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HISTAMINE*

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Histamine has attracted much attention because it has dramatic pharmacological effects in very small doses and because it is widely distributed in animal tissues. These two fundamental facts were clearly established by Dale and his colleagues between 1910 and 1927. This early work has often been reviewed (Feldberg and Schilf, 1930; Gaddum, 1936, etc.) and will only be briefly summarized now.

Pharmacological Actions

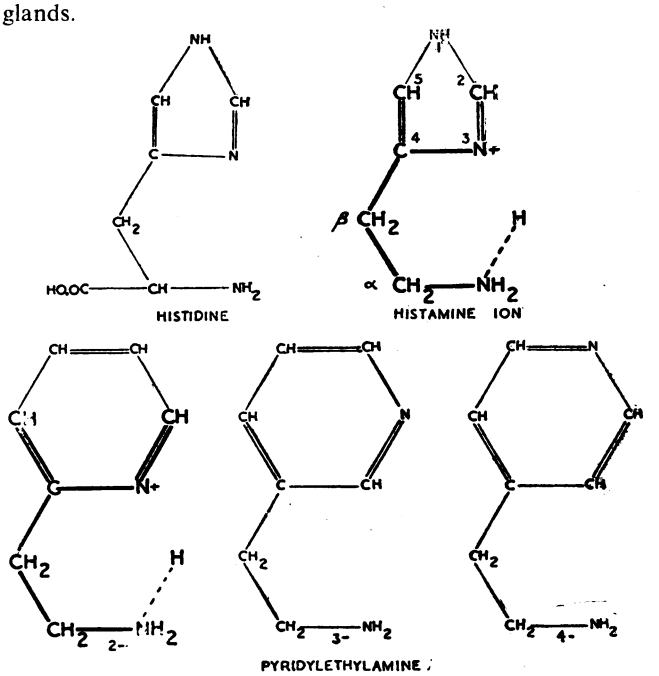
Histamine causes contraction of most plain muscle. This effect is particularly marked if the muscle comes from a guinea-pig; and this animal provides various preparations which can be used to detect histamine in concentrations down to about 10^{-7} or 10^{-8}. If a high concentration is left in contact with the muscle the contraction is not maintained. The muscle relaxes, although still in contact with histamine; even after the solution has been changed the muscle remains for a time insensitive to further applications of histamine though still sensitive to other drugs.

The contraction of plain muscle is well shown in isolated preparations and also when histamine is injected intravenously. In some species of animals large doses kill by this action, but the form of death varies, probably owing to differences in the distribution of the plain muscle. Guinea-pigs are killed by the action of histamine on the bronchi; rabbits by its action on the pulmonary arteries; and dogs by its action on the hepatic veins.

In some animals, including cats and men, histamine has a powerful action on the capillaries, which dilate. This is well shown by injecting histamine into skin, when it produces the well-known triple response which is called urticaria because of its resemblance to the effects produced by the sting of a nettle. The simple explanation of this resemblance has recently been discovered by Emmelin and Feldberg (1947), who find that nettle-stings actually contain histamine in a concentration of about 0.1% together with acetylcholine in a concentration of 1%. The urticaria is due to the histamine, and the pain is due to the two drugs acting together. The intradermal injection of either of them alone is painless.

When large doses of histamine are injected intravenously in cats the capillaries dilate so much that the blood pressure falls to very low levels, and so much plasma leaks out that the blood becomes very concentrated. These changes are the cause of death in cats. Histamine thus causes a form of shock which has much in common with shock due to other causes. The enthusiasts concluded that shock was always due to the release of histamine, but they were certainly wrong; there are many kinds of shock.

When histamine is injected subcutaneously, so that it is slowly absorbed, the mucous membrane of the stomach secretes large quantities of acid juice poor in enzymes. The use of this fact as a test of gastric function is the main clinical application of histamine. Histamine also stimulates the lacrimal glands, the salivary glands, the pancreas, and the glands of the intestine, but not the sweat glands.



Histamine is a base (β-iminazolylethylamine) which can be obtained from histidine by decarboxylation. It is soluble in water and ethyl alcohol, but not in ether. It can be extracted from alkaline watery solutions by various solvents such as amyl alcohol and re-extracted from the amyl alcohol with acid. It is extremely stable in acid solutions. When titrated with acids it shows two dissociation constants corresponding to *p*H 5.9 and *p*H 9.7 (Levy, 1935). Under physiological conditions it therefore behaves as a univalent cation. According to Niemann and Hays (1942), the structure of this cation is as shown above. Ionization is associated with the uptake of a proton attached to the amine group by means of a hydrogen bond (shown by a dotted line) and chelation occurs, which means that an unstable ring is formed.

