The release of histamine / by W.D.M. Paton.

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The Release of Histamine*

By W. D. M. PATON, London

Introduction

Although histamine has been known to be a pharmacologically active constituent of the tissues for nearly 30 years, and was indeed synthesized 20 years earlier still, its physiological role is still obscure. One method of studying its physiology is to attempt to rid a tissue of histamine and to examine the changes of function which result, a procedure corresponding to a section of a nerve or the ablation of a gland in order to elucidate the physiology of some nervous pathway or endocrine system. It has, of course, long been known that histamine can be mobilized from the tissues. But for many years the only procedures available were those of producing an anaphylactic reaction, the injection of certain toxins, proteolytic enzymes or venoms, direct mechanical, thermal or radiation trauma, or the injection of peptone. Some of these procedures are lethal and none of them is very effective in specifically depleting a tissue of its histamine; none of them has been shown, for instance, to bring about the reduction of histamine content in any of the tissues of an animal over a prolonged period. It is probable that peptone could, in principle, have been used successfully for this purpose. But its effectiveness has always been limited by its lack of potency, enforcing the use of doses up to 1 g./kg. body weight, and by the fact that it is liable to be contaminated with other materials including histamine itself.

The appearance in recent years of drugs which can specifically deplete at least some of the tissues of the body of their histamine content has allowed an approach to the physiology of histamine in this direction. Although removal of histamine from all tissues is

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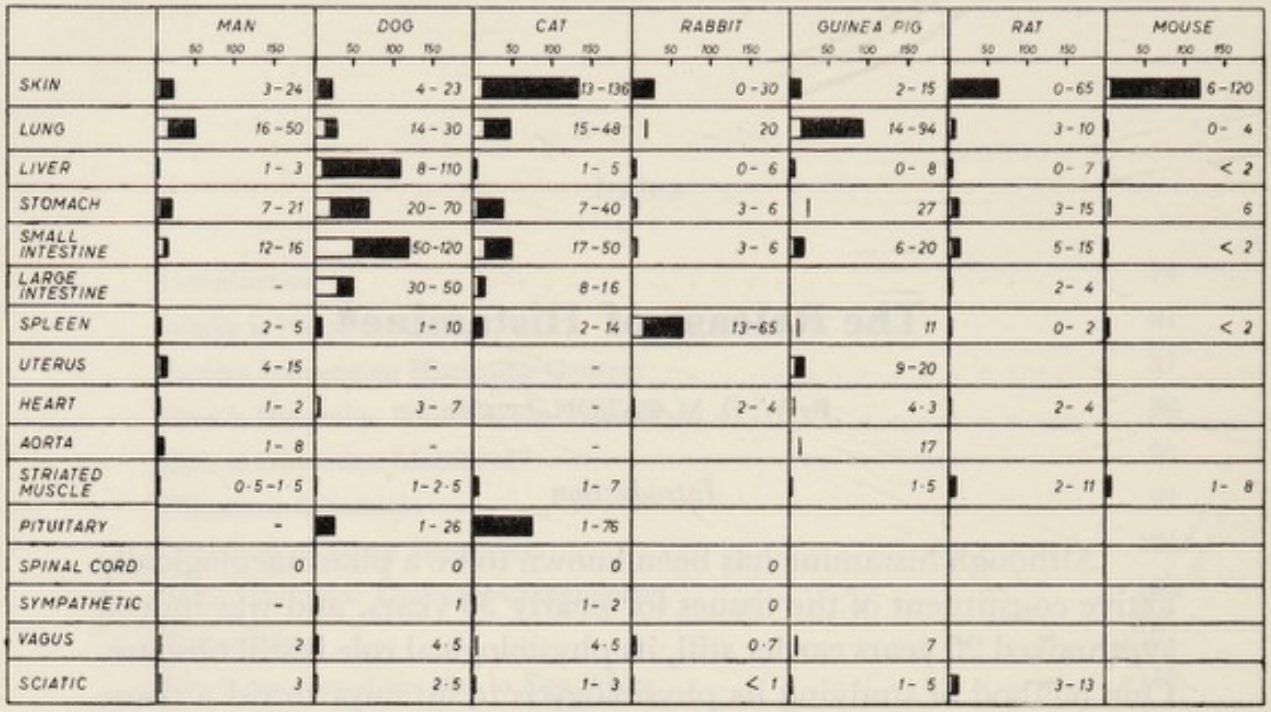


Fig. 1. Diagram of the histamine content of various mammalian tissues. At the top of each column is the scale of histamine content (divided at 50, 100 and 150 μg./gm. tissue, histamine as base). The figures in each rectangle give the range of histamine contents recorded; the blocks are filled in over this range, to show the variability of content of some tissues. Where only average values or a single value has been obtained, a vertical line has been drawn instead of a block. – The sources of the data are given, for each species, at the end of the References p. 148.

certainly not possible at present, it can be done in skin, to a less extent in muscle, and somewhat unpredictably in certain other tissues. Further, the characteristics of the release process and the nature of the substances which produce release must be regarded as throwing light on the way histamine is bound and mobilized in the tissues, even though we cannot interpret all the information at present available.

It is convenient to review the material by dealing with four main questions which are involved in discussing the release of histamine.

(1) Where is the histamine in the tissues?
(2) How is it bound in the tissues where it occurs?
(3) How is it mobilized from its binding and what are the effects of histamine so mobilized?

In addition further problems arise as to the possibility of modifying the release processes, and as to their relationship to or possible participation in anaphylactic and allergic responses.

Much of the material discussed is dealt with in the recent review by *Parrot* and *Reuse* (1954) and in the important Ciba Foundation Symposium on Histamine (1955) whose proceedings are now published.

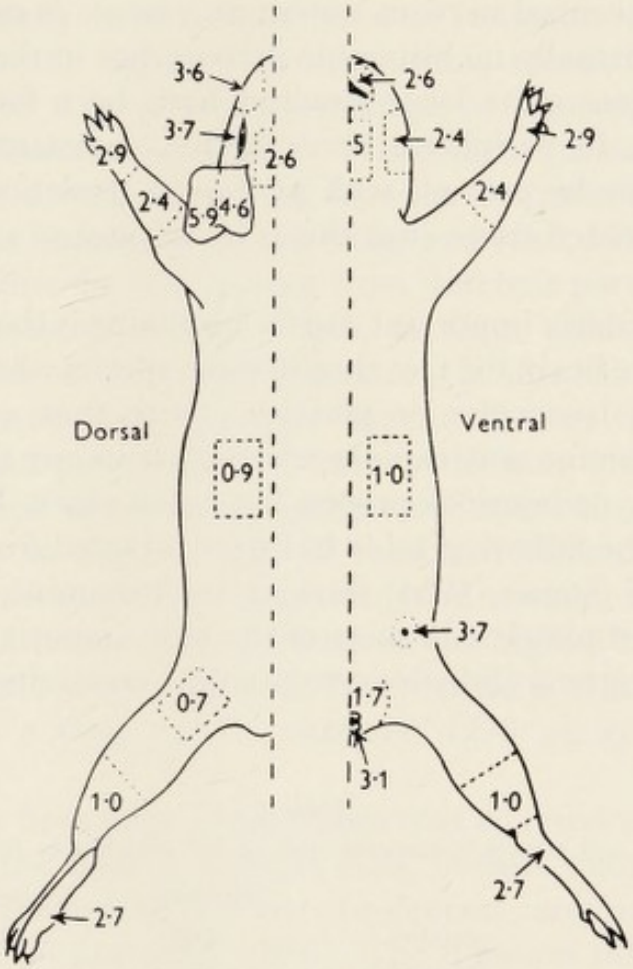


Fig. 2. Histamine content of various regions of guinea-pig skin, referred to ventral abdominal skin as unity. The distribution should be compared with that of the bluing in fig. 6. (From *Feldberg* and *Miles*, 1953: by permission of the Journal of Physiology.)

Where is Histamine Found?

There are now a number of surveys of histamine content in the tissues on a macroscopic scale. Fig. 1 has been constructed to summarize these observations on the tissues where histamine content is for one reason or another important. The sources of information for histamine content are given at the end of the References p. 148. Reviewing this information it seems clear that the skin, the lungs and the stomach and intestine are fairly consistently rich in histamine in nearly all species. Both in the skin (Fig. 2) and in the alimentary

tract (*Douglas* et al., 1951) there are considerable regional variations. In the dog the liver is another major histamine containing organ. In addition to these sites the non-pregnant uterus, the spleen, the striated muscles, and the heart and aorta may contain important amounts. An interesting situation exists as regards nervous tissue. So far as the central nervous system as a whole is concerned there is normally virtually no histamine present, but in the pituitary and adjacent regions quite large amounts have been found in the cat and the dog. In peripheral nerve again a substantial amount of histamine may be present, with perhaps a tendency to be higher in non-myelinated tissue (but this is by no means a regular observation).

A particularly important site for histamine is that in the blood. Its importance lies in the fact that in those species where histamine is contained in leucocytes or platelets, there then exists a means whereby histamine may rapidly accumulate in any tissue where an inflammatory or injurious process has taken place. Because of this importance the following table has been adapted from the work of *Humphrey* and *Jacques* (1954) showing the histamine content of the leucocytes and platelets in some of the most important species; the figures for serotonin (5-hydroxytryptamine-creatinine-sulphate) are also given.

Table I

Mean histamine and serotonin contents (μg) of 10^9 platelets or leucocytes.

		Histamine	*Serotonin*
Man	platelets	0.06	0.25
	leucocytes	c. 15	—
Dog	platelets	0	1.7
	leucocytes	<0.3	—
Cat	platelets	0.12	0.9
Rabbit	platelet	3.5	7.5
	polymorph	1.0	<0.01
Guinea-pig	platelet	0.18	0.21
	polymorph	0.1	<0.01
Rat	platelet	0	0.4
	leucocyte	16	—

[Adapted from *Humphrey* and *Jaques* (1954)]

Considerable debate has taken place as to whether the eosinophil cells in the blood carry histamine or not. It seems probable now that the basophil cells are the more important although the eosinophils may carry histamine, possibly during its detoxification and

disposal. The earlier findings by *Code* and his colleagues that cortisone could cause a fall both in blood histamine and in the eosinophil count in man has now been amplified by the observation that the basophils also fall. For a discussion of this problem the Ciba Symposium on Histamine of 1956 should be consulted (p. 399–415).

These results on the distribution of histamine need to be reconsidered in the light of the important recent evidence that histamine is to a large extent localized in mast cells (*Riley* and *West*, 1953; *Graham* et al., 1953). The evidence is now convincing that there is a strong association between the mast cell content of a tissue and its histamine content (*Riley* and *West*, 1953, 1956). The parallelism exists for skin coming from different parts of the body, or from animals of different ages, or from animals treated or untreated with a histamine liberator; it also holds when the histamine content and mast cell content of other tissues, such as the capsule and parenchyma of liver or the lung are compared in different species. In addition aggregates of mast cells, such as occur spontaneously in dogs or man in mast cell tumours, or in mice when the skin is painted with carcinogens, are also shown to have a very high histamine content. Indeed the highest histamine content ever described for a tissue so far recorded is that of a mast cell tumour from a dog, which contained 1290 μg./g. of histamine (*Riley*, 1956).

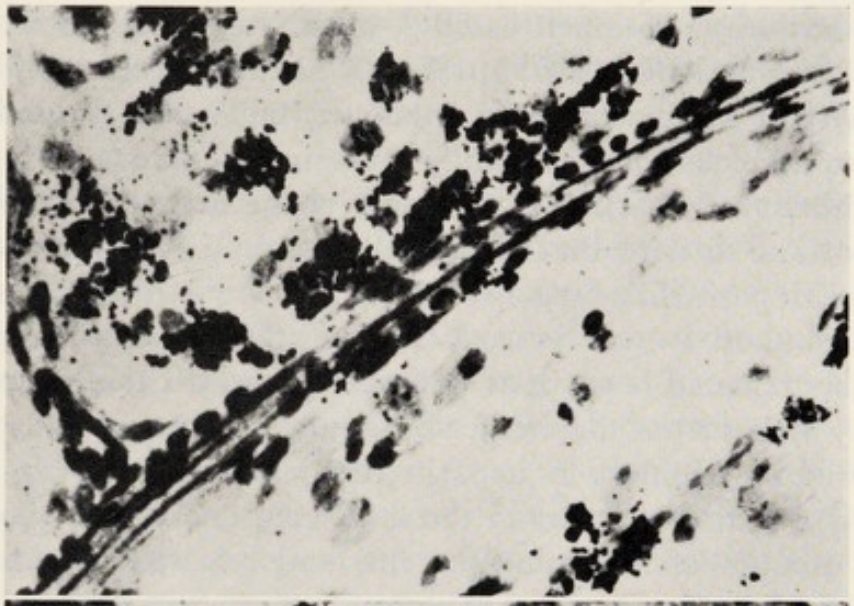
It is clear from these observations that an important amount of histamine in the body is in mast cells, already known to contain considerable amounts of heparin (for general account of mast cells, see *Michels*, 1938 and *Riley*, 1955). The question then arises as to whether histamine is only located in mast cells or whether some other cellular site exists. This is a difficult question to answer so long as the comparison of histamine content and mast cell content rests largely on a count of the mast cells present. With such comparisons there is an inevitable error and it would be quite possible for there to be a significant amount of histamine outside the mast cells without it being possible to demonstrate it. Indeed if the histamine content outside the mast cells bore some definite relation to the number of mast cells present it would be very difficult indeed to distinguish the two sources of histamine by methods in use thus far. This difficulty makes the interpretation even of the results on skin, for which the case of histamine being mostly in mast cells is best, very difficult. In the old experiments by *Harris* (1924) in

which the epidermis (from which mast cells are absent) were separated from the dermis, the bulk of the histamine was found in the epidermal layer. Attempts to repeat his work have led to quantitatively different results, although both *Paton* (1956) and *Mongar* (1956) have found histamine in the epidermal samples so obtained. An uncertainty rests in the possibility that during scalding mast cells in the outer part of the dermis may fragment and release histamine which diffuses into the epidermis in the brief period before it is removed. Attempts to establish the presence or absence of histamine in the epidermis by serial section have not given decisive answers, simply because it is technically very difficult to obtain a skin section consisting of epidermis only and in which the dermal papillae are not cut. So far as skin is concerned, therefore, one must regard the bulk of the histamine as being present in the layers in which the mast cells are most abundant but one cannot exclude there being some residue of histamine outside these cells. In another site, however, there is fairly good evidence that histamine may be bound in the absence of mast cells; this is in the pyloric mucosa of the hog stomach where the histamine content is comparable to that of the submucosa, although the mast cells are considerably fewer (*Riley* and *West*, 1956). *Mota* et al. (1956) also report a dissociation of histamine and mast cell content in the rat in the duodenum, ileum and rectum. Finally, *Freeman*, *Marx* and *Marx* (1956) failed to observe in rat and rabbit tissues as satisfactory a parallelism of mast cell count and histamine content as expected, and varying patterns were found when different organs were compared. One can only conclude at present that the mast cells are very important carriers of histamine, and may account for the bulk of it in a number of tissues, but that it cannot be said yet that they are the *only* histamine carriers in any tissue, and in the stomach and intestine at least there are certainly other binding sites.

