ON HISTAMINE IN COTTON DUST, AND IN THE BLOOD OF COTTON WORKERS

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(With 5 Figures in the Text)

Introduction

The possible relationship of respiratory disease in cotton workers to their employment, and in particular to the dusty conditions under which the strippers and grinders often operate in the card rooms, has long been a problem of considerable importance. In the past 6 years several relevant investigations have been carried out in these laboratories.

The presence of histamine, or of a "histamine-like substance"—so like it that for convenience we have long called it histamine—was detected in cotton-dust extracts, prepared from card-room dust, by Heap *et al.* (1932) by pharmacological methods. Such methods are of the order of one thousand times as sensitive as the most delicate of the known chemical tests, and also more specific. The further examination by Macdonald & Maitland (1934) of cotton, coir, and esparto-grass dust indicated that cotton dust was much richer in histamine than the others, and that in two dusts which were fractionated in accordance with particle size, the histamine was most plentiful in the finest fraction consisting of particles under 20μ in length.

These findings have been considerably amplified by Prausnitz (1936), whose report should be seen for a consideration of the whole problem, and a description of the classical symptoms of "stripper's asthma" in its various stages (p. 8). His method of histamine extraction, using larger scale apparatus, was less complete and gave a lower yield than the Soxhlet extraction previously employed, but was less open to possibilities that the histamine was not preformed. The yield he obtained was of the order of 0·003–0·02 mg. per 100 g. of husks, kernels, and delinted seed, but the card-room sliver was histamine-free. Much higher figures have been reported previously.

Prausnitz, in his report, concludes that "stripper's asthma" is a true allergic supersensitiveness to the protein of the dust, and that the role of histamine in causing the typical disease was doubtful. This paper presents work carried out along two lines: (*a*) the confirmation of the presence of histamine by its separation, purification, and chemical identification; and (*b*) the investigation of the blood histamine of card-room workers, and of controls.

It is believed that the results justify this further attention to the histamine side of the problem.

SEPARATION OF CRYSTALLINE HISTAMINE

Two methods have been followed by one of us (E. H.) for the chemical identification of the histamine. Most of the steps have been checked by biological tests.

(1) 10 kg. of fine cotton dust were boiled for 12 hours with 3 per cent HCl. Extraction of the concentrated filtrate with 2 parts of ether and 1 of 3 per cent HCl separated the tarry materials into the ether layer and the basic substances into the acid. The method of Best *et al.* (1926–7) for the extraction of histamine from ox liver was then applied, but it was found that the depressor substance had been absorbed on the precipitate formed when H_2SO_4 is added to give a 5 per cent solution prior to the addition of phosphotungstic acid, and on the crystals obtained by the concentration of this solution. The amount of depressor substance there, however, did not seem sufficient to make re-extraction of these two precipitates worth while; in all the numerous stages of the extraction, a certain proportion of the histamine is inevitably lost.

(2) A further 5·5 kg. of fine dust were similarly treated until after the basic lead acetate precipitate had been filtered off.

It was found at this stage that acidification of the solution with concentrated HCl gave a dense precipitate of small white crystals. After filtration, a portion was tested with H_2S for lead but none was present.

The acid solution was evaporated down when a few more crystals were deposited. These were filtered off and the filtrate taken to dryness *in vacuo*. Three portions of about 40 c.c. of 98·5 per cent alcohol were distilled off *in vacuo* to dry the residue completely and to remove most of the HCl. The residue was extracted with 98·5 per cent alcohol, made just acid to litmus with *N* H_2SO_4 by boiling under a reflux for $1\frac{1}{2}$ hours. The supernatant liquid was decanted off and filtered. This was repeated three times and at the end of the fourth extraction the whole contents of the flask were filtered. The combined filtrates were heated on a water-bath to remove the alcohol. Water was added to the residue and the H_2SO_4 removed as barium sulphate. The solution was still acid, so it was neutralized with NaOH and made just acid again with *N* H_2SO_4. The solution was concentrated and left for a week. At the end of this time one or two large crystals that looked like sodium sulphate had formed. These were filtered off and any sulphate ions removed as barium sulphate. The neutral filtrate was made up to a convenient volume—60 c.c.