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Histamine may be adsorbed in two different ways (see diagram). Adsorption on charcoal is increased when the solution is alkaline; this is presumably because charcoal adsorbs the free base (Phelps and Peters, 1929). On the other hand, adsorption on fuller's earth or magnesium trisilicate is increased when the solution is acid; this is presumably because these substances adsorb histamine ions in exchange for other cations.

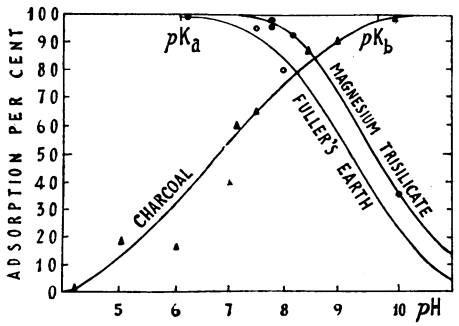


Diagram showing adsorption of histamine. In alkaline solutions adsorption on charcoal is increased, but adsorption on fuller's earth or magnesium trisilicate is decreased.

Several workers have studied the pharmacological activity of methylhistamines. Vartiainen (1935) found that if one methyl group is attached to the amino-nitrogen the resulting compound is more active than histamine in some tests and less active in others. If two methyl groups are attached to this group the compound still has histamine activity, but is less active. If three methyl groups are attached the compound becomes a quaternary base which acts like nicotine and has no histamine activity. Alles, Wisegarver, and Shull (1942) studied the compound formed by attaching a methyl group in position α. This compound has very little histamine activity; the most interesting thing about it is that it is not attacked by the enzyme histaminase (or diaminoxidase). They point out that this effect is similar to that of attaching a methyl group in the corresponding position in sympathomimetic amines, which are thus protected against aminoxidase.

There are three possible pyridylethylamines, depending on the position of the side-chain in the pyridine ring. The compound whose formula is shown above (2-pyridylethylamine) has effects like those of histamine and is almost as active. It is therefore suggested that the essential group for histamine activity is that shown large [CH : NC(CH_2.CH_2.NH_2) : CH]. The other two pyridylethylamines do not contain this group and do not act like histamine. Their pharmacological effects are like those of sympathomimetic amines, whose essential structure is phenylethylamine. In these compounds the pyridine ring behaves as if it were a benzene ring (Walter, Hunt, and Fosbinder, 1941; Niemann and Hays, 1942).

Antihistamines

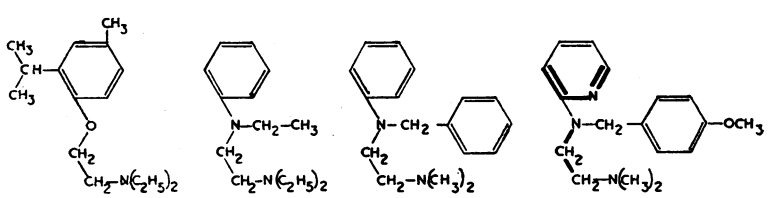
(Bovet and Walthert, 1944; Feinberg, 1946; Loew, 1947.) It has long been known that many of the pharmacological effects of adrenaline are opposed to those of histamine. These two substances act as physiological antagonists, and the body uses them as such. The injection of histamine releases adrenaline and, according to Staub (1946), the injection of adrenaline releases histamine. He found that, when 0.2 mg. of adrenaline was injected intravenously in man in nine minutes, the plasma histamine rose from 10 to 150 μg. per litre. This work seems to confirm a suggestion made long ago by Dale, who thought that the vasodilator effect of small doses of adrenaline might be due to release of histamine. Experiments which appeared to show that adrenaline did release histamine from the lungs turned out to be inconclusive (Dale and Richards, 1927), but the work of Staub supports the theory on which the experiments were based.

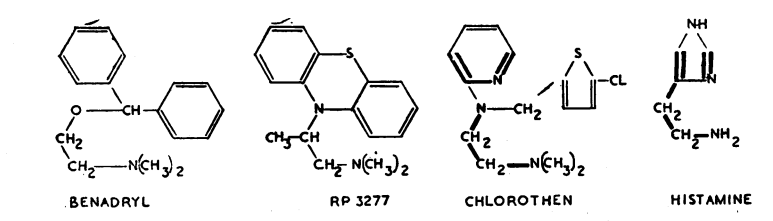
Another class of substance which is opposed to histamine comprises the substances that destroy it. Formaldehyde (Kendall, 1927) destroys histamine *in vitro* and may oppose its actions on an isolated organ. According to Garan (1938) CO_2 has a similar action, possibly due to the formation of a carbamino compound. Nitrites also destroy histamine *in vitro* (Gaddum and Schild, 1934). There is no evidence that they do this *in vivo*; if there were, it might explain the fact that nitrites inhibit smooth muscle. The enzyme histaminase is presumably the main factor which does destroy histamine in the body; but it seems to be ineffective when injected, and it is difficult to see how it can possibly be effective by the mouth (cf. Feinberg, 1946).