The mast cells themselves deserve a good deal more study. They appear not to represent a single homogeneous population in the tissues but exist in perhaps two main types: one smaller and staining densely and orthochromatically, the other larger with metachromatic granules (*Riley*, 1953). There is a tendency for the smaller dark cells to be found in the adventitia of the blood vessels, and the larger cells round capillaries or free in the tissue spaces. A possible interpretation is that the cells are formed in association with the blood vessels; and that, as they mature, they migrate

outwards to fulfill some function in the connective tissue. Further, the mast cell population differs in its response to histamine releasing agents (Fig. 3). Thus if a rat is treated with Compound 48/80, the proportion of mast cells which become degranulated increases as the dose of the drug is increased, implying that the cells differ in

3a



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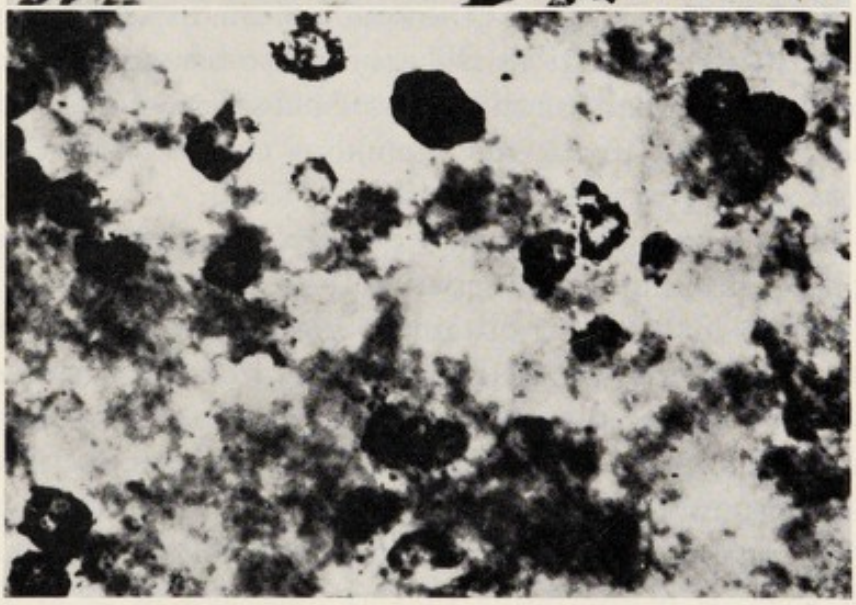


Fig. 3a. "Acute dosage" experiment. Mesentery 3 hours after intraperitoneal injection of compound 48/80, showing shattered mast cells swelling and increased basophilia of the adventitial cells. Very little histamine remained in this tissue. Toluidine blue. ×580. (From *Riley* and *West*, 1955). – *Fig. 3b.* "Subacute dosage" experiment. Subcutaneous connective tissue on third day of intraperitoneal injection of the histamine-liberator. One exceptionally large mast cell near the top remains unaffected. The remaining mast cells, in a slightly different tissue plane, all show varying stages of swelling and degranulation-"ghost" cells. This is a reversible process. Toluidine blue. ×350. (From *Riley* and *West*, 1955.)

their sensitivity to it; indeed, some of the mast cells resist the drug completely even when very large doses are given. Further, it is interesting that if the administration of a histamine liberator is continued, restitution of some of the mast cell population occurs even during the continued presence of the drug. Finally, it is worth noting that in the mouse even vigorous treatment with Compound 48/80 may leave as much as 50% of the subcutaneous mast cells intact (*Riley* and *West*, 1955a, 1955b). The cause of the differential sensitivity of the mast cells is unknown, but it is a reminder that they are not cells with constantly uniform reactions.

Although this article is primarily concerned with histamine it is important to note that mast cells certainly contain other substances. Heparin has long been known to be a constituent of the mast cell, and it was indeed the fact that chemical histamine liberators released heparin as well that prompted the investigation by *Riley* and *West* as to whether histamine was also present in mast cells. But in addition to heparin there is now evidence that 5-hydroxytryptamine occurs in the mast cell, perhaps in about 1/10 of the amount of histamine present, and released by procedures which disrupt the cell such as the action of the histamine liberators (*Bhattacharya* and *Lewis*, 1956). There are also a number of enzymes, including phosphatase, succinoxidase, phosphoramidase, histidine decarboxylase, and an enzyme resembling chymotrypsin (*Montagna* and *Noback*, 1947; *Schayer*, 1956; *Benditt*, 1956).

It is therefore misleading to think of the mast cells solely as reservoirs of histamine and heparin. Indeed the possibility cannot be excluded yet that their histamine content is incidental to some other more important function. This, however, is rendered less likely by the recent discovery (*Schayer*, 1956) of histidine decarboxylase in the mast cell, and the ability of the mast cell to form and bind histamine.

The distribution of histamine has been followed to the intracellular level. It is interesting that as long ago as 1938 *Trethewie* showed that it was possible to obtain, from dog liver, particles in cell-free extracts which contained histamine and which would release this histamine on heating; he made similar observations for acetylcholine. His words are worth quoting: "the cell cannot be regarded as a "diffusion-tight compartment" in the interior of which histamine or acetylcholine is freely diffusible. There is no experimental evidence for the assumption of a "precursor" or

"complex". The internal structure of the cell and not the integrity of the outer cell membrane determines its capacity for holding in its substance these active principles. As long as the internal structure even of part of a cell is intact, acetylcholine or histamine is prevented from diffusing readily." The exploitation of these results had to wait until the technique of differential high-speed centrifugation of cellular fractions developed a good many years later, but it has now been shown by several workers that if histamine-rich tissues are homogenized and differential centrifugation performed, then a "large granule" fraction can be obtained which usually holds the bulk of the histamine in the tissue (*Hagen*, 1954; *Copenhaver* et al., 1953; *Mota* et al., 1954; *McIntosh*, 1956). Although it was at one time thought that these granules originated in the parenchyma of the tissues employed (dog lung or liver) the recognition that mast cells are rich in histamine has made it more probable that the granules originate from mast cells embedded in the tissues concerned (*Mota* et al., 1954; *West*, 1955). Although this is not absolutely certain it is substantiated by the finding of heparin also in some of these large granule fractions. So far as mast cell histamine is concerned, therefore, our conception at present must be that it is contained in granules within these cells shielded by a specific membrane comparable to that which is known to control access to ordinary mitochondria.

Factors Influencing Histamine Content

The causes of the wide variation in histamine content of various tissues are largely unknown. Intake of histamine in food, or formation by bacteria in the intestine and subsequent absorption seem normally to be unimportant. There is evidence, however, that age can exert a profound influence. *Feldberg* and *Kellaway* (1937) found that the histamine content of cat lung rose from about 5 μg./g. with kittens of weight 1 kg. or less, to 40–100 μg./g. with animals of four to five kg. body weight. *Riley* and *West* (1953) found that the histamine content of the lung of man, cow and cat, liver of cow, and skin of cat was 3–20 times higher in the adult than in the foetal or very young animal. *Haeger*, *Kahlson* and *Westling* (1953) found that the histamine content of cat intestine was 2–3 times that of the cat foetus, although that of stomach wall was the same. On the other hand, *Rocha* e *Silva* (1940) records a fall in

histamine content of rabbit abdominal skin from values of 10 to 25 μg. near birth to about 5 μg./g. at a body weight of 2 kg. Important changes take place as birth approaches and at weaning. Although early foetal tissue is very low in histamine, guinea-pig lung may contain about 80 μg./g. at birth (*Trethewie*, 1947) and rat skin 30 μg./g. (*Hardwick*, 1954). Thereafter a fall takes place over some weeks interrupted, at least in the rat, by weaning at about 20 days with an accompanying increase of 200–300 % in skin histamine content. It seems likely that after these changes following birth, a steady rise in histamine content occurs in some, though possibly not all tissues. *Misrahy* (1946) raises the possibility that with lapse of time a progressive differentiation in histamine distribution in the body occurs.

Hormonal influences, too, appear to control tissue histamine. Adrenalectomy in the rat leads to increases in the histamine of skin, lung, liver, small intestine, spleen, heart and striated muscle (*Rose* and *Browne*, 1940; *Marshall*, 1943; *Geiringer* and *Hardnick*, 1953) but not in the stomach (*Haeger*, *Jacobson* and *Kahlson*, 1952). It also increases histamine binding in rat skin (*Schayer*, 1956). The administration of cortical hormone, on the other hand, lowers histamine content (*Rose*, 1939) and decreases histamine binding by rat skin and other tissues (*Schayer*, 1956). In addition, hypothyroid states tend to lower skin histamine, and hyperthyroidism to increase it in skin, liver and muscle (*Gotzl* and *Dragstedt*, 1940; *Hoffmann* and *Hoffmann*, 1944; *Feldberg* and *Loeser*, 1955). It is interesting that contrasting results have been found on the effect of these procedures on the mast cell count. Mast cell counts are stated to be increased by hypothyroidism or the administration of cortisone, and restored to normal in hypothyroid subjects by thyroxin administration. Oestrogens also increase their number (*Arvy*, 1955).

Our understanding of these processes is clearly deficient, but their importance for the physiology of histamine and for the understanding of the influence of age or endocrine balance on allergic reactions needs no emphasis.

How is Histamine Bound in the Tissues?

A primary element in any theory of histamine release is a statement as to how it is normally bound in the tissue. Of necessity the theory of release and the theory of binding are closely related.

A number of suggestions has been made as to how histamine is held in the body. It might be supposed that it is held behind some cellular barrier in such a way that it cannot diffuse out until the barrier is removed or broken down. Such a concept merely states the problem but does not contribute towards answering it. A more specific proposal was that histamine is bound by a bond corresponding to a peptide linkage, so that it could be freed from its attachment by the action of proteolytic enzymes. This idea originated from work by *Rocha* e *Silva* on the anaphylactomimetic properties of trypsin. But its persuasiveness has been lessened by the discovery of many other substances, whose proteolytic activity is clearly nil, which can yet release histamine both more promptly, in larger amounts, and with smaller doses, than can any proteolytic enzyme yet described. Some interest, as we shall see, has been taken in whether these substances might activate proteolytic enzymes in the body, but no decisive evidence of this has yet been found. Against the peptide bond theory, too, is the remarkable lability of histamine binding, such that it can be freed from the tissues by a wide range of simple bases, or by merely stroking the skin. No peptide linkage yet characterised is as fragile as this. A different proposal has been that the histamine is bound in some way as a complex of ionic nature with a tissue acid. This notion was stimulated by the observation (*Mac Intosh* and *Paton*, 1949) that the histamine liberators caused the appearance in the blood of animals receiving them, not only of histamine, but also of heparin, a highly sulphonated polysaccharide providing a possible acidic counterpart to the basic histamine molecule. It is in fact possible to show that histamine and heparin will form a complex. Heparin indeed will do this still more readily with a number of histamine liberators (*Mac Intosh* and *Paton*, 1949; *Mota* et al., 1953). This fact prompts a theory of histamine release that the liberator simply displaces histamine from its attachment to heparin in the tissues, presumably the mast cells. But if a tissue acid is sought with which such ionic binding might occur, heparin is not the only possible candidate. Another possibility is the widely spread nucleic acid of the body. With ribose nucleic acid, for instance, histamine will form not only a complex but a visible precipitate; and histamine liberators will form this precipitate with a readiness corresponding to their ability to mobilize histamine from the tissues (*Paton*, 1956). The solubility of the histamine-nucleic acid complex is considerably lower than that of the hist-

amine-heparin complex so that nucleic acid would indeed represent a more effective way for immobilizing histamine than would heparin; with the latter it is doubtful if a substance could ever be brought out of solution. In addition, there are a number of other phosphates which must be considered. *Darlow* (1956) has found that typical histamine liberators will form complexes with adenosine triphosphate (ATP), sodium triphosphate, glycerophosphate, glucose-l-phosphate and fructose-l,6-diphosphate. Although the reaction with histamine itself is not examined, these observations suggest that histamine might be attached not only to heparin or nucleic acids, but also to any of the numerous phosphate compounds present in the body. It is worth noting that ATP and adrenaline appear to be stoichiometrically associated in the adrenal medulla (*d'Iorio* and *Eade*, 1956); thus for another amine, not too dissimilar from histamine in many ways, an evidence for an association of the kind suggested already exists.

It must be admitted that our concepts of how histamine is bound are still speculative. But it is clear that the idea of some ionic binding, leading either to a complex or to an actual precipitation of histamine in some intracellular particle, is a possibility which promises to be fruitful.

How is Histamine Mobilized?

This review is concerned principally with the drugs known as histamine liberators and their actions. Before discussing these in detail, it may serve to clarify the picture to review some of the other agents which are known to release histamine.

First is the anaphylactic or allergic reaction. Here a previous exposure to an antigen is required; the histamine release from the tissue sensitized takes place only when the second exposure occurs after a certain minimum length of time; and antibodies of some sort can be demonstrated.

Second may be mentioned the wide range of toxins, venoms and proteolytic enzymes, which have been fully reviewed in earlier papers (see *Feldberg*, 1945). Some of these are very effective histamine releasing agents, but they commonly cause profound tissue damage, and may lead, for instance, to the appearance of cellular debris in perfusion fluids.

Third is a group of large molecular weight materials which show remarkable species selectivity. These include dextran, active

in the rat; egg albumen, active chiefly in the rat; polyvinylpyrolidone, active in the dog; horse serum, active chiefly in the cat. To this group may also belong, although this is quite uncertain, the interesting anaphylatoxin obtained by incubating guinea-pig serum with agar or certain other substances. This has recently been restudied by *Rocha* e *Silva* and will be discussed in some detail, together with the other substances mentioned, in view of their widespread use and their interesting properties theoretically.

Fourth is a number of surface active materials including lysolecithin (*Feldberg*, 1945), the detergent Tween 20 (*Krantz* et al., 1948) and bile salts (*Schachter*, 1952). These materials are highly surface active and have been shown under various conditions to be able to produce anaphylactoid reactions in the cat or in the dog and in some cases at least to produce a rise in histamine either in the blood of the whole animal or in the perfusate flowing from an isolated organ. Although the action of these substances is not clearly understood, their strong interfacial activity makes it probable that they function chiefly by lysing some limiting cell membrane. Although a substance like lysolecithin, which can be derived from natural sources, may be of physiological importance, the evidence for this is now less strong than when it was discussed by *Feldberg* in 1945.

Fifth is the group of fairly simple monobasic compounds, of which the simple amine octylamine may be taken as the prototype. These were found by *Mongar* and *Schild* (1953), using minced guinea-pig lung, to be effective in releasing histamine when used in quite low dilutions. They are much less active when given to intact perfused organs, when injected intravenously, or when injected intradermally into intact skin (*Feldberg* and *Mongar*, 1954). The relationship between these compounds and the histamine liberators is of some importance and will be dealt with later.

Histamine-Liberators

Finally there are the substances which *MacIntosh* and *Paton* (1947, 1949) termed histamine liberators. The earliest definite evidence for an action of this type was obtained by *Anrep* and his colleagues (*Alam* et al., 1939) in a study of the effects of curare on muscle. They were able to show that curare injected into a perfused muscle caused the appearance of histamine in the venous blood; that injected into a whole animal rises in systemic plasma histamine

occurred; that the muscle lost histamine during this release process; and that repeated injections yielded progressively smaller releases. *Mac Intosh* and *Paton* found that this property of histamine release was very widely diffused over a large number of bases, some of very simple structure. Some of these compounds have proved to be suitable for general purposes of histamine release, especially propamidine (one of the therapeutic diamidines used in trypanosomiasis) and the substance known as Compound 48/80. The name histamine liberator serves a useful purpose in characterising compounds having this particular action among the whole range of general histamine releasing agents or "histamine releasers". A number of properties common to the histamine liberators serves to distinguish them in one way or another from the histamine releasers mentioned earlier. (1) They act the first time they are administered to an animal. (2) They do not cause major damage to the tissues. (3) When their activity is compared on intravenous injection into a cat, or by intradermal injections into rat, rabbit, guinea-pig or man, or on isolated perfused skin of the dog or the cat, or on isolated perfused muscle of the cat, or on minced lung preparations, their potency is approximately uniform throughout, suggesting some consistent mode of action (4). With the more specific members of the group, histamine release appears to be nearly the only action, and if they are tested again after a pronounced release has taken place their physiological effects become steadily smaller. This may be compared, for instance, with the action of bile acids or of octylamine. With these substances given intravenously, considerable changes of blood pressure, for instance, are seen, due either to the adrenaline-like action of the molecule (in the case of octylamine) or to a direct action on the heart (in the case of bile acids); these changes may be so great as to obscure any histamine liberating action, although indeed none of the typical action of the histamine liberator can be seen even allowing for these additional changes. (5) The histamine liberators characteristically act, when given intravenously in the whole animal, in a strikingly prompt way, probably in a matter of seconds.