10 c.c. of this were taken, 3 g. solid NaOH were added and the mixture extracted six times with redistilled amyl alcohol using 20 c.c. for each extraction. The addition of 3 g. solid NaOH to 10 c.c. of the concentrated solution gave a very heavy precipitate which made the separation long and difficult. It was found better to do the separation in a medium size centrifuge tube and after each extraction to spin it at a low rate for a few seconds on the centrifuge and to pipette off the supernatant liquid. The combined extracts were then extracted with *N* H_2SO_4 five times, using 20 c.c. for the first and 10 c.c. for

subsequent extractions. This was repeated until all the initial solution had been extracted. This method of amyl alcohol extraction was part of the extraction used by Koessler & Hanke (1920) for the quantitative colorimetric estimation of histamine in protein and protein-containing material. This removes the histamine from the histidine almost completely. The combined H_2SO_4 extracts were reduced in bulk and the H_2SO_4 removed as barium sulphate. A small portion of the concentrated H_2SO_4 extract was tested for histamine and found positive, using Hunter's (1928) modification of the Pauly reaction.

After the positive Pauly reaction, sufficient sodium sulphate solution was added to the solution to precipitate the excess barium completely and the mixture digested on the water-bath for 1 hour. The precipitate was filtered off and washed with hot water. The filtrate was reduced in bulk to about 50 c.c. and exactly neutralized with NaOH. It was then evaporated to dryness *in vacuo*.

The perfectly dry residue was treated with 30 c.c. of methyl alcohol and 1·5 g. caustic potash. The alkaline mixture was treated with 400 c.c. of redistilled chloroform and left in the ice-chest for a day, when it was filtered through a small folded filter paper and the residue washed with 400 c.c. of hot chloroform. A few drops of 37 per cent HCl were added to the chloroform extract and distilled *in vacuo* to remove the chloroform and methyl alcohol. Water was added to the residue and distilled off *in vacuo* to remove the methyl alcohol completely and most of the HCl.

The residue was taken up in a small amount of water and divided into two parts.

A saturated solution of sodium picrate was added to the first portion drop by drop. A cloudy precipitate formed, from which, on standing, drops of oil appeared on the bottom of the flask. More sodium picrate solution was added and the solution left to crystallize slowly. The oil solidified and small, hexagonal, pale yellow crystals formed on it. The mother liquor was pipetted from the crystals on to a watch-glass and allowed to crystallize. A pale yellow feathery-shaped crystal composed of small hexagonal plates was formed. This was carefully extracted from the mother liquor and dried first on filter paper and then in the hot-air oven. Some of the first set of crystals were also dried in the same manner and a melting-point was taken of each.

The first set of crystals which were contaminated with the solidified oil melted with decomposition at 205–210° C. The second set melted with decomposition at 237–239° C. This agrees quite well with the melting-point for histamine picrate given by Best *et al.* (1926–7). They give the melting-point of histamine dipicrate (recrystallized twice from water) as 241° C. with decomposition.

The crystals obtained were feathery and composed of small apparently monoclinic crystals. This is in agreement with the observations of Itallie & Steenhauer (1925), who describe histamine picrate as pale yellow feathery

crystals, and Takalasi *et al.* (1931), who describe histamine picrate as monoclinic and melting at 232–233° C. Unfortunately there was insufficient material for a recrystallization, or a mixed melting-point with pure histamine picrate.

To the second portion of the final extract a few drops of 37 per cent HCl were added and the solution allowed to crystallize. White crystals were obtained which melted with decomposition at 235–240° C. This agrees with the melting-point (233–240° C.) given for histamine hydrochloride by Klein & Boser (1932).

Here again the amount of hydrochloride obtained was insufficient to allow of a recrystallization and a mixed melting-point with pure histamine hydrochloride.

From the above results the substance in cotton-dust extracts which is pharmacologically the same as histamine is also chemically the same, as shown by the Pauly reaction and by the fact that its salts are identical in appearance and melting-point with those of histamine.

The biological detection of histamine in cotton dust thus receives chemical confirmation.

HISTAMINE IN BLOOD

A method for the estimation of histamine in blood was published recently by Barsoum & Gaddum (1935). The presence of histamine in whole blood cannot be demonstrated, but by their technique with Gaddum's modification, according to which concentrated HCl is substituted for normal acid, an isotonic and neutral solution can be obtained and its histamine content assayed on one or more biological indicators—an isolated segment of guinea-pig ileum in oxygenated Ringer-Tyrode, as suggested by Guggenheim & Löffler (1916), is probably as good and convenient as any, and we have used it in all cases.