It is possible to immunize the body against histamine by injecting an antigen prepared by coupling histamine to a protein (cf. Feinberg, 1946). This discovery would have made more stir than it did if it had not been overshadowed by the discovery of drugs which probably act as competitive antagonists, combining with the tissues in the same way as histamine and keeping the histamine off.

Perhaps the first competitive antagonist that should be considered is histamine itself. Its power of desensitizing tissues to its own action has already been mentioned. In the search for other antihistamines Edlbacher, Jucker, and Baur (1937) tested most of the amino-acids and found that histidine, arginine, and cysteine had some effect on high concentrations. It seems natural that histidine should act in this way as its molecule differs from that of histamine only by the presence of a carboxyl group. The molecule of arginine can be drawn to look like that of histamine, but it is not so easy to suggest why cysteine should act in this way.

The discovery of the really potent antihistamines is mainly due to Bovet and his colleagues in the Pasteur Institute in Paris. This laboratory had been interested for some years in a series of drugs with an action antagonistic to adrenaline (Bovet, 1943). The molecules of adrenaline and its allies contain phenylethylamine. The anti-adrenalines studied in Paris were tertiary amines containing phenoxyethylamine. The introduction of oxygen between the benzene ring and the side-chain, together with the attachment of alkyl groups to the amino-nitrogen, seemed to convert drugs which acted like adrenaline into drugs which opposed adrenaline. Ungar, Parrot, and Bovet (1937) found that some of these drugs also acted against histamine. The first of the new antihistamines to attract attention was 929F, or thymoxyethyldiethylamine (Bovet and Staub, 1937). The more active drugs discovered later were mostly modifications of this original substance. The formulae of some of these substances are here shown.





In 1571F (Staub, 1939) the oxygen in 929F is replaced by NC_2H_5 and thymol by phenol. In "antergan" (Halpern, 1942) the NC_2H_5 is replaced by $N.CH_2.C_6H_5$ and the diethylamine group is replaced by dimethylamine. In "neoantergan" (Bovet *et al.*, 1944) the benzene ring is replaced by pyridine, and a methoxy group appears. This interesting drug is also known as pyranisamine or "anthisan." "Pyribenzamine" has just the same molecule as neoantergan except for the absence of the methoxy group.

A large number of other antihistamines have been synthetized in the last few years, including benadryl, "antistin," chlorothen, RP 3277, hetramine, and others, but neoantergan appears to be more active and specific than most of the other antihistamines which have been discovered more recently.

Various theories have been advanced to explain the action of these drugs, but the most probable is that they act by competition, blocking up the sites of action of histamine. The results of quantitative studies of the antagonism are compatible with this view. The slope of the logarithm of the dose-effect curve appears to be unaltered by antihistamines (Schild, 1947b). The antagonism between benadryl and histamine seems to be simple (Wells *et al.*, 1945). Halpern and Mauric (1946) have studied the antagonism between antergan and histamine and got more complex curves, but their results can be fitted by a formula of the type proposed by A. J. Clark (1937) to express the relationship in the case of other drugs which are thought to act by competition.

Bovet believes that not only the antihistamines but anti-adrenalines such as phenoxyethyldiethylamine act in this way. It has been objected (Loew, 1947) that the molecules of antihistamines are so different from those of histamine and from one another that they would not be expected to combine with the same groups in tissues. The manner in which the above formulae are drawn represents an attempt to answer to this objection. It has already been concluded from the study of the pyridylethylamines that the essential pharmacodynamic group of histamine is the skeleton shown in thick lines in its formula. Neoantergan contains this skeleton, but the two parts of it are separated by a nitrogen atom with other groups attached to it. This separation of the side-chain from the ring is comparable with the similar separation in the molecules of the synthetic anti-adrenalines. It is noteworthy that the nitrogen in the ring in neoantergan is in the same position as it is in histamine. Several other powerful aintihistamines, such as pyribenzamine, hetramine, and chlorothen, contain a nitrogen in the same position. Some of the other molecules do not fit into this picture quite so well, but none of them are so widely different from neoantergan as to disprove the theory of competitive antagonism. This is not, however, the only possible theory.

These antihistamines appear to antagonize all the actions of histamine, with the notable exception of its action on the gastric juice, which is little, if at all, affected. Their activities have been measured in various ways, the most satisfactory being probably that proposed by Schild (1947a), according to which the activity is expressed in terms of the pA_2—the negative logarithm of the molar concentration which is just sufficient to make the tissue half as sensitive to histamine as it was before. Using guinea-pig's intestine, Schild measured the pA_2 for neoantergan, benadryl, pethidine, and atropine acting against both histamine and acetylcholine. Reuse (1948) has confirmed Schild's results for neoantergan and also obtained estimates for antergan, antistin, and RP 3277. These results agree with those of other workers obtained by different methods in showing the outstanding activity and specificity of neoantergan. Most of the antihistamines are fairly powerful antagonists for acetylcholine; many of them have a local anaesthetic effect and mixed effects on the central nervous system, which is depressed in man but excited in most other animals (Loew, 1947).