Some of the features of the action of histamine liberators deserve additional discussion. The first is that just already mentioned, their speed of action. When they are injected into a cat anaesthetized with chloralose, they produce a characteristic delayed depressor response, which occurs because the drug is ineffective

until it has released histamine from the tissues (Fig. 4). There is, therefore, a latency in its action (compared to that of histamine itself) corresponding to the time taken for the blood to circulate from the point of injection of the drug, i.e. a vein, through the

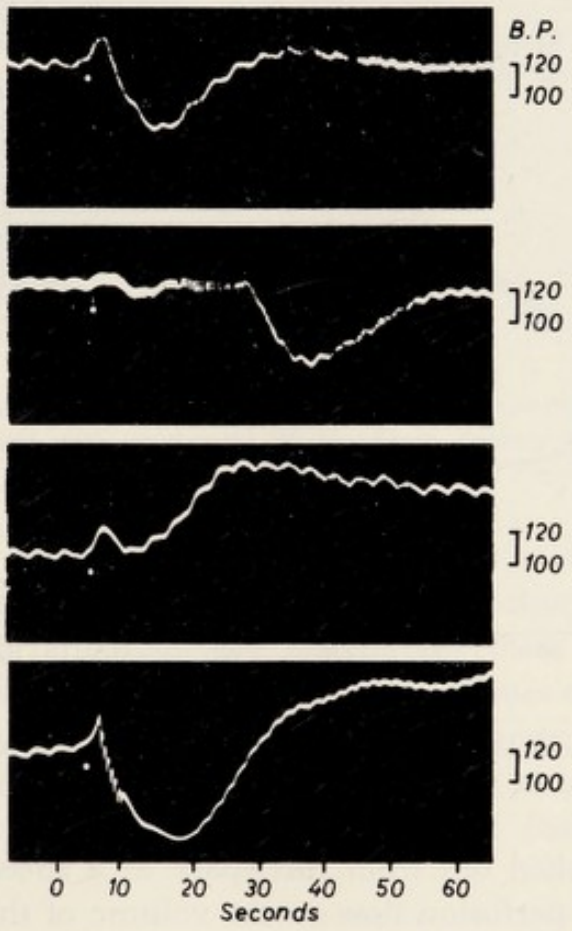


Fig. 4. Effect of histamine, compound 48/80, and octylamine, on the blood pressure of the cat under chloralose. The time of injection (intravenous) is marked with a dot. Note the latency of action of compound 48/80. (From *Paton*, 1956.)

heart and lungs and the arterial system to the tissues and then to return again to the heart and lungs and out into the systemic circulation carrying histamine with it. If this latency is compared with the known circulation time in the cat (*Gray* and *Paton*, 1949) it is necessary to suppose that histamine release takes place in a few seconds at most. The fall in blood pressure due to the released histamine takes place at just about the same time as blood is beginning to make its second circulation. This is probably the most accurate estimate of the speed of histamine release. But a similar conclusion as to the rapidity of the process follows some experiments on perfused organs (*Feldberg* and *Paton*, 1951). In muscle in particular the characteristics of the release process confirm rather precisely to the idea that the histamine is freed rapidly into the extracellular space

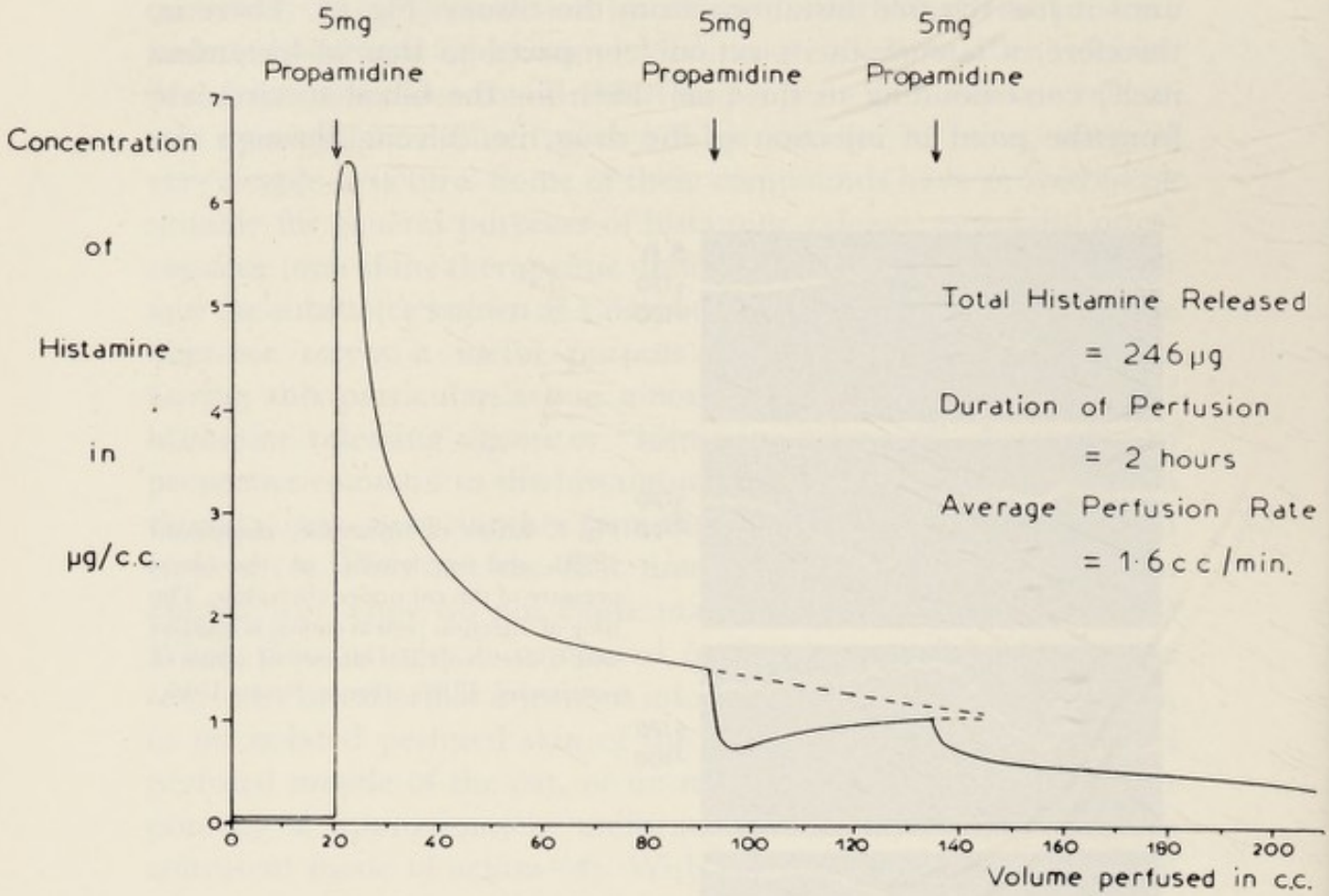


Fig. 5. Release of histamine from cat's isolated perfused skin. (From *Feldberg* and *Paton*, 1951, by permission of the Journal of Physiology.)

of the muscle and is then washed out from this space at a speed depending on the ratio of the perfusion flow to the volume of the space. The form of the release curve is the same with a single injection as with an infusion of liberator. From these experiments, the release has been termed "explosive", and contrasts with some of the other release processes such as those by proteolytic enzymes and the large molecule substances referred to above. This speed of action in intact organs may deserve some attention, since in the recent work on mast cell preparations or intracellular granules in vitro, a rather longer period is needed for the full action of the liberating agent to be displayed; sometimes as much as 30 minutes may be required, a time longer than can be accounted for by diffusion difficulties. This may not represent any basic objection to mast cells or intracellular granules being concerned in histamine release, but provides a warning against extending in vitro results directly to phenomena in the whole animal.

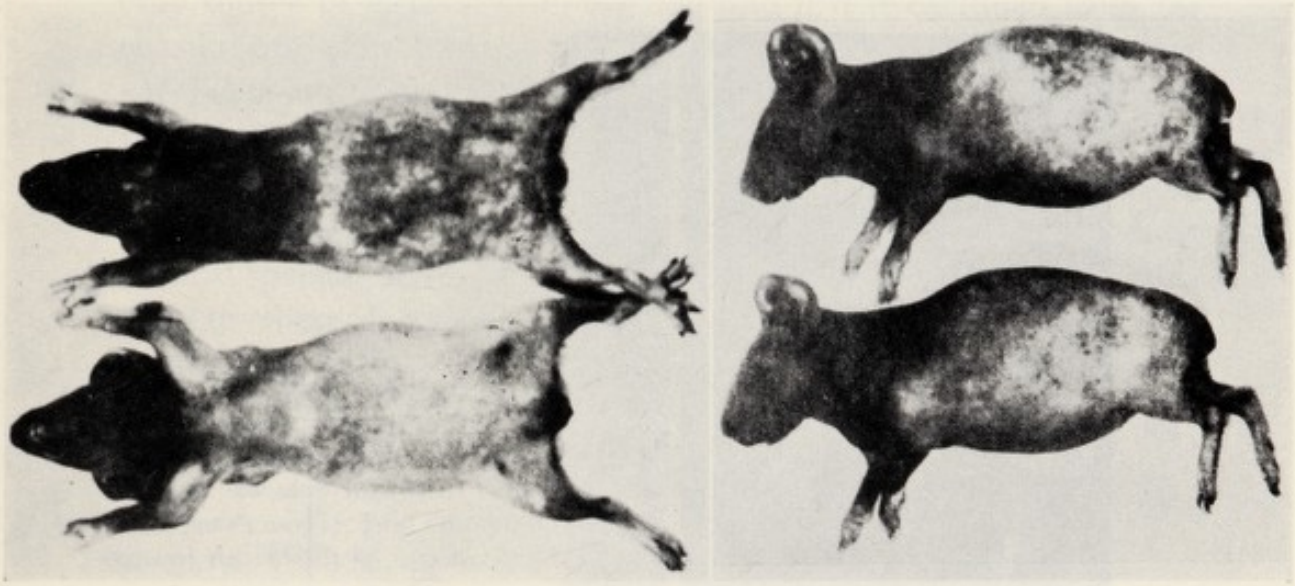


Fig. 6. The distribution of bluing in a guinea-pig's skin when dye is injected after treatment with compound 48/80. (From *Feldberg* and *Miles*, 1951, by permission of the Journal of Physiology.)

A second feature about the histamine liberators concerns the tissues of which they are most active. The skin is far the most important tissue for histamine release by these substances (Fig. 5). This is partly because of the tissues on which they work, skin contains the highest histamine content in nearly all species; partly because the fraction of the tissue histamine which can be mobilized is virtually 100% (*Feldberg* and *Paton*, 1951). Thus in the whole

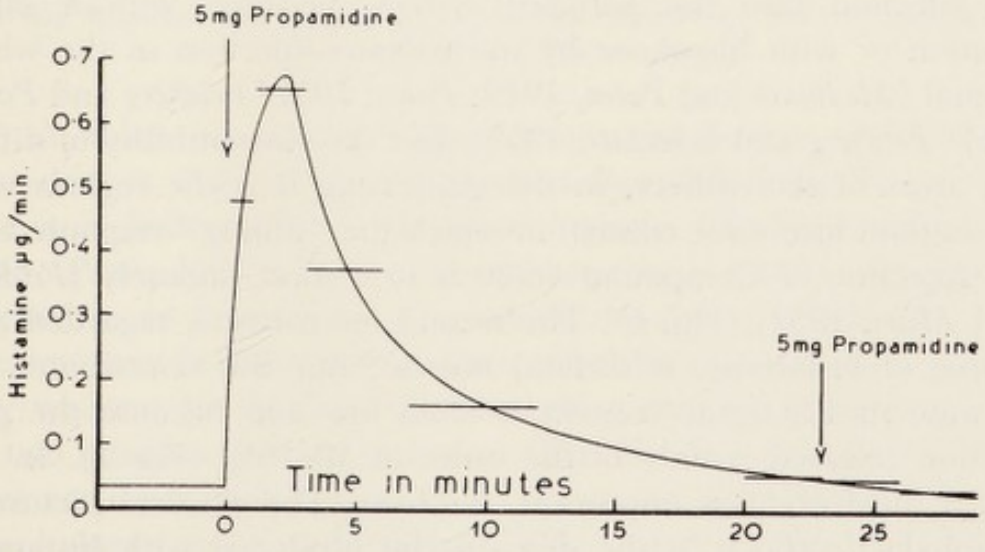


Fig. 7. The release of histamine from cat's isolated perfused gastrocnemius in response to a single injection of histamine liberator. (From *Feldberg* and *Paton*, 1951: by permission of the Journal of Physiology.)

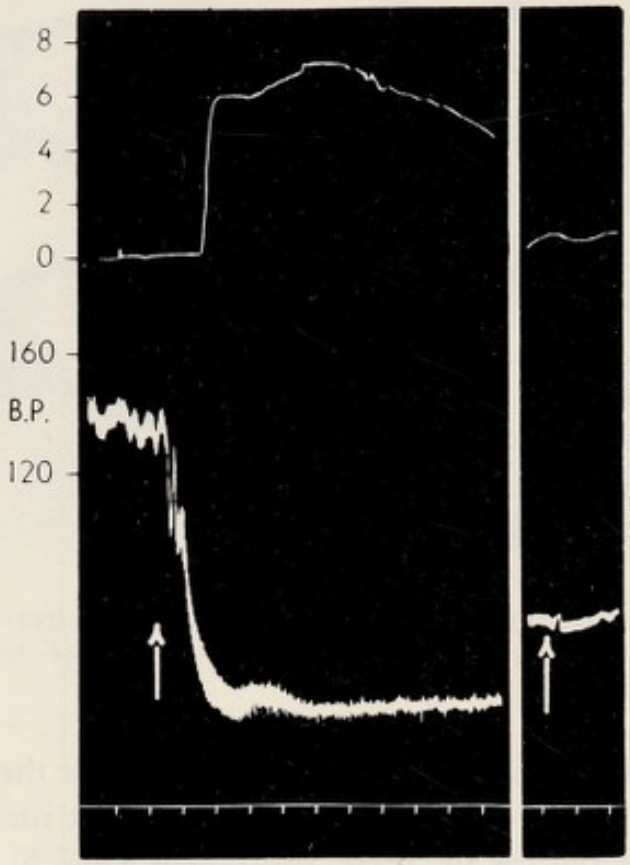


Fig. 8. (a) Effect of 48/80 (1 mg./kg. intravenously) on portal pressure, above, and arterial blood-pressure, below. In (b) the dose was repeated 60 minutes later. (From *Paton*, 1951: by permission of the British Journal of Pharmacology.)

animal large amounts of histamine can be mobilized from the skin, amounts fully adequate to produce circulatory shock and other gross signs of histamine poisoning. The liberators are effective on all skin preparations whether the drug is given intradermally, by injection into the perfused system, perfused with a saline solution or with blood, or by intravenous injection in the whole animal (*McIntosh* and *Paton*, 1949; *Paton*, 1951; *Feldberg* and *Paton*, 1951; *Feldberg* and *Schachter*, 1952). But the susceptibility of different areas of skin differs; in the guinea-pig it is the regions with the highest histamine content in which the "bluing" response after the injection of Compound 48/80 is seen most intensely (*Feldberg* and *Miles*, 1952) (Fig. 6). The second main tissue, regarded as a source of histamine, is skeletal muscle; but this contributes less because its histamine content is often low and because the proportion released is only of the order of 50–70% (Fig. 7). In the dog a third organ is important, the liver. The classical picture of anaphylactic shock in the dog can be produced with histamine liberators, i.e. engorgement of the liver, a rise in portal pressure, and a circulatory shock resulting (Fig. 8). But the liver is not the

only source of histamine in the dog and if it is excluded from the circulation, skin and muscle make an important contribution (*McIntosh* and *Paton*, 1949). With other tissues, such as the intestine and the lung, release of histamine is slight and can usually only be observed if the concentration of liberators is raised to the order of 0.1 or 1 mg./ml. It appears that the histamine in skin and muscle in most species, and in the liver of the dog, is accessible in a specific way to these histamine liberators, whereas histamine in other tissues is fixed in some slightly different manner.