Because of the small amounts of histamine which have to be estimated, a sensitive indicator in a small bath is desirable. We have used a bath for the gut which requires only 2 c.c. of Tyrode, and have found that with a sensitive muscle, such as that used for the record shown in Fig. 1, the response is measurably different in the sensitive range for increments of 0·005–0·01 c.c. of $H/5$ (a solution of histamine containing 0·2γ per c.c.). Such a muscle is therefore sensitive to differences of 0·001–0·002γ H in the 2 c.c.—a change in concentration of the order of 10^{-9}.

It was felt that a comparison between the normal blood-histamine figures and those for card-room workers, many of whom are daily exposed to a histamine-containing dust—a dust which Prausnitz has shown to be so fine that ventilation, however much improved, cannot give complete protection against it—might yield interesting information.

Barsoum & Gaddum give the figures of 0·03 and 0·04γ per c.c. (for convenience we prefer to take such figures in terms of γ per litre) as the histamine equivalent of human blood. A number of male university students were taken as normals, and in the first eight the blood histamine was found to vary between 21 and 63γ per litre. Such variation made it necessary to examine a con-

siderable series in order to know what limits might be regarded as normal. Over one hundred bloods were therefore assayed, and the results are given in

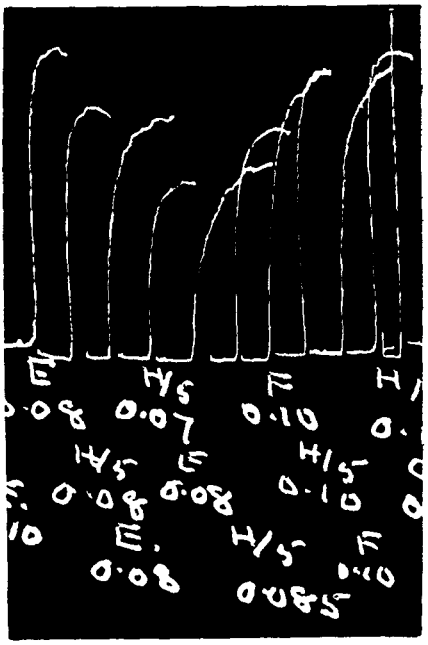

Fig. 1. Assay of blood histamine E (2·5 c.c., E represents 10 c.c. blood) against a histamine solution $H/5$ (0·2γ per c.c.) on sensitive segment of guinea-pig ileum. Comparing the first six contractions from left to right, E 0·08 > $H/5$ 0·08 > E 0·08 > $H/5$ 0·07 < E 0·08 < $H/5$ 0·085. From this we assume E 0·08 = $H/5$ 0·08, i.e. $E = H/5$, or 0·2γ per c.c., and the blood contains 0·05γ per c.c. or 50γ per litre.

the form of a distribution curve (Fig. 2). A few were re-examined at intervals, and these are tabulated (Table I) to show that, even when this interval is considerable, normal blood histamine does not vary greatly. Such a measure of agreement, with a fair range of blood histamines, gives the experimenter considerable confidence that he is measuring with fair accuracy a fairly constant blood constituent.

Table I. *Repeated estimations in normals*

Initials	Blood histamine in γ per litre: 1st estimation	2nd estimation	Interval days
G. R. C.	51	52	14
A. D. L.	21	21	23
G. K. T.	28	28	19
R. T. G.	70	67	19
D. B.	27	30	128
T. H. R.	38	33	23
S. E. G.	39	27	19
A. D. M.	19	19	5

The distribution curve (Fig. 2) was compiled during the collection of the figures. It suggested—especially at a middle stage of its preparation—that the histamine equivalent of a "normal" blood tended to fall into one of two

classes—between 20 and 40 or between 50 and 70γ per litre. Any such differentiation became less definite with increasing numbers of estimations, and it is improbable that a statistician would accept Fig. 2 as indicating the existence of two classes. It was felt, however, in many cases that one could foretell from the appearance of the donor whether his blood-histamine level would be high or not, but this, of course, does not guarantee the existence of two separable groups.

