is on steroid therapy.

Blood: W.B.C. = 6,600/c.mm. Eosinophils 700/c.mm.

Skin tests: Immediate reaction to house dust.

Respiratory function: V.C. = 3.7 l. FEV_1 = 2.3 l.

This patient responded well to steroid therapy following admission to hospital in status asthmaticus. She was admitted five times to hospital over a two year period with acute asthma. On each occasion respiratory function was normal at

time of discharge.

Subject 9. D.B. (M) Birth 27.4.56. Schoolboy.

Infantile eczema developed in this boy when he was 8 months old and has been troublesome since then. Bronchial asthma first noted when 3 years old but not really troublesome till 5 years of age. Although not seasonal the asthma was more troublesome in the summer months. There is a family history of hay fever and asthma.

Blood: W.B.C. = 7,300/c.mm. Eosinophils 1500/c.mm.

Skin tests: Showed immediate reactions to house dust, grass pollens and hormodendrum.

Respiratory function: V.C. = 2.4 l. FEV_1 sec. variable the best recorded being 1.5 l.

Nasal swabs: Coagulase positive staphylococcus aureus.

The atopic eczema responded well to local corticone and measures to deal with the staphylococcal carrier state. He was desensitised to grass

pollens which relieved the summer exacerbations of asthma but he still required occasional courses of corticotrophin gel to control the asthma.

Subject 10. Dr. N.W. Birth 8.11.26. Physician.

This patient has suffered from atopic eczema and asthma throughout his life. The eczema is still troublesome but usually easily controlled by topical steroid. The asthma has not been severe in recent years but he requires to use some form of broncho-dilator daily.

Blood: W.B.C. = 8,000/c.mm. Eosinophils 940/c.mm.

Skin tests: Show immediate positive reactions to house dust, grass pollens and cat dander.

Respiratory function: Show a variable degree of airways obstruction.

Nasal swabs: Negative for staph. aureus.

This doctor is a pharmacologist and volunteered

for the C^{14} labelled histamine investigations.

Subject 11. N.W. Birth 18.9.43. Salesman.

This patient gives a history of asthma and eczema which dates from childhood. The atopic eczema tends to be worse in the Spring but the asthma is a non-seasonal complaint usually being aggravated by bronchial infections.

Blood: W.B.C. = 6,500/c.mm. Eosinophils 690/c.mm.

Skin tests : Grass pollens, cat fur, egg and milk all gave marked immediate positive reactions.

Respiratory function: V.C. = 4.8 l. and the best FEV_1 = 3.5 l.

Nasal swabs: Coagulase positive staph. aureus.

This patient's atopic eczema improved considerably when the staphylococcal carrier state had been dealt with. He was advised to be desensitised to grass pollens.

Subject 12. M.R. Birth 31.3.28. Unemployed.

This patient has hypercholesterolaemia and has

had several myocardial infarctions. He was known to have had asthma in childhood in India and this recurred in 1963. Since then he has had occasional mild attacks of asthma requiring admission to hospital on one occasion

Blood: W.B.C. = 5,900/c.mm. Eosinophils = 625/c.mm.

Skin tests: Immediate positive reactions to house dust and grass pollens.

This patient was used only for the investigations with C^{14} labelled histamine during his admission with acute asthma. The major problem here is the hypercholesterolaemia.

Statistical analysis of the Vital Capacity and Forced Expiratory Volume 1 Second reading after the infusion of 3 μc. C^{14} histamine dihydrochloride in six asthmatic subjects and seven controls.

1. The comparison of two means.

$$S^2 = \frac{1}{n_1 + n_2 - 2} \left\{ S(x-\bar{x})^2 + S(x^1-\bar{x}^1)^2 \right\}$$

$n_1 = 6$ $\quad$ x = VC or FEV_1 (Asthma)

$n_2 = 7$ $\quad$ x^1 = VC or FEV_1 (Control)

S^2 = variance

Vital Capacity

10 Minutes VC

$$S^2 = \frac{1}{11} \left[578.86 + 694.84 \right]$$

$$= \frac{1}{11} \times 1273.70 = 115.79$$

$$\therefore S = \sqrt{115.79} = 10.76$$

20 Minutes VC

$$S^2 = \frac{1}{11} \left[397.2 + 650.22 \right]$$

$$= \frac{1}{11} \times 1047.42 = 95.22$$

$$\therefore S = \sqrt{95.22} = 9.7580$$

40 Mimutes VC

$$S^2 = \frac{1}{11} \left[958 + 1557.06 \right]$$

$$= \frac{1}{11} \times 2515.06 = 228.64$$

$$\therefore S = \sqrt{228.64} = 15.120$$

60 Minutes VC

$$S^2 = \frac{1}{11} \left[148 + 679.34\right]$$

$$= 75.21$$

$$\therefore S = \sqrt{75.21} = 8.6724$$

Forced Expiratory Volume (FEV) 1 Second

10 Minutes FEV_1

$$S^2 = \frac{1}{11} \left[327.40 + 801.50\right]$$

$$= \frac{1}{11} \times 1128.90 = 102.62$$

$$\therefore S = \sqrt{102.62} = 10.129$$

20 Minutes FEV_1

$$S^2 = \frac{1}{11} \left[199 + 1200.80\right]$$

$$= 127.25$$

$$\therefore S = \sqrt{127.25} = 11.278$$

40 Minutes FEV_1

$$S^2 = \frac{1}{11} \left[595.40 + 1250.80\right]$$

$$= 167.83$$

$$\therefore S = \sqrt{12.953}$$

60 Minutes FEV_1

$$S^2 = \frac{1}{11} \left[329.54 + 1210.84\right]$$

$$= 140.03$$

$$\therefore S = \sqrt{140.03} = 11.832$$

2. t-Tests on VC and FEV_1 second of Asthmatic Subjects and Controls.