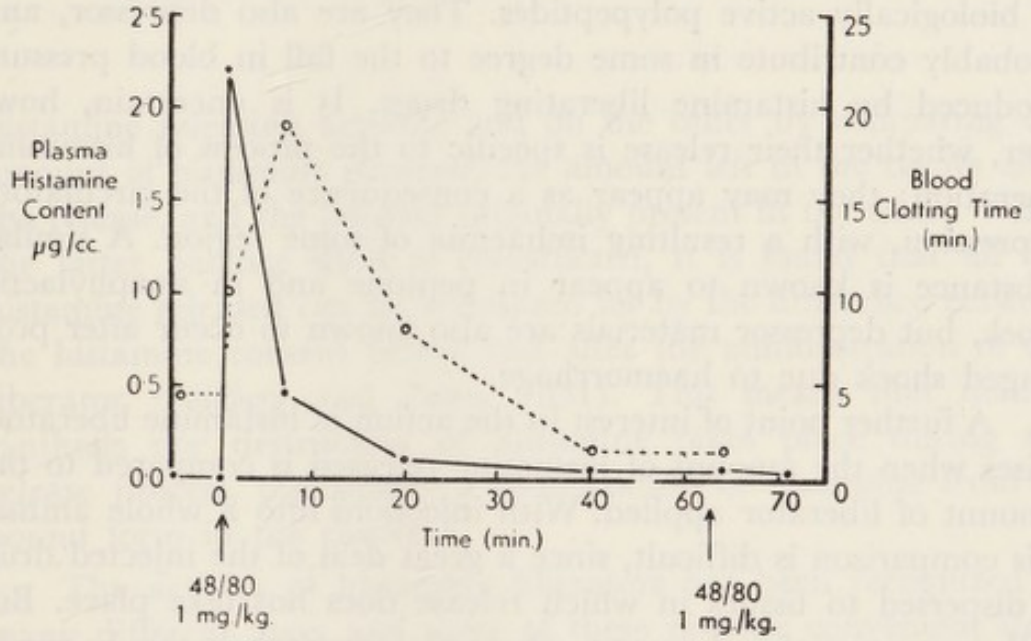


Fig. 9. Histamine release and delay in clotting time produced by 48/80 in the anaesthetised dog. Repetition of the injection had no effect. (From *Paton*, 1951: by permission of the British Journal of Pharmacology.)

A third important feature about the action of histamine liberators is that the blood becomes less coagulable in the dog, due it is believed to a release of heparin (*McIntosh* and *Paton*, 1949; *Paton*, 1951). The release of heparin is slower than that of histamine, so that the time at which the defect of coagulation is greatest may be about ten minutes after the injection of the liberator, whereas the peak of the histamine content is obtained one or two minutes after the injection (Fig. 9). The coagulation defect does not seem, however, to be as intense as with peptone or with anaphylactic shock, and *Quivy* (1955) using another method has had some difficulty in demonstrating it. The position is complicated by the fact that many of the histamine liberators form insoluble

complexes with heparin, interfering with its anticoagulant activity just as can toluidine blue or protamine (*McIntosh* and *Paton*, 1949; *Garcia Arocha, et al.*, 1953, *Mota, et al.*, 1952). As a result, one must expect rather variable effects, according to the amount of heparin released, the dose of histamine liberator given, and the solubility of the complex between them. As well as heparin there may also appear in the blood one of the so-called "slow reacting substances". These are recognized by their production on the guinea-pig ileum preparation of a rather sluggish contraction resistant to atropine and antihistamines; such substances probably belong to the group of biologically active polypeptides. They are also depressor, and probably contribute in some degree to the fall in blood pressure produced by histamine liberating drugs. It is uncertain, however, whether their release is specific to the process of histamine liberation; they may appear as a consequence of the circulatory depression, with a resulting ischaemia of some region. A similar substance is known to appear in peptone and in anaphylactic shock, but depressor materials are also known to occur after prolonged shock due to haemorrhage.

A further point of interest in the action of histamine liberators arises when the amount of histamine released is compared to the amount of liberator applied. With injections into a whole animal this comparison is difficult, since a great deal of the injected drug is dispersed to tissues in which release does not take place. But with tests on perfused organs, where the comparison can be made more satisfactorily, it is found that compound 48/80, for instance, may release many times its own weight of histamine, probably of the order of 10–50 times on a molecular basis. Even with less active substances, such as propamidine, a ratio larger than 1 : 1 is obtanied (*Feldberg* and *Paton*, 1951; *Paton* 1951).

The studies of the action of histamine liberators have incidentally excluded some mechanisms by which they might be thought to operate. It could be suggested for instance that they inhibited diamine oxidase in the tissues, so allowing histamine to accumulate. Alternatively it might be supposed that they promote a synthesis of histamine in the tissues. These hypotheses are excluded, on the one hand by finding that there is no correlation between the ability of the drug to inhibit amine oxidase and its ability to release histamine (a striking example is that of isonicotinic acid hydrazide, which is a very effective antihistaminase but quite devoid of

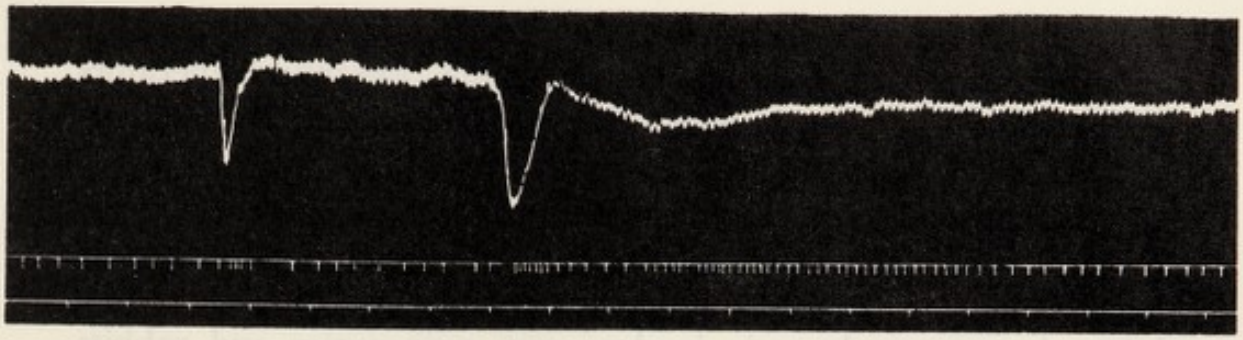


Fig. 10. Effect of histamine (10 μg.) and propamidine (3 mg.) on the flow of lymph from thoracic duct of the cat under chloralose. Upper tracing, blood pressure; middle tracing, record of drops of lymph; lower record, time in minutes.

histamine releasing action); and on the other by comparing the amount of histamine released, the amount left in the tissues after the release, and the amount originally present in the tissue. When the latter balance sheet is constructed, it is found that all the histamine released can be accounted for by the difference between the histamine content before and after the administration of the liberator (*Feldberg* and *Paton*, 1951). This means that neither synthesis nor destruction of histamine takes place during the release process, but simply a mobilization of histamine from its bound form in the tissues.

The activity of histamine liberators has been recognized in many different ways and some of these provide convenient signs of such an action, as well as recapitulating the general physiological actions of histamine.

(1) Together with a fall in blood pressure, there is, with the larger doses, a marked haemoconcentration of the blood and signs of sympathoadrenal activity, often giving rise to a secondary rise in blood pressure and a strong tachycardia.

(2) In the anaesthetized animal, if the thoracic duct is cannulated there is a pronounced increase in the flow of lymph (*Paton*, 1954) (Fig. 10).

(3) In unanaesthetized animals, such as the dog, cat, and rat, histamine liberators produce intense generalized itching, together with oedema and erythema of the muzzle and ears and around the eyes and of other parts of the body (*Paton* and *Schachter*, 1951; *Feldberg* and *Talesnik*, 1953; *Feldberg* and *Miles*, 1953; *Smith*, 1953).

(4) In the anaesthetized cat, and still more strikingly in the unanaesthetized dog with a gastric fistula, histamine liberators elicit a flow of pure acid gastric juice (*McIntosh* and *Paton*, 1949; *Paton* and *Schachter*, 1951).

(5) Administered intradermally in man, histamine liberators produce a typical erythema, wheal, flare and itching. In animals local oedema is less readily demonstrated, but blueing of the injected area can be seen in dogs, rat, guinea-pigs and rabbits if a suitable dye is injected shortly after the intradermal injection is made.

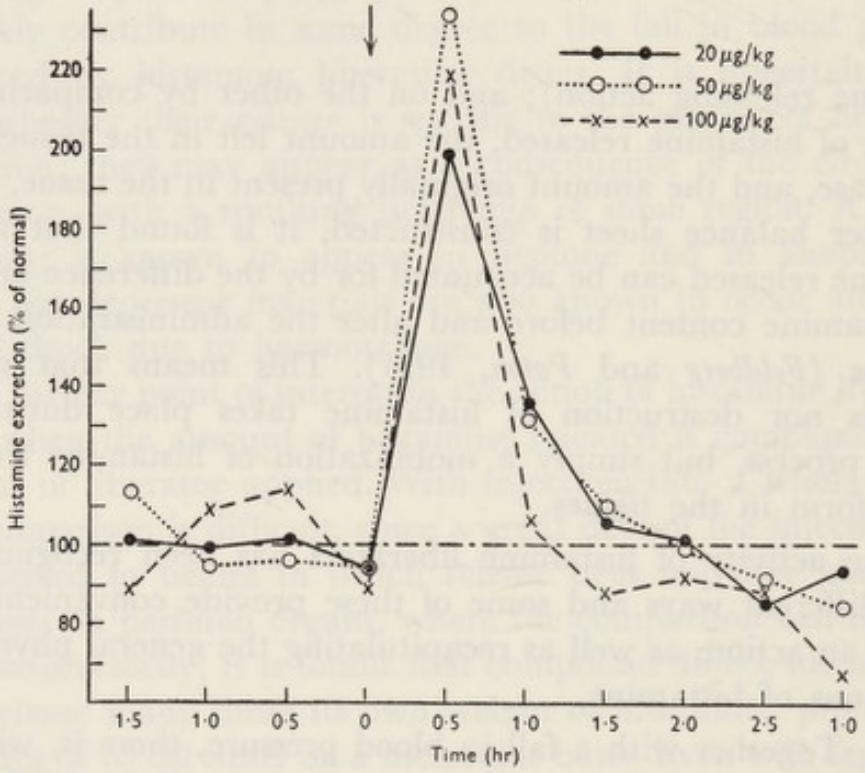


Fig. 11. Effects of intraperitoneal injection of different doses of 48/80 on the excretion of histamine in the urine. Injection made at the time shown by the arrow. (From *Wilson*, 1954: by permission of the Journal of Physiology.)

(6) An increase in the urinary excretion of histamine can be demonstrated after the administration of a histamine liberator. The amount excreted does not, of course, correspond to the amount released. The work of *Adam* and his colleagues (1954) indicates that of histamine infused in man to a brachial vein, only about 1% can be collected in the urine. *Wilson* (1954) found in rats that perhaps 7% of parenterally administed histamine appeared in the urine. *Wilson* has used the urinary excretion to test various

histamine liberators (Fig. 11) and finds that with representative compounds, an abrupt rise in urinary excretion takes place which terminates within one to 1½ hours. The interest of this finding is that it indicates a way of detecting the presence or absence of histamine release under clinical conditions.

Depletion of Tissue Histamine

The potentialities for ridding a tissue of its histamine appeared when *Feldberg* and *Paton* were able to show, in experiments on perfused skin, that virtually all the histamine content (99%) of the skin could be displaced. Following this *Feldberg* and *Talesnik* (1953) made a study of rats receiving repeated injections of 48/80 or propamidine in increasing dose in such a way as to produce the greatest mobilization of histamine compatible with survival of the animal. At first, the subcutaneous injection in 1 ml. of 48/80, 500 μg, or propamidine 2 mg., was used. By this method a considerable reduction of the histamine content of the skin at the site of injection could be achieved, the content falling from a mean value of about 33 μg/gm. to between 3–4 μg/gm. During the subsequent 40 days the histamine content slowly returned to its normal level. Control injections of saline showed a reduction of perhaps 30% when the content was measured 90 minutes after the injection, followed by a return to normal values within a day; this result was no doubt due to a temporary increase in the fluid content of the skin at the injection site. With these subcutaneous injections the changes in skin histamine content were purely local, skin in other regions was unaffected, and there were no systemic signs of histamine liberation. Intraperitoneal injections, however, were more effective. With 500 μg of 48/80, general symptoms appeared and this dose might be lethal. A course of treatment was evolved starting with 150 μg once or twice on the first day, working up to 500 μg. on the eighth day by gradual steps. With this method, the typical results of histamine release (itching, erythema, oedema, cyanosis and prostration) were seen on the first two or three exposures but towards the end of the course the animals no longer reacted so strongly, and might even be able to tolerate a dose of 600 μg. which is normally lethal. The reductions in histamine content produced are shown in the following table, in which some results obtained by *Mota et al* (1956) by a similar method are also included.