Other substances found to have some antihistamine action include peptamines containing histamine (Rocha e Silva, 1943), iminazole (Morris and Dragstedt, 1945), picrotoxin (Anrep *et al.*, 1939), and nicotinamide (Halpern and Daïnow, 1944).

Antihistamines can provide evidence for or against theories which attribute physiological changes to the liberation of histamine. Such evidence is of real value only when one of the more specific drugs such as neoantergan is used. Experiments of this type support the view that many of the phenomena of anaphylaxis are due to the release of histamine. On the other hand, benadryl has been found ineffective in protecting animals against trypsin (cf. Loew, 1947). This does not invalidate the evidence that trypsin liberates histamine, but does suggest that its actions are not all due to the release of histamine.

The Estimation of Histamine

Practically all our knowledge of the behaviour of histamine in the body is based upon biological assays. Colorimetric methods have been devised, but they have been found to be comparatively laborious, insensitive, and unspecific. In the early work histamine was generally detected by its effect on the cat's blood pressure, but the favourite test to-day depends on the guinea-pig's intestine. Other suitable tissues are the guinea-pig's uterus or bronchi, the hen's rectal caecum, and the human skin. In all such tests the extract of the tissue is compared with a solution of histamine and the doses adjusted until the two solutions cause equal effects. For really accurate assays the size of the effects can be measured and the result calculated statistically (Schild, 1942).

Crude tissue extracts contain many pharmacologically active substances besides histamine, and when such extracts are used the result means very little. At one time it was customary to use such extracts and to speak vaguely of histamine-like substances. The introduction of improved pharmacological techniques has made these tests more specific, and if proper precautions are taken it is now possible to be reasonably certain that the result of an assay does give an estimate of the concentration of histamine itself.

In order to do this it is necessary to subject the extract to chemical or physical procedures which remove or destroy all other substances that would affect the test. The estimation is thus comparable with a colorimetric estimation in which tissues are ashed or extracted before the final test is applied. In a method which Barsoum and I (1935a) devised in Cairo for the assay of histamine the tissue is minced and extracted with trichloroacetic acid, which precipitates the proteins. The trichloroacetic acid is removed from the extract with ether, and strong hydrochloric acid is then added and the mixture boiled for 1½ hours. This destroys other substances, and generally gives a pharmacologically pure solution after the HCl has been evaporated off. This extract is tested in comparison with histamine on guinea-pig's ileum. We found that when the concentration of histamine was very low there was enough potassium in this final solution to interfere with the test, so we devised a technique involving alcoholic extraction to get rid of this potassium when necessary. Code (1937a) improved this method by the discovery that extraction with ether was unnecessary because the HCl destroyed the trichloroacetic acid. Since then various other methods of extracting histamine for pharmacological tests have been used (cf. Minard, 1940; McIntire *et al.*, 1947).

By such methods it is generally possible to obtain pharmacologically pure extracts, but until confirmatory tests are made it is unsafe to assume that this result has been achieved. The best confirmatory test is to make parallel quantitative assays. The extract is compared quantitatively with histamine by several different methods. If all the results agree quantitatively it is probable that they give an estimate of histamine (cf. Chang and Gaddum, 1933). Histamine can be distinguished from N-methyl-histamine in this way; the methods used so far would not distinguish it from N-dimethyl-histamine.

The effects of antihistamines can also be used in evidence, provided that the experiment is properly designed.

Large amounts of antihistamines will desensitize tissues not only to histamine but also to other substances such as acetylcholine and potassium. The effect of antihistamines is therefore convincing only when they antagonize extracts in the same concentrations that they antagonize histamine. One sound way of arranging the experiment is to obtain a series of equal responses of the guinea-pig's gut with the extract and with histamine given in alternate doses. A small concentration of neoantergan is then added to the bath for a minute and the alternate dosing continued. The result is that the response to histamine is greatly depressed or abolished and then gradually recovers as successive doses are given. If the active principle in the extract is histamine its effects will be depressed to the same extent and for the same time as the effects of the standard solution of histamine. This type of test can be used to distinguish histamine from many substances, but Schild (1947b) has shown that it does not distinguish histamine from N-methylhistamine, although these two substances can be differentiated by the method of parallel quantitative assays. This result suggests that antagonists can be used to distinguish between drugs acting at different sites in tissues but not between two drugs which act at the same site.

Evidence for the identification of histamine in extracts can also be obtained by exposing the extracts to various solvents, and destructive agents such as histaminase; but it must be remembered that this enzyme has not been prepared in the pure state, and that preparations containing it may contain other enzymes as well. Evidence depending on the use of this enzyme is not conclusive by itself.