Distribution graphs for the histamine content of the blood

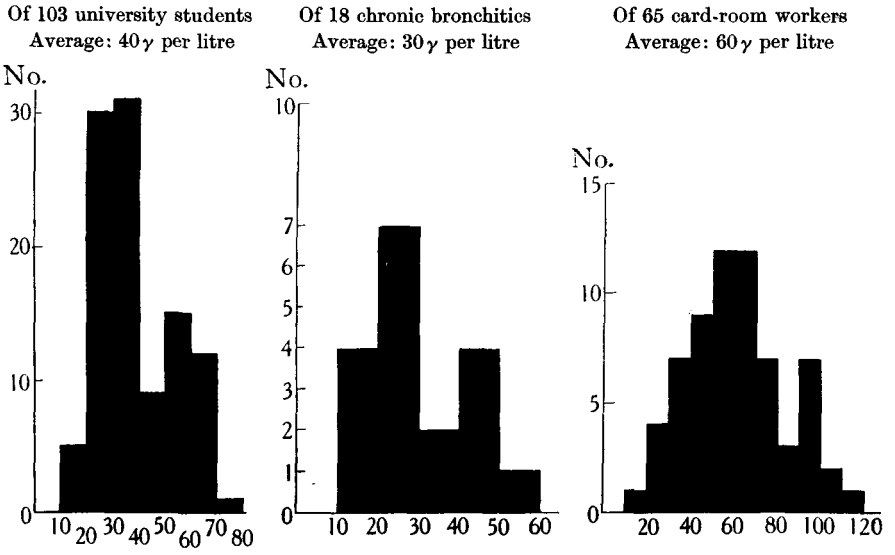

Fig. 2 Fig. 3 Fig. 4

University students obviously do not form an ideal control for card-room workers. They are younger, come from a more favoured section of the population, and lead very different lives from factory hands. The ideal control would be workers of similar ages from some other rather dusty occupation, but to get such in adequate numbers presented difficulties. We have, however, carried out tests on a number of hospitalized chronic bronchitics, older than the card-room workers, but whose respiratory disease was not directly attributable to dusty occupations. The distribution of the blood histamines in this group is shown in Fig. 3. The figures are not above those for students.

Through the kindness and co-operation of the officials of the Amalgamated Association of Blowing, Card, and Ring Room Operatives, blood samples for histamine assay were collected from over seventy card-room workers in the Bolton and Oldham areas. These men were by no means equally exposed to histamine-containing dust. Conditions vary with different cottons and different mills. Some had been at work on the day on which they were giving blood samples; others were on rather broken time, working only 3 days a week or less; many had been so incapacitated by respiratory disease that they had

been off work for some time. Some of this last section had made attempts to begin again but had been unequal to the work; others had tried to turn their hands to other occupations; still others were clearly unequal to any manual labour. Among the workers the stage of respiratory distress varied enormously, but scarcely one was free from at least some "tightness in the chest" and trouble on Monday afternoons; the worst were often late in arriving because of their difficulty in getting along from the mill, and especially up a stair; almost all were interested in our tests and feared that the dust would eventually be too much for them.

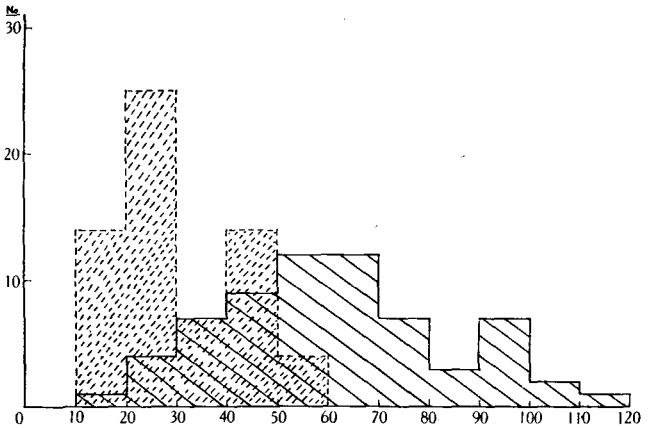

Fig. 5. Distribution curves for the blood histamines of 65 chronic bronchitics (calculated from Fig. 3) and the 65 card-room workers of Fig. 4. Only 25/65 of the range is common to the two groups.