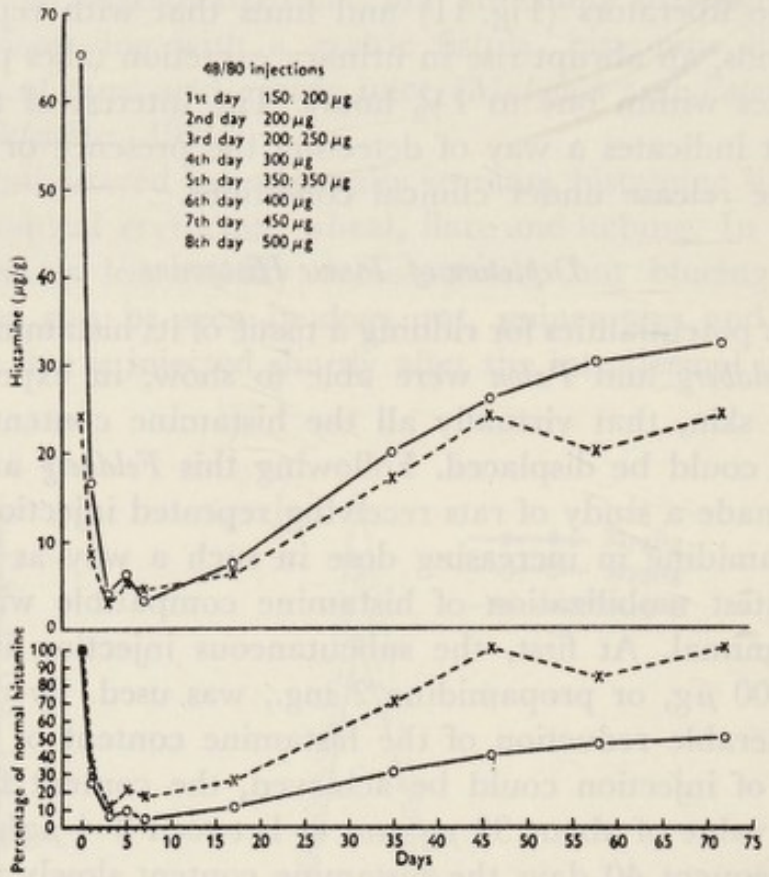


Fig. 12. Effect of intraperitoneal injections of 48/80 on histamine content of rat's skin from abdomen (×---×) and feet (o——o). Upper graphs histamine content expressed in µg./g. skin; lower graphs in percentage of normal values. Eight days treatment with increasing doses of 48/80 as indicated. (From *Feldberg* and *Talesnik*, 1951: by permission of the Journal of Physiology.)

Feldberg and *Talesnik* found that in stomach duodenum and liver, the histamine content was not changed and remained at about 11 µg./gm. for the stomach, 7 µg./gm. for duodenum and 0.5 µg./gm. for liver. With the course of treatment outlined, recovery of histamine content of skin, muscle and heart took about 40 days or more, although the recovery of histamine in the skin of the feet was much slower and had hardly reached 50% of the original value in 75 days (Fig. 12). *Riley* and *West* have made similar observations for the reduction of the histamine content of the tissue of the omentum, mesentery, and subcutaneous tissue in the rat. *Mota et al.* correlated their results with the presence of mast cells in the tissues concerned and the extent to which they were attacked by the liberator. They found that in the tissues in which a successful release took place, there was a notable reduction in the mast cell content and that in a tissue, such as the fundus

Table II
Histamine Depletion in the Rat

Tissue	Normal animals		48/80 treated animals		% Histamine loss
	Histamine μg/gm	Mast cells	Histamine μg/gm	Mast cells	
Skin (foot)	65	—	±5	—	92
Skin (abdomen)	24	—	±5	—	79
Striated muscle					
Diaphragm.	8.5	—	1.6	—	81
Gastrocnemius	2.5	—	0.8	—	68
Heart muscle	2.2	—	1	—	55
(From *Feldberg* and *Talesnik*, 1953)					
Skin (foot)	61.0	++++	8.6	+	86
Skin (dorsum)	7.9	+++	5.4	+	81
Tongue	14.5	+++	2.9	+	80
Oesophagus	4.5	++	1.9	0	58
Cardia	3.0	+	1.0	0	67
Fundus	15.1	+	14.8	0	2
Duodenum	14.8	0	14.2	0	4
Ileum	5.5	0	5.4	0	2
Rectum	2.5	0	2.8	0	—12
Liver	0.5	+	0.4	0	20
(From *Mota*, *Beraldo*, *Fevis* and *Junqueira*, 1956)					

of the stomach or the duodenum, in which there was a content of histamine greater than could be accounted for by mast cell content, histamine release did not take place.

A similar analysis of depletion was made in the cat by *Smith* (1953) and the following table shows the mean results obtained.

To produce these changes an initial injection of 1 mg./kg. of 48/80 was given, followed by 0.5 mg./kg. increments every second or third day until more than 30 mg./kg. had been given over a period of two to three weeks. Doses higher than 6 mg./kg. produce severe prostration. During the treatment most animals lost up to 1 kg. body weight and their health deteriorated. In several animals there was serious gastric ulceration, and haemorrhages in the gastric mucosa and sometimes in the duodenum; haemorrhagic changes elsewhere were also seen. It was noticed that as a result of treatment with 48/80 not only did the external signs of response to 48/80 become slighter, but also the effects of 48/80 on the blood pressure were much reduced. Thus whereas 50 μg./kg. normally produces a precipitous fall in blood pressure, in a treated animal, even if it had been kept well hydrated during treatment, no fall in blood pressure occurred, and there might be a small

Table III

Histamine Depletion in the Cat

Tissue	Histamine μg/gm		% Histamine depletion
	Normal	48/80 treated	
Skin: ear	96	12	87
abdomen	22	5	77
Skeletal muscle:			
diaphragm	2.6	1.9	27
gastrocnemius	2.1	1.5	29
Stomach: corpus			
mucosa	29	20	31
sub-mucosa	19	12	37
Muscularis externa	12	11	8
Stomach: pyloric region			
mucosa	23	23	0
sub-mucosa	17	18	— 6
Muscularis externa	10	10	0
Duodenum	40	35	12
Ileum	22	19	14
Colon	11	11	0
Liver	2.9	2.7	7
Pancreas	3.3	2.5	24
Spleen	8.1	9	—11
Lung	35	22	37
Kidney	1.2	1.5	—25

(From *Smith*, 1953)

rise. It was interesting that the response to histamine infusions also changed and a distinct pressor response, abolished by adrenalectomy was found. *Smith* also found that the gastric secretion produced by injection of 48/80 diminished in treated animals.

Brocklehurst, *Humphrey* and *Perry* (1955) in work on the allergic responses obtainable in the rat and guinea-pig, point out that even the most stringent measure for reducing the histamine content of rat's skin will not bring it much below about 3 μg./gm. This was true even though 48/80 was combined with octylamine, with hyaluronidase, with the induction of an allergic reaction, or with all these procedures together. They also noted that in the guinea-pig, whose skin histamine content is a good deal lower than of the rat, the proportionate reduction of histamine content produced by 48/80 is also much smaller. They suggest from this that there may be in all species a fraction of skin histamine, perhaps 3 μg./gm. magnitude, which is insusceptible to mobilization by chemical releasing agents. This does not square with *Feldberg* and *Paton*'s

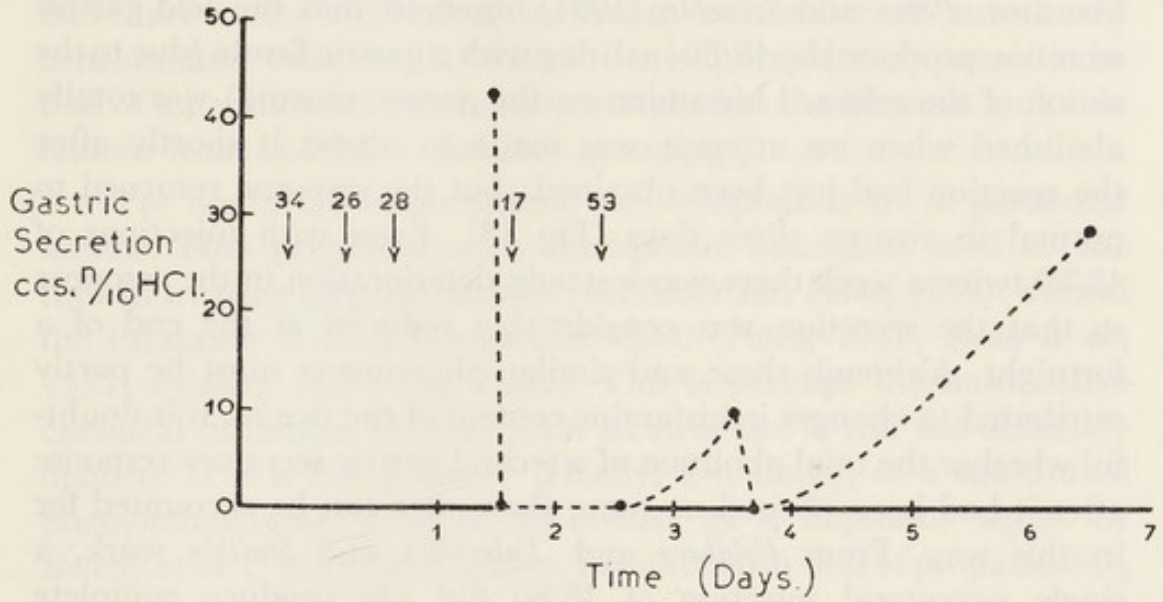


Fig. 13. Graph of gastric secretion produced by injection of 10 mg. compound 48/80 into the unanaesthetised dog with a gastric fistula. The inserted figures are the responses to 1 mg. histamine acid phosphate at times marked by the arrows. (From *Paton* and *Schachter*, 1951: by permission of the British Journal of Pharmacology.)

results on perfused skin, where the histamine content could be reduced to undetectable levels; but it may well be true for the whole animal, in which the histamine content of the tissue may be replenished from other regions in the body. The possibility of depletion of the tissues with histamine liberators as it stands at present may be summarized as follows. That of skin may be lowered by 80–90%, that of muscle by perhaps 30%, that of lung, oesophagus and gastric mucosa of the fundus of the stomach by comparable amounts, but the histamine of other parts is relatively resistant. The process of depletion is not, however, a completely harmless one and serious changes take place in the animal, possibly due to the continued high level of histamine free in the body, possibly from other causes.

The finding that a major portion of the histamine in the body can be removed by treatment with liberators probably explains a good many of the phenomena of refractoriness which have been described. From the earliest work it was found that after a large dose of liberator had been given, subsequent injections became progressively less effective. *McIntosh* and *Paton* (1949) found that intradermal injection of histamine liberator rendered subsequent injections ineffective for up to about 24 hours at the same site and this provides a very simple method for identifying a histamine-

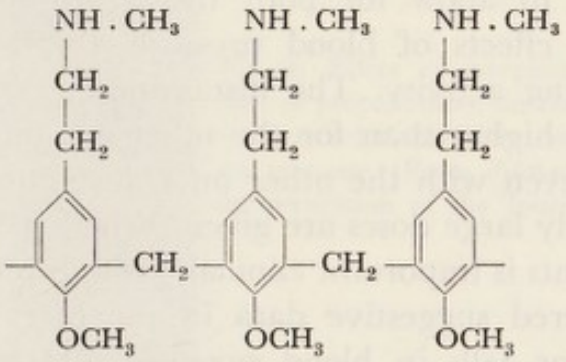
liberator. *Paton* and *Schachter* (1951) observed that the acid gastric secretion produced by 48/80 in th dog with a gastric fistula (due to the action of the released histamine on the gastric mucosa) was totally abolished when an attempt was made to repeat it shortly after the reaction had just been obtained; but the response returned to normal in two or three days (Fig. 13). Even with injections of 48/80 twice a week there was a steady deterioration in the response so that the secretion was considerably reduced at the end of a fortnight. Although these and similar phenomena must be partly attributed to changes in histamine content of the tissues, it is doubtful whether the total abolition of a second gastric secretory response after it had been elicited no more than once can be accounted for in this way. From *Feldberg* and *Talesnik*'s and *Smith*'s work, a single parenteral injection of 48/80 did not produce complete depletion, and it needed a course before the animal became unresponsive to the liberator. It seems possible, therefore, that as a result of injection of liberator, other protective mechanisms come into play. One of these might, indeed, be the mobilization of heparin in the tissues, which could then, by binding the liberator in a complex, render it less effective. Adaptation to the effects of histamine might occur, although this is known not to happen so far as gastric secretion is concerned. Finally some other hormonal reaction, such as the secretion of adrenaline, might come to counter the effects of any released histamine. Whatever the cause, it seems advisable not to attribute all the signs of refractoriness to a depletion of histamine in the tissues alone, without some corroborative evidence.

Some Histamine Liberator Compounds

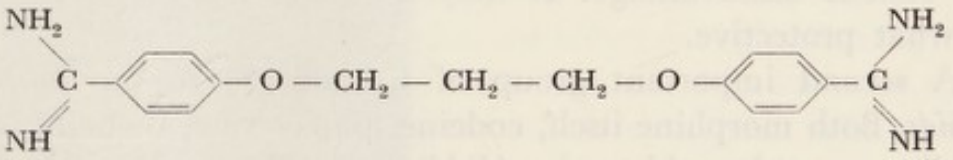
Since 1947 some scores of simple chemical substances have been found to release histamine from mammalian tissue. A number of these are of interest chiefly from the point of view of the characteristics of chemical structure which confer this property. In general it appears that any molecule in which two basic groups (such as amine, amidine, guanidine, isothiourea or quaternary groupings) are separated by an aliphatic chain of five carbon atoms or more or by some corresponding aromatic skeleton, is liable to have noticeable activity. In the simple aliphatic series it appears that the peak of activity is maximal when the carbon atoms are roughly 15 Å units apart. In addition to these relatively simple molecules

there are other molecules, including a number of substituted benzamidine compounds, where the requirement appears to be that of an additional polar group attached to the benzene molecule remote from the basic amidine grouping.

For use in the whole animal two compounds are of particular service. First propamidine, a therapeutic diamidine used at one time in treating trypanosomiasis (*McIntosh* and *Paton*, 1949); second the substance termed Compound 48/80 (*Paton*, 1951; *Dews et al.*, 1953; *Feldberg* and *Paton*, 1951). This is perhaps the most active chemical histamine liberator, but its structure is still not definitely defined. It is a low polymer (chiefly, 2–4 units), of a substituted phenylethylamine nucleus discovered as a depressor agent by *Baltzly* et al. (1949); its structure, together with that of propamidine, is shown below.



Compound 48/80 (general type of polymeric structure)



Propamidine

The main pharmacological action of these two compounds is that of histamine release. Propamidine is effective in doses of about 0.1 mg./kg. and Compound 48/80 in doses from 10 μg./kg. It has to be remembered, however, that almost any pharmacologically active amine is liable to possess some other actions when given in higher dose, and these two substances are no exception. With higher concentrations Compound 48/80 may produce itself a slight contraction of smooth muscle and may in addition anta-

gonize the contractions produced by other stimulants. It also can block autonomic ganglia when used in high dosage (*Gertner*, 1955) and has a rather feeble neuromuscular effect. But except with the highest doses used at the end of a course designed to deplete tissue histamine, it is doubtful whether these other pharmacological actions play any significant part in their effects.

In addition to the compounds mentioned there are a number of drugs in which the property of histamine liberation accompanies other actions of importance. Thus among *quaternary salts* are found a number of active compounds and indeed curare and d-tubocurarine were among the earliest for which histamine release was demonstrated (*Alam* et al., 1936; *Schild* and *Gregory*, 1947; *McIntosh* and *Paton*, 1949; *Reid*, 1950). D-tubocurarine is comparable to propamidine in potency. It has been shown to produce many of the effects of histamine liberators, although in analysing its action it is necessary to allow for both the neuro-muscular blocking action and (if effects of blood pressure are being studied) its ganglion blocking activity. The histamine releasing action of d-tubocurarine is higher than for the other commonly used muscle relaxants, but even with the other ones, histamine release can be produced if fairly large doses are given. Whether histamine release by these relaxants is important clinically is a disputed point. There are only scattered suggestive data in the literature pointing to bronchospasm or falls in blood pressure due to d-tubocurarine and in general it seems, at least in anaesthetic practice, not to be a serious disadvantage. It may be that anaesthesia is itself somewhat protective.