$$t = \frac{\left[\bar{x}-\bar{x}^1\right]}{S} \sqrt{\frac{n1\ n2}{n1 + n2}}$$

For 11 degrees of Freedom

t 0.05 = 2.201

t 0.02 = 2.718

t 0.01 = 3.106

Vital Capacity

10 Minutes VC

$$t = \frac{3.14 - (15.17)}{10.761} \times 1.7892 = \frac{18.31}{10.761} \times 1.7892$$

$= 1.701 \times 1.7892 = 3.0434$

Significant at t 0.02 level

20 Minutes VC

$$t = \frac{2.28 - (-23.2)}{9.758} \times 1.7892 = \frac{25.48}{9.76} \times 1.7892$$

$= 2.610 \times 1.7892 = 4.6698$

Significant at t 0.01 level

40 Minutes VC

$$t = \frac{4 - (-24.4)}{15.12} \times 1.7892$$

$= 1.878 \times 1.7892 = 3.3601$

Significant at t 0.01 level

60 Minutes VC

$$t = \frac{0 - (-16.67)}{8.6724} \times 1.7892$$

$= 1.92 \times 1.7892 = 3435$

Significant at t 0.01 level

FEV_1 second

10 Minutes

$$t = \frac{5.29 - (-15.5)}{10.12} \times 1.7892$$

$$= 3.675$$

Significant at t 0.01 level

20 Minutes

$$t = \frac{1 - (-24.8)}{11.278} \times 1.7892$$

$$= 2.28 \times 1.7892 = 4.079$$

Significant at t 0.01 level

40 Minutes

$$t = \frac{1.29 - (-29.2)}{12.953} = \frac{30.49}{12.953} \times 1.7892$$

$$= 2.354 \times 1.7892 = 4.2117$$

Significant at t 0.01 level

60 Minutes

$$t = \frac{-1.29 - (-22.83)}{11.832} \times 1.7892$$

$$= 1.82 \times 1.7892 = 3.2563$$

Significant at t 0.01 level

Conclusion

The change in VC and FEV_1 second at 10, 20, 40 and 60 Minutes following the infusion of C^{14} histamine is statistically significant when the asthmatic subjects are compared to controls.

TABLE 11

A. The change in Vital Capacity in six asthmatic subjects after an intravenous infusion of 50 µg. histamine dihydrochloride.

Vital Capacity in litres

Time in Minutes	Subject					
	1	2	3	4	5	6
0	3.50	3.25	3.65	3.00	4.70	3.30
5	3.10	2.50	2.55	2.75	4.45	3.00
10	2.55	2.50	2.45	1.60	4.00	2.95
20	3.40	2.85	2.70	2.85	3.60	3.00
40	-	3.20	3.35	2.95	3.75	3.30

B. The change in Vital Capacity after an intravenous injection of 5 mg. phentolamine followed by an intravenous infusion of 50 µg. histamine dihydrochloride.

Time in Minutes	Subject					
	1	2	3	4	5	6
0	3.45	3.20	3.00	2.80	4.70	3.15
5	3.35	2.95	2.95	2.95	4.35	3.10
10	3.30	2.95	3.25	2.95	4.50	3.25
20	3.70	2.35	2.95	3.10	4.15	3.00
40	3.80	2.90	3.15	2.95	3.85	3.10

For 5 degrees of freedom $t_{.05} = 2.57$

$t_{.02} = 3.36$

$t_{.01} = 4.03$

At 0 min. $t = 1.95$; 5 min. $t = 2.57$; 10 min. $t = 4.45$

20 min. $t = 0.95$; 40 min. $t = 1.06$.

At 5 and 10 minutes there is a significant difference in the Vital Capacity at the 5 per cent and 2 per cent levels respectively. Thus phentolamine significantly inhibits the fall in Vital Capacity due to the histamine infusion at 5 and 10 minutes.

TABLE 12

A. The change in FEV_1 second in six asthmatic subjects after an intravenous infusion of 50 µg. histamine dihydrochloride.

FEV_1 in litres

Time in Minutes	Subject					
	1	2	3	4	5	6
0	1.90	2.40	2.25	1.65	2.20	2.50
5	1.35	2.05	1.55	1.65	2.15	2.30
10	1.20	2.05	1.20	1.60	2.05	2.10
20	1.60	2.25	0.85	1.15	1.75	2.15
40	-	2.35	2.20	1.70	1.90	2.20

B. The change in FEV_1 second after an intravenous injection of 5 mg. phentolamine followed by an intravenous infusion of 50 µg. histamine dihydrochloride.

Time in Minutes	Subject					
	1	2	3	4	5	6
0	1.70	2.35	1.85	2.00	3.10	2.55
5	1.80	1.80	2.10	2.15	2.60	2.55
10	1.90	1.90	2.00	2.30	2.75	2.35
20	1.90	1.75	2.15	2.30	2.75	2.15
40	1.85	1.90	1.90	2.35	2.35	2.45

For 5 degrees of freedom $t_{.05} = 2.57$

$t_{.02} = 3.36$

$t_{.01} = 4.03$

At 0 min. $t = 1.55$; 5 min. $t = 2.69$; 10 min. $t = 3.29$

20 min. $t = 5.81$; 40 min. $t = 1.66$.

At 5 and 10 minutes there is a significant difference in the FEV_1 second at the 5 per cent level and at 20 minutes the difference is significant at the 1 per cent level, so that phentolamine significantly inhibits the fall in FEV_1 due to the histamine infusion.

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