The division of these operatives into such classes as

(*a*) working whole time in the card room,
(*b*) working part time there,
(*c*) working elsewhere, and
(*d*) not fit for work,

meant such reduction of the numbers in our groups as would make the figures obtained from them of doubtful significance. We have accordingly, rather regretfully, taken them as a single group—they are probably a reasonable cross-section of that portion of the population which has earned or is earning its living in card rooms. A study of the distribution figure for this cross-section (Fig. 4) leaves one in no doubt that there is very definitely raised blood histamine in card-room workers as compared with either university students or elderly chronic bronchitics. To emphasize this differentiation Figs. 3 and 4 are combined as Fig. 5, the chronic bronchitics of Fig. 3 being multiplied by a factor to make them equal in number to the card-room operatives. In Fig. 5 only about one-third of the field is common to the two groups; almost two-thirds of the group of card-room workers have a higher blood histamine than elderly chronic bronchitics.

A few of the card-room workers presented themselves for a second blood-

histamine assay. The two figures obtained for these and the intervals between the assays are set out in Table II. Without exception rather widely different

Table II. *Repeated estimations in card-room operatives*

Initials	Blood histamine in γ per litre		Interval days
	1st estimation	2nd estimation	
J. W.	63	33	90
J. H.	66	42	75
T. C.	56	95	27
W. H.	30	44	27
W. B.	58	75	75
W. R. B.	90	52	27

values were got for these men on the two occasions on which they were examined. Had such discrepancies been obtained in Table I we would have felt most unhappy about our technique or about the reliability of the method. Yet the routine of collection and assay had continued unaltered. While one is unwilling to base substantial theories on such a small series of patients it seems clear that the blood-histamine level is much more variable in card-room workers than in our controls. Such variation is probably quite significant, and may be related to the extent of the exposure to the histamine-containing dust, and to the interval between the collection of the blood and the last asthmatic attack.

Discussion

In the light of the Prausnitz report, one might regard the presence of histamine in cotton dust as a mere incidental to card-room respiratory disability—possibly almost as an unfortunate red herring innocently drawn across the trail in 1932. The confirmation of the presence of histamine in the dust and the probable presence in the blood of the card-room worker of more histamine than is usually found in human blood make one hesitate to suggest that the protein alone is responsible, especially when we remember the possible role of histamine in allergic phenomena.

Daly *et al.* (1935) have brought forward a great deal of evidence that a histamine-like substance is released from the perfused lungs of the sensitized guinea-pig in anaphylactic shock, and corroborating assays of this substance against histamine on a variety of biological indicators—cat's blood pressure, guinea-pig ileum and guinea-pig lung, yield "strong presumptive evidence that the substance released from the lungs is histamine". May it not be that "stripper's asthma" differs from most asthma—as it certainly does—in that the two factors are present, absorption of histamine and of the allergen which "touches it off"? On some such hypothesis it might even be possible to develop a working hypothesis for the difficult "Monday sickness". In the week-end, in the absence of exposure to the specific protein, there may well be some accumulation of the histamine in lung tissues—from the blood and/or the alveoli—and this may be liberated by Monday's contact with allergen. During the rest of the week there may be less accumulation, and less severe symptoms follow. This, at any rate, may be more acceptable than Prausnitz's

suggestion that the card-room worker, having breathed deeply of uncontaminated air in the week-end, has to learn painfully each Monday to breathe shallowly while at work. The suffering experienced by many workers every Monday would surely make such repeated lessons unnecessary.

Much work on such points needs to be done. It would be interesting to follow the variations in blood histamine in selected sufferers from "Monday sickness" in some detail. The figures we have given for bronchitics require extension, and a fresh series for asthmatics not exposed to cotton dust is needed. It is hoped to undertake further work on these lines in the near future.

Summary

Crystalline histamine picrate and hydrochloride have been prepared from cotton dust. The melting-points and crystalline structure found are in agreement with those obtained by other workers.

The blood histamines in sixty-five card-room workers have been estimated and compared with the figures found in 103 students and eighteen elderly chronic bronchitics. The figures for the three groups are shown in distribution figures, and the card-room workers have, on an average, a higher blood histamine than both groups of controls. It has, however, to be admitted that the differentiation of card-room asthma from other respiratory diseases on the basis of the level of blood histamine cannot be guaranteed in individuals.

Acknowledgements. It is a pleasure to put on record our sincere thanks to all who have so cheerfully given samples of their blood for these estimations, to the Medical Superintendents of Crumpsall and Withington Hospitals for facilities in their institutions, and especially to the officials of the Amalgamated Association of Blowing, Card, and Ring Room Operatives in Manchester, Bolton and Oldham for their interest and courtesy in finding us members for examination.

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