A second important group of substances are the *morphine alkaloids*. Both morphine itself, codeine, papaverine, thebaine, and pethidine, can release histamine (*Feldberg* and *Paton*, 1951; *Schachter*, 1952), although this histamine release only accounts for a component of the depressor potency of the substances (Fig. 14). Histamine liberation probably accounts for some of the anaphylactoid reactions produced by morphine and its analogues, and for the itching and urticaria. In the case of morphine a central pruritogenic effect is also known. The lethal action of morphine in bronchial asthma, (although not in a true cardiac asthma) is also probably to be attributed to histamine release in a subject particularly sensitive to it. Other centrally active drugs known to release histamine include strychnine and atropine.

A number of drugs used for their vasodilator action also can mobilize histamine. Arfonad (Trimetaphan) originally employed as a ganglion blocking agent, certainly releases histamine in doses such as are used clinically. It represents an interesting combination

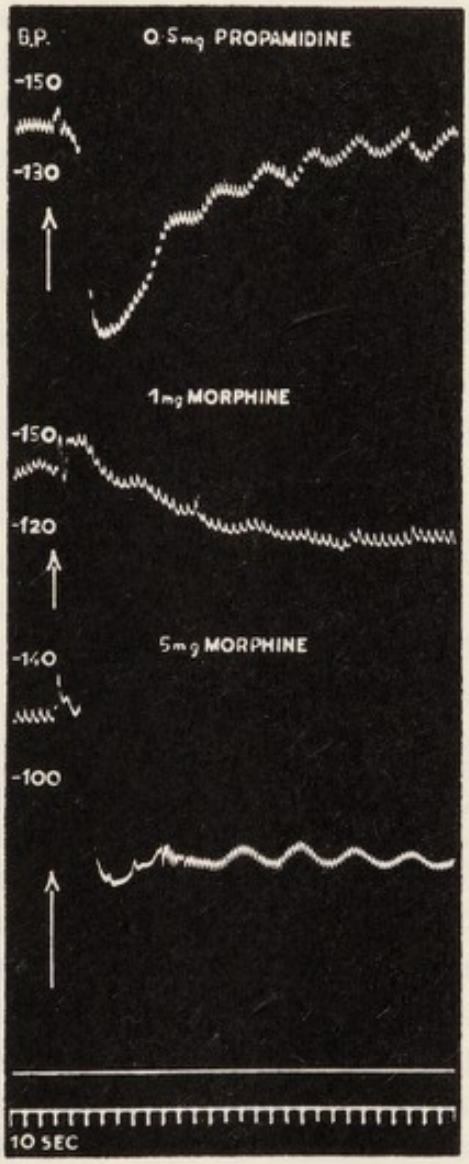


Fig. 14. Effect of morphine, compared with that of propamidine, on the blood pressure of the cat under chloralose. Note delayed depressor response. (From *Feldberg* and *Paton*, 1951: by permission of the Journal of Physiology.)

of ganglionic and histaminic effects, which may indeed have some clinical advantages, provided that histamine liberation is not fundamentally deleterious. The use of arfonad for analytic purposes, of course, is rendered much more complicated by this combined action. Tolazoline (priscol) often used to produce cutaneous vasodilation, can also release histamine (*Schachter*, 1952) although only in rather large doses, probably beyond any achieved in practice. Apresoline (Hydrallazine), which has had some vogue in

hypertension, can also release histamine; this is not a strong feature of its action but since the molecule seems to have no single powerful action it may well contribute, with a number of other moderate activities, to a total depressor effect.

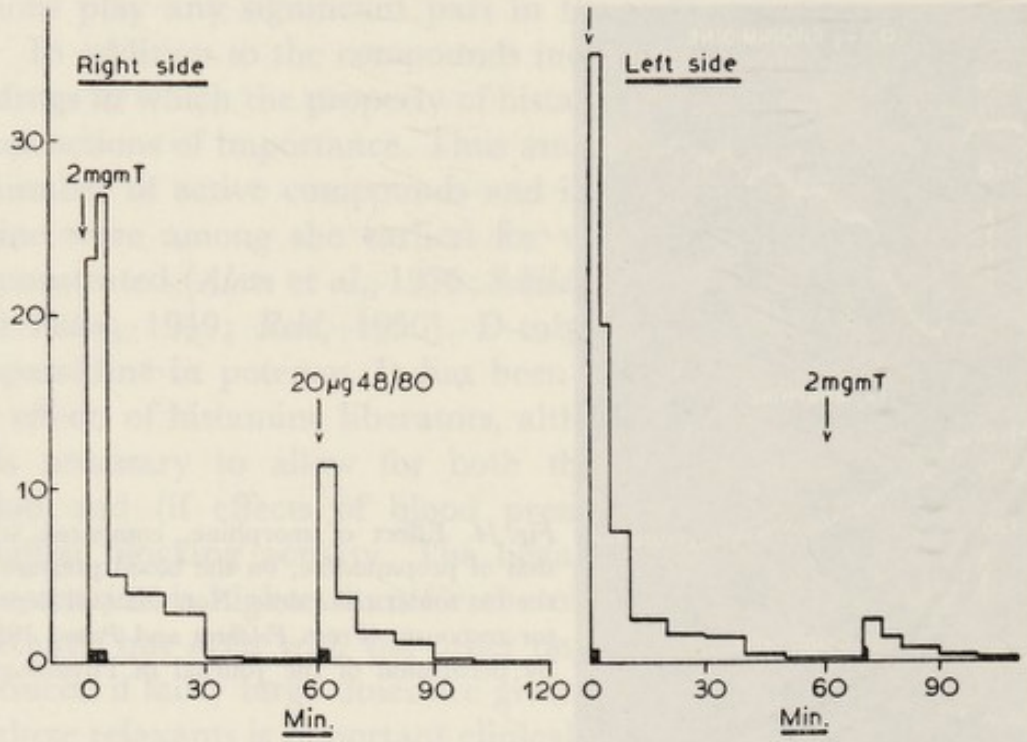


Fig. 15. Comparison of the histamine output by 48/80 and tryptamine (T) from perfused skin preparations of the hind legs of the same cat. The black areas above the base-line indicate periods of pronounced vasoconstriction. (From *Feldberg* and *Smith*, 1953: by permission of the British Journal of Pharmacology.)

Particular interest attaches to compounds of natural origin which can release histamine. The oldest known is, of course, peptone and it appears that those peptones which are prepared from proteins rich in basic amino acids are the more effective. This may well correspond to the general fact that it is basic compounds as a whole which possess the ability to release histamine. It is possible to obtain protein hydrolysates which are free of histamine liberating activity but a certain amount of care is necessary in doing this. A recent attempt (*Eldridge* and *Paton*, 1953) to purify the active principle of peptone led to disappointing results. This suggested that in fact the histamine releasing activity was diffused over many peptides or even amino acids in the peptone, rather than being concentrated to a higher degree in any one of them. As a result the amino acids themselves were

tested for histamine releasing action, as a result of which it was found that the basic amino acids, particularly arginine and lysine were able to release histamine, although those of lower or acidic isoelectric point were totally inactive. This provides a rather convincing example of the way in which it is only basic compounds which display the property of histamine release, whereas substances of similar structure and corresponding acidic strength are devoid of activity. Protamine and polylysine have also been shown to be active (*Garcia Arocha* et al., 1953).

Another substance occurring naturally of considerable interest is hydroxytryptamine, which, with tryptamine, was shown by *Feldberg* and *Smith* to be able to release histamine from mammalian tissues (Fig. 15). This derives additional importance from the recent evidence that tryptamine may occur in mast cells. The possibility opens up of a pharmacological "feed-back" system, whereby hydroxytryptamine might be able to release not only histamine but more of itself from mast cells and so initiate a self-intensifying mast cell degranulation and histamine release. But neither hydroxytryptamine nor tryptamine (*Reid*, 1951), are very active in this respect and it is doubtful whether they would act as histamine liberators under physiological conditions. Gastrin, the extract from gastric tissue which is capable of producing a flow of gastric juice by a still unknown mechanism, has also recently been found to be a histamine liberator of rather modest activity (*Smith*, 1954). Although gastrin is now well distinguished from histamine, and the possibility that it might be able to mobilize histamine in the stomach mucosa would provide an elegant explanation for its action, in fact its activity seems to be too slight to account for its properties. A further group of substances capable of histamine release are extracts of crustacean muscle. There is no doubt that in these extracts there are other principles which through eliciting a sensitization reaction may cause undesired effects after eating. But there is also a substance present which on its first administration to mammalian tissue will cause the release of histamine (*Paton*, 1954). This has been found for mussel and lobster extracts of various kinds. Since it has also been shown that histamine liberators can promote a flow of lymph, it seems clear that the lymphagogue action of extracts of crustacean muscle described many years ago by *Heidenhain* as lymphagogue action of the first order, is to be attributed to this histamine releasing activity. It is of interest that

Broadbent (1953), in a study of the mechanism of action of itching powder, obtained evidence that there is contained in the fine hairs of which the powder consists a histamine liberating substance. Finally, the antibiotics of the polymixin group are effective histamine-releasing agents (*Bushby* and *Green*, 1955; *Norton* and *de Beer*, 1955).

The Responses of the Rat to Egg White

The observation by *Selye* in 1937 that the injection of egg white could produce oedema of the rat was followed in 1947 and 1948 by the demonstration by *Leger* and *Masson* that the active principle in egg white was ovo-mucoid, a substance belonging to the group of glucoproteins. The whole molecule is required for action, and the sugar moiety alone is inactive. The reaction has now been widely used for the study of anaphylactoid responses in this animal or for the testing of so-called "antiphlogistic" substances. It is curious to find that in 1931 *R. L. Webb* noted that egg-white could destroy mast cells in the rat's peritoneal cavity, an observation which had to wait 25 years to be understood.

The main analysis of the physiological changes produced in the body by egg white has been by *Halpern* and *Briot* (1949) (Fig.16). These workers used egg white given intraperitoneally usually in a dose of 1.5 ccs. It is worth noticing that they record that 14% of their animals did not respond to egg white and were excluded from their experiments, showing that the reaction to egg white is not quite as general as may be imagined. The effect produced in the animal under these conditions takes about 15–40 minutes to develop. The limbs are first affected becoming red and then swelling so that the oedema is maximal 90–100 minutes after the injection. The oedema affects the paws chiefly but also the whole limb leading to an increase of 25% in weight. The muzzle is also affected especially the lips which become cyanosed and the nose; the tongue can be enormously swollen up to three times the normal size and occasionally oedema of the glottis is produced. Oedema of the perineal region is also common. The oedema fluid is relatively rich in protein and was estimated to have about 3.5 to 4.5% protein content. The oedematous areas become rapidly blued if an appropriate dye (in this case T. 1842) is injected. At the same time a considerable haemoconcentration occurs so that the count of red cells may increase as much as 57% (mean increase 36%)

although one day later the haematocrit is normal. In addition there is a substantial fall in body temperature of several degrees Centigrade (Fig. 16a).

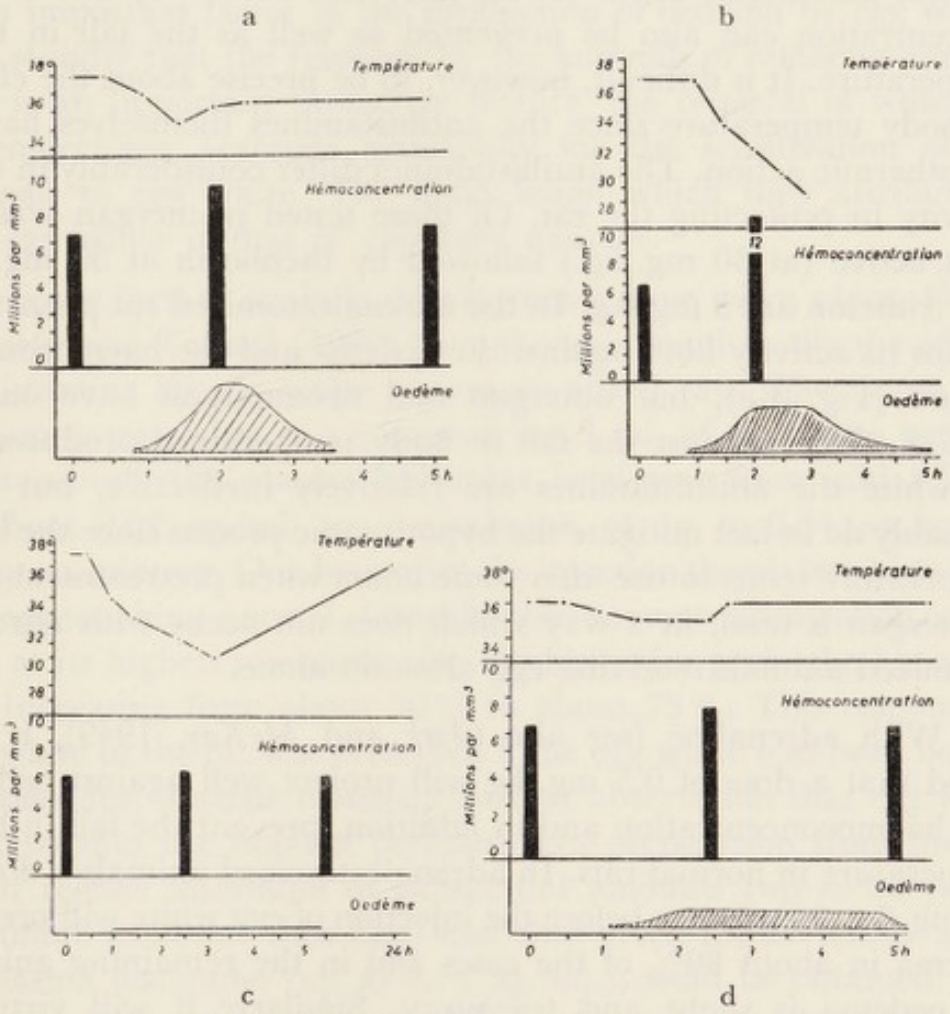


Fig. 16. Comparison of the effect of egg albumin in the rat, (a) normally; (b) after adrenalectomy; (c) after adrenalectomy and treatment with promethazine; (d) after adrenalectomy and treatment with adrenaline. (From *Halpern* and *Briot*, 1949).

If the animal is adrenalectomized three or four days before the exposure to egg albumen and not treated with any additional hormones, the normal dose of egg white produces a much more intense oedema together with respiratory difficulty, attributed to oedema of the tongue and glottis. The haemoconcentration becomes still greater and the red cell count increases by an average of 72% (Fig. 16b). The fall in body temperature now becomes very great indeed and many reach levels as low as 27° C. The general effect is also more notable and the animals become completely inert, chilly and cyanosed, losing all signs of vitality.

The principal antagonists to this syndrome are the antihistamines and adrenaline. Various antihistamines are capable both of producing or virtually abolishing the oedema, as *Leger* and *Masson*, and *Brown* et al. (1948) have also observed; the haemoconcentration can also be prevented as well as the fall in body temperature. It is difficult, however, to be precise about the effects on body temperature since the antihistamines themselves have a hypothermic action. The antihistamines differ considerably in their activity in protecting the rat. Of those tested phenergan was the most active (at 30 mg./kg.) followed by thephorin at 50 mg./kg. and trimeton at 15 mg./kg. In the adrenalectomized rat phenergan retains its activity both against the oedema and the haemoconcentration (Fig. 16c), but antergan and neoantergan have only a modest effect; against the fall in body temperature produced by egg-white the antihistamines are relatively ineffective, but they probably do in fact mitigate the hypothermic process since the body temperature tends to rise after some hours when pretreatment with phenergan is used, in a way which does not occur with adrenalectomized animals receiving egg albumin alone.