Behaviour of Histamine in the Body

It seems likely that the histamine in the body is derived originally from histidine by decarboxylation. Histidine is one of the essential amino-acids, and it is generally supposed that the iminazole group which it contains is not synthetized in the animal body but absorbed from the intestine. Histidine may be converted into histamine by bacteria either in the food before it is eaten or possibly in the intestine. When animal tissues are eaten they also often contain histamine derived from the animal. Histamine is absorbed by the mucous membrane in the mouth, but most absorption occurs in the ileum. However, large doses may be eaten with surprisingly little effect, for reasons to be discussed later.

Histamine may also be formed from histidine in the body by the enzyme histidine decarboxylase (see Blaschko, 1945), which is present in the liver and kidneys of certain small animals. It is not known what proportion of the histamine normally present in the body is derived from this source and what proportion is absorbed from the intestine, but the fact that histidine decarboxylase appears to be absent in many species of animal suggests that it does not play an important part in providing the body with histamine.

Numerous estimates have been made of the amounts of histamine in animal tissues, with varying results (Feldberg and Schilf, 1930; Gaddum, 1936; Rocha e Silva, 1946; etc.). There are usually particularly large amounts in the lungs and skin, but most tissues may contain quite large amounts in some species at some times. The blood has attracted particular attention because it is easy to obtain successive samples of it. Most of the blood histamine is in the cells. The plasma usually contains so little that it is difficult to estimate it, but some does seem to be there in a pharmacologically active form. Emmelin (1945), for example, took pairs of guinea-pigs of which one normally had a high concentration of histamine in its plasma and the other a low concentration. When he established a cross-circulation between two such guinea-pigs bronchoconstriction occurred in the animal with an initial low plasma histamine and not in the other animal. This experiment seems to show that the concentration in the plasma does play a part in regulating the normal functions of the body. Code (1937b) came to the conclusion that most of the blood histamine is in the granulocyte cells. This view was confirmed by the fact that in myeloid leukaemia when the number of granulocyte cells was very high the blood histamine reached astronomical proportions. Later work suggests that this is not true of all species of animal and that most of the histamine in rabbit's blood is in the platelets (Minard, 1940). In any case, estimations of total blood histamine are often difficult to interpret; a fall of blood histamine may be due to the disappearance or destruction of histamine-containing cells.

Observations on the blood histamine are also complicated by the fact that when histamine is liberated into the blood by one organ it may be rapidly absorbed, destroyed, or excreted by another. Anaphylaxis in rabbits causes a fall in the histamine content of the lungs, spleen, and blood, and it is not known what happens to the histamine in these circumstances. The injection of histamine itself in rabbits has been found to cause a fall in the blood histamine apparently due to the loss of leucocytes or platelets (Rose and Browne, 1941). Such observations help to explain why estimations of histamine in the blood have not led to as rapid advances in the study of histamine as seemed possible at one time.

It has long been obvious that the histamine in the tissues cannot all be free; something prevents it acting under normal conditions. If all the histamine in a cat's lungs were liberated it would kill the cat. The methods generally used for the estimation of histamine do not estimate free active histamine but only the total amount that can be extracted from the tissues. When tissues are ground up in saline part of the histamine is released, but part of it appears to be bound to cellular debris, from which it can be released by procedures, such as heat, which coagulate the proteins (Trethewie, 1938).

When histamine is injected intravenously most of it disappears from the blood within a minute or two and is taken up by the tissues and particularly by the kidney (Rose and Browne, 1938; and cf. Emmelin, 1945). Various alkaloids disappear from the blood equally quickly. It is unlikely that this phenomenon is entirely due to the combination of the drug with the specific receptors on which it acts. It is thus probable that histamine can combine with tissues in at least two ways, one of which is specific and produces pharmacological effects, and the other of which is unspecific and merely removes the drug from the blood. According to Phelps (1935) the pharmacological effects depend on the combination of free histamine base with the tissues, and according to Rocha e Silva (1946) the unspecific combination of histamine with the tissues depends on a mechanism which will be discussed later. But first consider the factors which release histamine from the tissues.

Release of Histamine from Tissues

A number of different methods have been used to study this question. Lewis (1927) showed that various injurious agents produced effects which could be attributed to the release of histamine or something like histamine. The best-known of these effects is the local triple response produced in the skin by intradermal injections, but Lewis and his co-workers also observed other effects such as vasodilatation in the skin of the face which could be attributed to the release of histamine into the general circulation (Lewis and Harmer, 1927). Various other similar methods have been used; for example, the released histamine has been detected by its effect on the gastric juice (Kalk, 1929;

Ungar, 1935; Feldberg and Holmes, 1941) and on the blood pressure (McIntosh and Paton, 1947).