With adrenaline (see also *Clark* and *McKay*, 1949), it was found that a dose of 0.5 mg./kg will protect well against oedema and haemoconcentration and in addition, prevent the fall in body temperature in normal rats. In adrenalectomized animals a dose of 1.5 mg./kg. 30 minutes before the injection of egg white will prevent oedema in about 80% of the cases and in the remaining animals the oedema is slight and transitory. Similarly it will virtually abolish the haemoconcentration, and even in these animals in which the fall in temperature is normally so great, it will prevent the hypothermia (Fig. 16d). *Halpern* and *Briot* pose, but do not analyse, the problem of whether adrenaline produces its beneficial effect by curring the capillary defect, or by having some effect on cellular metabolism.

They extended their studies on sympathetic amines to test first the semicarbazone of adrenochrome but failed, even with doses of 20 mg./kg., to modify the toxic effects of egg white. Noradrenaline, in fairly high doses, is about 300 times less effective than adrenaline although a distinct beneficial effect can be obtained. Ephedrine hydrochloride, in a dose of 150 mg./kg., 200 times greater than the effective dose of adrenaline, had no protective

action. They also tested Rutin (one of the candidates for the role of Vitamin P), but found that it had no beneficial action.

Halpern and *Briot* conclude from this study, first, that histamine is an important factor in the production of oedema by egg white: and seondly that the response by the adrenals of releasing adrenaline is an important protective feature, the removal of which by adrenalectomy accounts principally for the sensitization of the animals to egg white. The main lesion which they attribute to the egg white is that of capillary damage.

Some further analytic developments have taken place. Firstly *Schachter* and *Talesnik* (1952) have shown unequivocally the release of histamine by egg white in non-sensitized rats. They were able to demonstrate that in a rat receiving 3 ml. of egg white per kilo intravenously the plasma histamine level rose from initial levels of about 0.02 μg./ml. to a maximum of up to 0.54 μg./ml. in about six minutes. One hour after the injection the plasma histamine was approaching normal. Considerable haemoconcentration occurred, at its highest sometime after the histamine peak, the haematocrit increasing from about 50% to about 75%. This response to egg white in the rat was preserved if the egg white had been boiled. *Schachter* and *Talesnik* made the further observation that egg white injected into the isolated perfused skin preparation from the cat could release histamine in substantial amounts (up to 30 μg. of histamine from one skin flap) and that given intravenously a rise in plasma histamine (up to 0.19 μg./ml.) could be obtained. The response in the cat, however, differed from that in the rat in that boiling the egg white completely destroyed this activity, implying that it was a protein component in egg white, rather than ovomucoid which was responsible. They tested dog's skin and whole animal in a similar way but were unable to show these phenomena in the dog.

In addition, the effect of ovomucoid on mast cells has been analysed by *Benditt* et al. (1954). They showed that 3 mg. of ovomucoid in a 200 g. rat given intravenously led to the distruction of the mast cells in the oedematous areas. They were also able to show that ovomucoid, as well as 48/80, could release histamine from strips of rat's skin. They took these results to indicate that histamine is indeed associated with mast cells in the rat and that ovomucoid acts by damaging them.

Finally *Feldberg* and *Talesnik* (1953) have shown that an animal of which the histamine of the skin has been depleted by previous repeated treatment with 48/80, so that it is no longer responsive to 48/80, has now become insensitive to egg white.

The conclusion from all this must be that the action of egg albumen in the rat represents primarily a histamine releasing process from mast cells in subcutaneous tissue, leading in turn to a generalized defect of capillary permeability resulting in oedema and haemoconcentration as well as other signs accompanying histamine shock. The cause of this action on mast cells is quite obscure; but it appears able to take place in vitro, and therefore presumably does not require any components in its action from the body at large. The effectiveness of adrenaline as an antidote and the sensitization of the rat by adrenalectomy imply that adrenaline release in response to histamine shock is of major importance in the body's reaction to it.

Histamine Release by Horse Serum

Although release by horse serum has usually been achieved by means of presensitization, it has recently become clear that in the cat it may act as a primary histamine releasing agent. *Feldberg* and *Schachter* (1952) tested it both in the dog and in the cat. In the dog, horse serum was not active on perfused isolated skin preparations unless the animal had been sensitized. But on cat's skin it produced a release in unsensitized animals and the release had the promptness which is seen with the histamine liberator compounds such as Compound 48/80. Cat, dog or rabbit serum failed to act. They also made the interesting observation that if the horse serum was boiled it lost its releasing power, but could now interfere with the action of normal horse serum. This antagonism of release by unboiled serum did not extend, however, to antagonism to Compound 48/80. Later *Schachter* found that rabbit tissues in general did not respond to horse serum unless previously sensitized, but that sometimes the liver released histamine on its first exposure.

It is interesting that *Lake* et al. (1953) found that various plasmas could produce, in other species, defects in permeability. Thus intradermal injections into rat's skin of plasma from several different species produced an intense capillary damage; in rabbit's

skin horse plasma was very effective; in guinea-pig's, rabbit's plasma was effective. These experiments on capillary permeability were not related to histamine release and it would be interesting to know whether, in fact, such release was taking place as with the other compounds discussed above.

Dextran, Dextran Sulphates, and Polyvinylpyrrolidone

Considerable interest has been taken in the observation that in the rat injections of dextran will produce the typical response of histamine liberation. This was first reported by a number of workers in 1951–1952 (*Vorhees, Baker* and *Pulaski*, 1951; *Morrison, Richardson* and *Bloom*, 1951; *Edlund, Löfgren* and *Vali*, 1952). The threshold dose given by the intraperitoneal route seems to be of the order of 50 mg./kg., although larger doses of about 150 mg./kg. are required to obtain a clear response in all animals. The syndrome produced is the familiar one of scratching, oedema, notably of the paws, muzzle, tongue and genital regions, sometimes dyspnoea and wheezing, and some degree of collapse and even a stuporose state. If a dye is injected, profound blueing of the oedematous regions occur. The reaction can also be elicited by intravenous injection in a similar dosage; it appears by two hours after the injection, dextran blood levels are roughly comparable whether the intravenous or intraperitoneal route is used. Differences occur between dextrans of different molecular weight, less than 10,000 are relatively ineffective. *Vorhees* et al. find that the time of onset of the anaphylactoid reaction, when the dextran is given intraperitoneally, is considerably influenced by the molecular weight, ranging from a latency of 15–30 minutes for a weight of 25,000 up to a latency of more than an hour for a weight of 170,000. The latter also found that the reaction was less severe with a larger molecular weight compound but this may be because the penetration into the circulation was so much delayed.

In analysing this response (*Briot* and *Halpern*, 1952, 1952) it has been found firstly that if the dextran is injected intracutaneously in a concentration of 10 μg./ml. or higher, a gross increase in the ability of a blue dye to leak through capillaries occurs. Further *Halpern* and *Briot* (1952) have shown that the effects of dextran injected locally are only pronounced in those regions where the oedema occurs when the substance is given systemically. Thus injection

of 1/10 ml. into the paw can produce an intense oedema. *Halpern* and *Briot* (1952) have also shown that dextran will release histamine from rat's skin in vitro.

A further important feature is the fact that the rat after adrenalectomy becomes very much more sensitive to dextran. Whereas normally a rat may tolerate successfully 0.5–3 ml. of the standard dextran solution, after adrenalectomy a dose of 0.05 ml. is regularly lethal by intravenous route. This sensitization by adrenalectomy extends to dextran given intravenously or intraperitoneally but the sensitivity to local injections into the skin is not changed. All the actions of dextran become more intense in the adrenalectomized animal. The oedema is more prolonged, cyanosis is intense, a marked haemoconcentration is produced and the body temperature may fall by as much as 7° C.

Several studies have also been made of how this reaction may be overcome. *Halpern* and *Briot* (1952) have shown that adrenaline is very effective in overcoming all the effects of dextran in the rat, whether the rat is adrenalectomized or not. Following their analysis of the effects of egg albumen in the rat in which similar observations were made, one may take their results as indicating that the response of the adrenal medulla is important in reducing the effect of dextran in the normal animal, and that the removal of the suprarenals sensitises the animal not so much by removing cortical hormone as by depriving the animal of the ability to secrete adrenaline in response to histamine release. Antihistamines have also been widely tested for antagonism to dextran oedema. Promethazine appears to be the most effective remedy and *Halpern* and *Briot* (1952) find that a dose of 100 mg./kg. will reduce all the effects, and increase the tolerated dose in an adrenalectomized animal by as much as twentyfold. Adrenaline in a dose of 1 mg./ kg. repeated twice was also effective, but less effective than promethazine. In these experiments mepyramine in a dose of 100 mg./kg. was ineffective, and this general tendency for most antihistamines to be useless was also found by *Morrison* et al. (1951) who tested pyribenzamine, benadryl, and trimeton, without success. These workers, however, found that thephorin in a dose of 50 mg./kg. produced about 90% protection. They also observed that procain and procain amide (150 mg./kg.) could also confer about 50% protection. *Morrison* et al. failed to reveal any effect with epinephrine in a dose of 40 μg./kg., but in

this dose its effect may have been too transient to show itself. Cortisone in a single injection was ineffective but pretreatment for five days previously had some protective action.

From these studies the general conclusion emerges that the response to dextran in the whole animal is not a true sensitization reaction. The objections to it being so regarded are the fact that such relatively large amounts of material need to be injected, that the reaction always occurs on first exposure and no sensitization period is ever required and that the rat is resistant to histamine and to anaphylaxis in general. Further it has been found impossible to demonstrate the formation of precipitins with dextran with any constituent in rabbit plasma. But the phenomenon therefore is a rather mysterious one and there is no established reason why the rat should be sensitive when the mouse, rabbit, guinea-pig, dog and pigeon have been found to be insensitive.

It was thought at first that in man these reactions do not occur but with further examination the position seems to be somewhat more complicated (see *Squire* et al., 1955 for an excellent review). The responses in man seem to be determined to a considerable extent by whether a normal unanaesthetised subject is used, or whether anaesthesia or a state of shock already exists. In normal subjects, healthy volunteers, very high reaction rates up to 50% have been obtained with some samples of dextran. The reactions include general urticaria, oedema of the eyelids, vomiting and headache, incontinence, pain in the loins and chest, vasomotor collapse and profuse sweating. Flushing of the face and pruritus have also been noted. These seem to correspond fairly closely to an acute anaphylactoid reaction and they have been relieved by antihistamine drugs. It has also been found that in normal subjects who do react, there is not long after the injection of dextran a substantial fall in circulating plasma volume, corresponding to a defect of capillary permeability. Although it is sometimes stated that reactions in man bear no relation to the rat, there can be little doubt that the character of the reaction is similar in type. If the patient is anaesthetised or in a state of haemorrhagic shock, however, then these reactions do not occur.

Attention has been given to how far this response might be a true allergic one. It has been shown that an individual receiving 1 mg. dextran intracutaneously may become sensitized and develop

antibodies to the dextran at the same time as acquiring an allergic type of skin sensitivity to the substance. On the other hand, the occurrence of these specific antibodies with a frequency adequate to account for the commonness of the reaction to dextran in a normal individual has certainly not been demonstrated. It has been suggested that there may be some cross sensitivity with antibodies related to the pneumococcal polysaccharides, or the blood group substances, with which the dextrans are related, but no correlation has been established with antibody reactions in subjects reactive to dextran. The possibility of sensitization to dextran exists since dextran is a contaminant of sugar as normally eaten and dextran is formed by certain streptococci occurring in the human throat, although it is not certain that these streptococci actually form dextran in the body. The interesting observation has been made that the tendency to reaction is greater the more recently an innoculation for TAB has been conducted. There are also signs that different dextrans differ in the frequency of reactions produced, and in particular the more highly branched dextrans or the dextrans of highest molecular weight seem to cause reactions more commonly. An unexplained difference appears to be that the American population is on the whole rather sensitive to Swedish or British dextrans compared to American dextrans, whereas American dextrans do not seem to be correspondingly active on Swedish or British populations.

Some somewhat different studies have been made by *Haining* (1955) on rabbit blood. He has observed that dextrans will release histamine from rabbit blood and that dextran phosphates, which are relatively inactive in the rat, are still more active than dextran on rabbit blood. With dextran of molecular weight 22,000 to 80,000, a concentration of 100 μg./ml. produced a moderate release and higher molecular weight dextrans (220,000 – 1000,000) were active at 20 μg./ml. Dextrans of molecular weight less than 14,000 were active at 20μg./ml. Dextrans of molecular weight less than 14,000 were inactive in a concentration of 0.5 mg./ml.

The sulphates, on the other hand, were effective at concentrations as low as 4 μg./ml. when the molecular weight was 440,000; at 100 μg./ml. with a molecular weight of 40,000 and inactive with molecular weights less than 10,000. *Haining* was able to inhibit the release of dextran from rabbit blood either by using inactive dextransulphates, heparin, or maltotriosulphate, where the anta-

gonism could be imagined to be competitive in nature; or by treating the blood before adding the releasing agent with sodium oxalate in a concentration of 0.021 M. He draws an analogy between the active dextran sulphates in their capacity to release histamine and in their capacity to precipitate fibrinogen; both these actions occur only when the molecular weight of a compound is above a certain limit, both reactions are limited by excess of dextran sulphate, and both reactions are inhibited by heparin or low molecular weight extracts. On the basis of his experiments he has suggested that the release process may involve a clumping of platetlets in rabbit blood which then gives rise to the mobilization of histamine. This is an interesting suggestion and may indeed account for the results seen with blood in vitro. It is difficult, however, to explain the phenomena in the rat since the platelets of the rat, unlike those of the rabbit, are virtually devoid of histamine. In the rat, as in man, it is leucocytes which are richest in histamine. But it is not easy to suggest, alternatively, that in rat and man, for some reason, dextran is able to clump selectively the leucocytes, in view of *Halpern*'s result that dextran works on isolated rat skin in vitro.

Polyvinylpyrrolidone (P.V.P.) can also produce an anaphylactoid reaction, but only in the dog. *Halpern* and *Briot* (1953) showed that after 0.25 ml./kg. of a 25% solution, there was itching, erythema, oedema, fall in blood pressure, and a shock-like state with haemoconcentration. The plasma histamine rose, and an acid gastric secretion occurred. After such an exposure the animals were resistant to P.V.P. for some days. Antihistamines could reduce all the effects described save the secretion of acid gastric juice. In further work (*Halpern* et al., 1955) a method devised by *Landis* for measuring capillary filtration rates was used to show that in a dog receiving P.V.P., the loss of fluid to the tissues increased two-fold, and was rich in protein. Promethazine (10 mg./kg.) could abolish these changes. *Behrmann* and *Hartman* (1955) have also studied capillary changes after macromolecular infusion into dogs. Gelatin had no action, but dextran and P.V.P. caused a low capillary resistance, thrombocytopaenia and hypoprothrombinaemia. They compare these effcts with those due to certain polysaccharides which produce platelet agglutination, deposits in the reticulendothelial system and haemorrhagic changes.