In some experiments the evidence has been the demonstration of a general rise of the histamine content of the circulating blood (Dragstedt and Mead, 1936) or of the blood coming from one particular organ (Anrep and Barsoum, 1935) or in the lymph (Dragstedt and Gebauer-Fülnegg, 1932). The release of histamine can also be demonstrated in tissues such as guinea-pig's lungs perfused with salt solutions (Bartosch, Feldberg, and Nagel, 1932) or by diffusion from tissues suspended in salt solutions (Schild, 1939) or from blood cells into plasma (Katz, 1940). Another possible method, to be discussed later, depends on estimating histamine in the urine.

The factors which control the release of histamine have recently been reviewed by Kellaway (1947), and it will therefore be unnecessary to discuss all the details here.

Histamine is liberated by trypsin, and this fact has been made the basis of an interesting theory regarding the way in which histamine combines with tissues. Rocha e Silva (1946) showed that crystalline trypsin liberates histamine from lungs or from blood cells. It has been shown by Bergmann that crystalline trypsin is a much more specific enzyme than had been supposed, and that it acts on the peptide links formed by the carboxyl groups of the basic amino-acids lysine and arginine. Rocha e Silva therefore suggests that the amine group in histamine combines with the carboxyl groups in these two acids, which themselves presumably form part of protein molecules in the tissues. In support of this very definite theory he finds that chymotrypsin, which attacks the carboxyl group of aromatic amino-acids, does not liberate histamine from cells. On the other hand, papain, which contains a mixture of proteolytic enzymes, does liberate histamine (Rocha e Silva and Andrade, 1943). There is evidence that proteolytic enzymes are liberated or activated in various forms of injury. This is one mechanism by which histamine may be released.

Rocha e Silva (1946) has suggested a theory of how this happens. It is well known that histamine is released in anaphylactic shock, together with other substances such as heparin (Feldberg, 1941; Dragstedt, 1941; Rose, 1947). At the same time leucocytes and platelets disappear from the blood. Rocha e Silva found that both peptone and extracts of ascaris caused similar effects in dogs, and obtained microscopical evidence that the leucocytes and the platelets were retained in the liver. He believes that these cells liberate or activate trypsin, or some similar enzyme, in the tissues and that this trypsin then liberates both histamine and heparin. Such experiments are complicated by the fact that heparin antagonizes the action of trypsin (Rocha e Silva, 1945).

A large number of other substances are known to liberate histamine, and it is clear that they do not all act in the same way. The effects of various forms of injury have been studied in detail by Feldberg, Kellaway, and their co-workers in Australia (Kellaway, 1947), who found that a number of different irritant poisons had various effects in common. The poisons studied included snake venoms, bee venom, peptone, toxins from staphylococcus and *Clostridium welchii*, and mercuric chloride. All these poisons caused a liberation of histamine, and some of them the liberation of other substances as well. For example, cobra venom also liberated adenosine compounds, which inhibit most smooth muscle, and a substance which causes a slow contraction of guinea-pig's isolated intestine and is known simply as the "slow-reacting substance." Another important substance liberated by cobra venom is lysolecithin, which is formed from lecithin by an enzyme. This substance is itself destructive of cells, causing haemolysis and the release of proteins, pigments, histamine, and small amounts of the slow-reacting substance (Feldberg and Kellaway, 1938). Some, at least, of the release of histamine by cobra venom must be the secondary result of the formation of lysolecithin.

Histamine is also liberated by various substances which do not produce obvious injury. For example, Alam *et al.* (1939) showed that curare liberates histamine, and this fact has been confirmed in experiments with tubocurarine (Schild and Gregory, 1947; Grob *et al.*, 1947). McIntosh and Paton (1947) have also found that stilbamidine and a series of other diamidines as well as diamines and diguanidines also release histamine in the body.

In most of the experiments discussed so far there was no question of any new formation of histamine. The appearance of histamine in the fluids in contact with tissues has often been shown to be accompanied by a disappearance of similar amounts of histamine from the tissues themselves. The work of Dekanski (1945) suggests that some other mechanism may play a part in the effects of the exposure of tissues to high temperatures. In the hope of following the fate of histamine liberated by injury he made extracts of whole mice. Anaesthetized mice were immersed in water at 60° C. for 10 or 30 seconds. During the first hour after this treatment the total amount of histamine which could be extracted from the whole body rose from an average value of 216 μg. per mouse to about double this value. The main site of this new formation of histamine appeared to be the skin, but it is not known from what precursors the histamine was formed.

The Fate of Histamine

Histamine is destroyed by some bacteria and also by the enzyme histaminase which is present in the body (Best, 1929). According to Zeller (1938, 1941) this same enzyme also destroys various diamines, such as putrescine and cadaverine, and should therefore be called diaminoxidase. The enzyme is inhibited by cyanide and acts by oxidation. One atom of oxygen is absorbed and one molecule of ammonia is formed when one molecule of histamine is destroyed by the purified enzyme; H_2O_2 is formed and the iminazole ring is destroyed. The most sensitive method of estimating the amount of enzyme in a solution depends on adding histamine and following its destruction by pharmacological tests. Other methods depend on the measurement of the oxygen consumption, the ammonia production, and the peroxide production.

The highest concentrations of the enzyme are in the intestinal mucous membrane, the kidney, and the placenta. The liver contains surprisingly little. In normal circumstances the blood contains very little, but during pregnancy there is an enormous increase of the histaminase content of the blood of women. This phenomenon was first described by Marcou (1937) and has been confirmed by many others (for references see Ahlmark, 1944; Anrep *et al.*, 1947). The enzyme is thought to come from the placenta.

Kapeller-Adler (1944) studied this phenomenon by means of a colour test depending on the formation of H_2O_2. The rise in the concentration of the enzyme in the blood during normal pregnancy could be demonstrated by this test when either histamine or cadaverine was used as substrate, but bloods taken from women suffering from eclampsia or pre-eclamptic toxaemia behaved in a curious way. The effect of these bloods on cadaverine was similar to that of blood from normal pregnant women, but their effect on histamine was much less. This observation seemed at first to suggest that the enzyme oxidizing cadaverine was

not, after all, identical with the enzyme oxidizing histamine. The evidence on this point has therefore been re-examined using the Warburg technique to measure the oxygen consumption as well as other tests. A highly purified preparation of the enzyme from pig's kidney was used in this work. The results showed that there were not two independent enzymes. When histamine and cadaverine were both present the combined effect on the oxygen consumption was less than the sum of their effects when either is present without the other. Cadaverine inhibited the destruction of histamine as measured by biological assay. The effects of various poisons and changes of *p*H produced similar effects with either substrate, but dialysis had an effect like eclampsia: it decreased the action of the purified enzyme on histamine and increased its action on cadaverine. Both these effects were reversed by flavine adenine dinucleotide; the effect of dialysis thus appears to be due to the removal of this substance. The relation of these observations to those on toxaemia obviously requires further investigation.

The eventual fate of most of the histamine in the body is probably to be destroyed by the enzyme histaminase, but a certain amount appears in the urine. The first clear quantitative evidence of this was obtained by Anrep, Ayadi, Barsoum, Smith, and Talaat (1944), who found that the activity of extracts of urine could be increased by acid hydrolysis. The histamine in the urine seems to be partly free and partly conjugated with other substances, from which it can be liberated by acid. These facts have been confirmed and extended in Edinburgh by Adam. He has been using a simplified form of the test devised by Anrep *et al.* to measure the histamine in human urine. In normal circumstances the concentration of histamine in the urine is comparable with that in the blood—about 50 μg. per litre. This is near the limit of what can be measured by the methods available. When histamine is given by the mouth to man about 1% of it appears as conjugated histamine in the urine, but when histamine is given by slow intravenous infusion it is free histamine which appears in the urine. These observations confirm results obtained by Anrep *et al.* in experiments on dogs.

The fact that large doses of histamine have very little pharmacological effect when swallowed may be partly due to this formation of conjugated histamine, but other factors may be more important. The mucous membrane of the gut contains particularly large amounts of histaminase, and this probably also plays a prominent part in protecting the body from the effects of the absorption of histamine. It has already been mentioned that other organs, such as the kidney, also contain histaminase, and the general result is that only a small amount of histamine normally appears in the urine. This proportion may be much greater when large doses of histamine are given. This is well shown in some experiments by Alexander (1944), who injected 3 mg. of histamine intravenously into each of a group of mice, and then followed its fate by estimating it in the urine and in extracts of the whole mouse. The percentage appearing in the urine in these experiments varied widely, but on the average it was 37.7. After 24 hours the histamine content of the mice had fallen practically to the normal value of 0.3 mg. per mouse, so that the other 62.3% of the histamine must have been destroyed in this time. During the first hour, when the mice were flooded with histamine, excretion in the urine was the main channel by which they got rid of it.

The main interest of estimations of histamine in the urine lies in the fact that they provide a possible means of studying the factors which cause the liberation of histamine in the body. Under the conditions of Adam's experiments there was no detectable rise in the blood histamine, although measurable quantities of histamine appeared in the urine. It thus seems likely that the liberation of histamine in the body may be followed by estimating it in the urine even when no changes occur in the blood histamine.

Histamine in Normal Physiology

It is natural to suppose that the local control of the circulation may depend on histamine. Vasodilatation is known to occur when the blood supply to a tissue is obstructed or when the activity of the tissue is increased. These phenomena may be partly due to the local release of histamine, but the evidence is conflicting. Workers in Cairo got evidence of an increase in the histamine content of blood coming from the voluntary muscles of a dog when the circulation was obstructed; when the muscles were stimulated the effect was much larger. They also did experiments with a heart–lung preparation, and found that when the heart worked harder it liberated more histamine (Barsoum and Gaddum, 1935b; Anrep and Barsoum, 1935; Anrep, Barsoum, and Talaat, 1936; Anrep, Barsoum, Talaat, and Wieninger, 1939). Some of these conclusions were supported by the results of Feldberg and Holmes (1941), who found that stimulation of voluntary muscles caused gastric secretion in cats which could be attributed to the release of histamine. Unfortunately other workers failed to confirm the experiments in Cairo (Code, Lovatt Evans, and Gregory, 1938; Emmelin, Kahlson, and Wicksell, 1941; Kwiatkowski, 1941). Emmelin and Emmelin (1947) found that benadryl in doses sufficient to reduce the effect of histamine did not reduce the reactive dilatation of the vessels in a cat's leg after occlusion of the circulation.

According to Anrep, Barsoum, Salama, and Souidan (1944) some at least of the negative results were due to the fact that the histamine is very rapidly washed away unless drastic measures are taken to control the blood flow when it is first released. It has also been pointed out that the muscles of the dogs in Cairo contained three to four times as much histamine as the muscles of the dogs used by other workers. It is possible that histamine did contribute to the hyperaemia in all the experiments, but that it was only when it was present in large amounts that it appeared in the venous blood. In any case the evidence in favour of histamine's function in the local control of the circulation cannot be regarded with complete satisfaction at present. It is very probable that various other substances are involved.

The question of the release of histamine by nerves has received less attention than it deserves. Ungar (1935) stimulated the posterior roots antidromically in dogs and found that the vasodilatation thus produced was accompanied by gastric secretion. He attributed this at first to the release of histamine into the general circulation, and suggested that these nerves should be called histaminergic. Kwiatkowski (1943) obtained more direct and convincing evidence in favour of this conclusion. He found that antidromic stimulation in cats caused the appearance of a histamine-like substance in the venous blood. Confirmatory results were obtained when a cat's leg was perfused with Tyrode's solution. High concentrations of histamine were also found in extracts of the nerves which cause these effects, and Kwiatkowski considered that this was analogous to the fact that extracts of cholinergic and adrenergic nerves contain high concentrations of the substances liberated at their endings. There are, however, various complications which suggest that the analogy is not a close one. Kwiatkowski himself found that degeneration caused

an increase in the histamine content of nerves, whereas it is known to have the opposite effect on their content of acetylcholine and sympathin. The work of Koshtojanz, Ryvkina, and Mitropolitanskaya (1945) indicates a more complicated situation. They compared the effects of a blow on the head with those of ether narcosis. Extracts of nerves taken from animals killed by this form of violence contained little or no histamine, whereas those obtained from anaesthetized animals contained large amounts. It is clear that there is still much to be learned about the histamine metabolism of nerves.

Therapeutic Applications

(Rose, 1947)

The realization that the release of histamine plays a part in various pathological conditions has stimulated the search for ways of counteracting its effects. The results of this search have already been briefly discussed; the most promising antidotes for histamine are the drugs, like neoantergan, which probably act as competitive antagonists. Their effect in anaphylaxis has led to their clinical use in allergy. They have been found effective in allergic urticaria. In various other dermatological conditions their main effect is to diminish itching. They are also effective in hay-fever and other forms of spasmodic rhinitis. There is evidence that when patients are specifically sensitive to drugs such as liver extracts or insulin the unpleasant effects can be suppressed with antihistamines and the treatment continued. Under these conditions the patient may be desensitized so that the administration of the antihistamine need not be continued indefinitely. Antihistamines may cause various toxic effects, of which the most troublesome at present seems to be drowsiness. Much work remains to be done before the full possibilities and limitations of these drugs are known, but it is already clear that they have some uses. Histamine thus provides another example of the way in which fundamental discoveries which were originally inspired by unorganized scientific curiosity may bear practical fruit which could not have been foreseen.

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A paper entitled "Some Aspects of Physique in Boys and Girls," by R. E. Roper, M.A., M.Ed., has been published (price 2s. 6d., post free) by the Research Board for the Correlation of Medical Science and Physical Education, Apothecaries Hall, Blackfriars Lane, Queen Victoria Street, London, E.C.4. Mr. Roper examined 1,852 children from State schools and day nurseries, a sample which excluded children at special schools. He has tabulated the age, height, weight, and other particulars of the physique and carriage of the children. He recommends that education authorities should consider employing chiropodists to work in schools, that special corrective classes should be available for children with minor defects, that a pair of large mirrors should be provided in all schools, that education authorities should provide travelling *x*-ray equipment to examine the children's physique, that each school should have a rest room for children with special needs, that physical exercise should not be omitted to provide more time for study when examinations approach, and he advocates fuller co-operation between physical education and the medical profession.