Anaphylatoxin

This term originated nearly 50 years ago to designate the substance obtained by incubating serum either with the specific precipitate or with certain polysaccharides such as agar, dextran, inulin or starch. The material was believed to be formed in anaphylaxis and to be responsible for the anaphylactic phenomena. The studies by *Dale* and *Kellaway*, however, led to the rejection of this theory largely because that work made it unnecessary to postulate any activation process taking place in the blood and because the properties of an anaphylatoxin did not correspond to those of the sensitization reaction in different organs and in different species. *Rocha é Silva* (*Rocha è Silva* and *Aronson*, 1952; *Rocha é Silva* and *Rothschild*, 1955; *Rocha é Silva*, 1956), however, has recently taken up the study of anaphylatoxin again. He has found that such a toxin, obtained by incubating guinea-pig serum with agar, will cause a considerable release of histamine from perfused guinea-pig lung, a release considerably greater than can be achieved with contact of sensitized lung with the antigen. *Rocha é Silva* finds that the toxin requires an activator present in normal plasma and that the activation is enzymatic in nature. Activation can be promoted by reduction of the ionic strength of the plasma and can be prevented by heating to a temperature of 60° C. Rendering plasma twice the normal tonicity will also prevent the activation process, as well as citrate or oxalate anion or calcium cation. The ability of a serum to produce anaphylatoxin is lost if the polysaccharide used is sulphonated, and indeed such sulphonated polysaccharides lessen the activation in the presence of normal agar. Heparin is likewise incapable of activating anaphylatoxin, and inhibits agar activation if there is a large excess present.

Anaphylatoxin itself has not been characterised. It appears to belong to the protein components in the plasma but the processes of fractionation of these proteins tend to produce spontaneous activation which complicates the analysis. The anaphylatoxin prepared from rat plasma has no proteolytic action and appears to be formed by a process not accompanied by proteolysis. Thus it is possible, for instance, to obtain an activated rat plasma fully active so far as its anaphylatoxic activity is concerned but devoid of bradykinin action. On the other hand if the serum is then incubated with the appropriate factor favouring bradykinin release,

bradykinin may appear in large amounts. Anaphylatoxin activation and bradykinin formation must, therefore, be distinguished. The different species vary as regards the potency of the anaphylatoxin produced; that from rat serum is from 5–10 times more active than that from guinea-pig or rabbit. The usual test is the contraction of the guinea-pig ileum obtained by it, a contraction which rapidly becomes desensitized with repeated administration. The rat itself is very insensitive to anaphylatoxin.

Anaphylatoxin appears to be distinguishable both from histamine liberating agents and from the antigen-antibody reaction. Thus *Rocha é Silva* has found that the anaphylatoxin activators injected intradermally produce no increase of capillary permeability (by the trypan blue test) in the rat, although histamine releasing agents or the antigen-antibody reaction will do this readily. He further found that if the rat was depleted of histamine then the blueing reaction to histamine liberating agents could be abolished although the antigen-antibody reaction was maintained. Finally the interesting observation was made that if the animal was treated with large amounts of the activators, then the response to the antigen-antibody reaction was reduced, but that to the histamine releasing agent was retained.

Histamine Release by Sympathomimetic Amines

Although perhaps the most important single drug for the relief of the symptomatology caused by histamine release is adrenaline, there is nevertheless some evidence that sympathetic amines may themselves cause release. In the 1914–1918 War during studies on shock, it was found that large doses of adrenaline could produce in the dog itself a shock-like state. Thus *Bainbridge* and *Trevan* found that 100 μg. of adrenaline intravenously caused a fall and then a rise of portal pressure with engorgement of the liver. The flow of lymph increased four or five times and became very concentrated. 0.5 mg. adrenaline injected into the portal vein produced the same effects. *Erlanger* and *Gasser* (1919) produced somewhat similar results. Since that time a number of deleterious effects have been recorded with adrenaline. *Selye* (1939) found that it was among the agents which could produce the alarm reaction. *Green* et al. (1948) found that adrenaline infusions in man for about an hour led to a considerable depression of the blood pressure

when the infusion stopped. *Freeman* et al in 1941 showed that adrenaline infusions at the rate of 3–16 μg./kg per minute for up to three hours led to a fall in plasma volume, a leakage of blue dye, a state of "shock" and haemorrhages in the endocardium and in the duodenal mucosa. *Blackett* et al. (1950) made the interesting observation that although with the infusion of renin it was possible to produce a sustained hypertension without any obvious harm to the animal, with adrenaline and noradrenaline the dose had to be increased steadily to maintain a given hypertensive response and the animals deteriorated. When the infusion ceased a severely shocked state with acute dilation of the intestine ensued.

A number of possible explanations exists for a failure of the peripheral circulation after adrenaline infusions. First might be the production of ganglion block by the drug (for which a good deal of evidence exists; *Bulbring* and *Burn*, 1942; *Marrazzi*, 1939; *Paton* and *Thompson*, 1953). But the circulatory state produced by adrenaline does not resemble that produced by an overdose of a ganglion blocking agent. Secondly, it might be supposed that adrenaline can release histamine from the tissues. This was an earlier suggestion in another connection, when *Dale* and *Burn* (1926) and *Dale* and *Richards* (1927) were testing the vasodilator action of adrenaline; the possibility of adrenaline releasing histamine from the lung was tested but finally rejected. In more recent years it has been claimed by *Staub* (1946) and by *Eicher* and *Barfuss* (1940) that rises in histamine content of blood can be produced by adrenaline and also by sympatol (*Baur* and *Staub*, 1948). The changes are not very large and the experiments were for the most part made on whole blood; thus if there had been any redistribution of the formed elements of the blood, this might easily produce the observed changes without there having been any true mobilization of histamine from tissues. A renewed attempt by *Mongar* and *Whelan* (1953) to settle this point yielded negative results. With infusions into the brachial artery they were unable to show any rise in histamine content of the venous blood, despite the use of large doses and occlusion of the limb to allow the adrenaline to act before being swept into the general circulation.

The preceding results would suggest that on the whole release of histamine does not provide a satisfactory explanation for the effects of prolonged adrenaline administration. On the other hand, it has been found that some of the other sympathomimetic amines

are quite distinct histamine liberators as shown both by the production of wheals when given intradermally, when given into human skin, and by the release of histamine from isolated perfused skin. Amphetamine, phenylethylamine, and tyramine are all active in this way, although ephedrine is inactive (*Paton*, unpublished). This suggests by analogy that in fact adrenaline may have some slight histamine-releasing action, but that it is difficult to demonstrate in the presence of other actions it possesses.

From the therapeutic point of view, the most important point about adrenaline shock is to avoid it. Noradrenaline is less unsatisfactory in this way and prolonged infusions can be given safely provided that the infusion is not abruptly terminated at the end.

The Relationship of Histamine Liberation to Anaphylaxis

The actions of the histamine liberators bear a striking analogy to some of the classical effects of anaphylaxis. Thus they cause the appearance of histamine in the blood with all the accompanying histaminic effects, and in the dog a defect of coagulation, accompanied by a rise in portal pressure and engorgement of the liver. Further, both in anaphylaxis and after the histamine liberator, there appears in the blood one of the so-called "slow reacting substances" (*Paton*, 1951; *Beraldo*, 1950). In addition, if the amount of histamine released is compared over a number of tissues a remarkable parallelism may be found; thus in 13 different tissues in which release of histamine is produced by Compound 48/80, d-tubocurarine, or antigen (the animal having been previously sensitized) the releases obtained were comparable with all three procedures, with percentage releases ranging from 2% to 40% (*Mongar* and *Schild*, 1952) (Fig. 17). Finally, there is evidence that the histamine which is released by the antigen-antibody reaction at least shares the same site as that which can be released by chemical liberators. A similar disruption of mast cells is achieved by both means (cf. *Riley*, 1953, *Mota* and *Vugman*, 1956). *Copenhaver* et al. (1953) and *Mongar* and *Schild* (1954) have found that sensitized liver or lung, which has been previously treated with antigen so that histamine release occurs, contains less histamine in its intracellular particles than tissues not so treated. Since the histamine in these particles is accessible to chemical liberators, then presumably the histamine released by both the antigen-antibody reaction and by

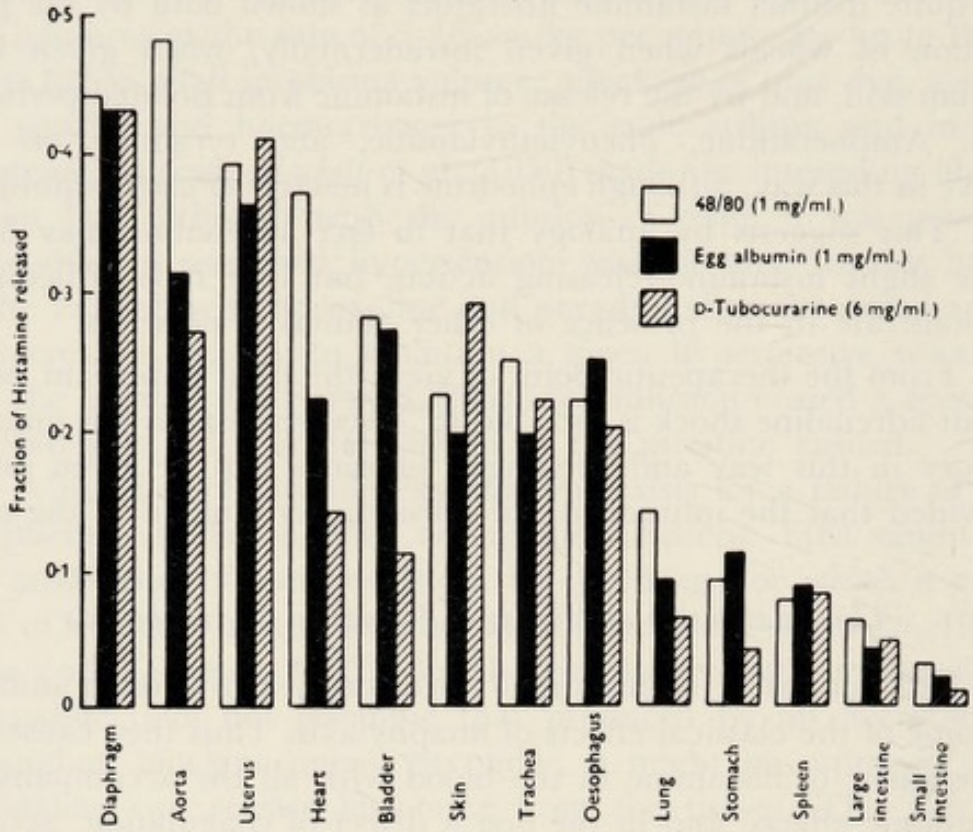


Fig. 17. Comparison between the histamine released by the histamine-liberators compound 48/80 and d-tubocurarine with that released by antigen in sensitized animals. (From *Mongar* and *Schild*, 1952: by permission of the Journal of Physiology.)

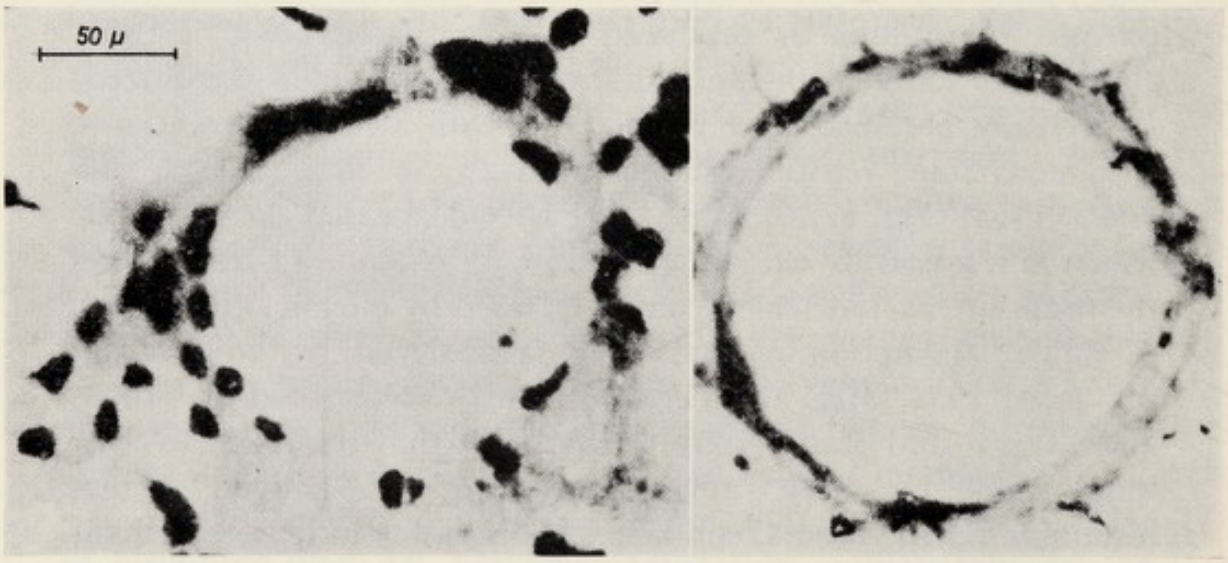


Fig. 18. Lung from a sensitized guinea-pig, stained with toluidine blue, injected with histamine (left), and after anaphylactic shock (right). (From *Mota* and *Vugman*, 1956).

liberators is in part common. Certainly mast cell disruption can be achieved by both (see Fig. 3 and Fig. 18). One can say, therefore, that there is a striking parallelism between the effects, on one hand, of the action of the simple basic chemical compounds known as histamine liberators, and on the other side of the undoubtedly more complex process resulting from the antigen-antibody reaction.

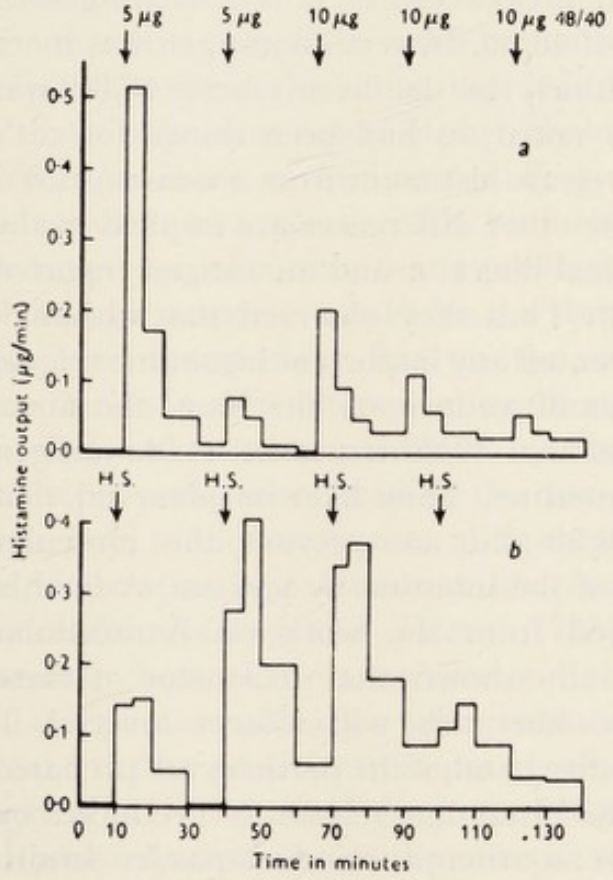


Fig. 19. Histamine output from perfused dog skin preparations. (a) effect of successive doses of 5 and 10 μg. of compound 48/80. (b) effect of successive doses of 0.5 ml. 50% horse serum (H.S.) on skin preparations of another dog previously sensitized. (From *Feldberg* and *Schachter*, 1952: by permission of the Journal of Physiology.)

Nevertheless, there are a number of major differences between the reactions. Thus in the experiments by *Feldberg* and *Schachter* (1952), horse serum applied to the skin of a sensitized dog produced a more sluggish release, and one which was potentiated by repeated administration, in a way quite unlike the response produced by 48/80. With 48/80 the whole histamine release is complete within half-an-hour, and with repetition of the injection a much smaller response or none is obtained. With horse serum in a sensitized animal the release continues slowly for as long as two hours and a repeated injection gives a much augmented effect (Fig. 19). There is too, a considerable difference in the speed of actions in

the whole animal. Whereas histamine liberators injected intravenously produce a nearly maximal action within one or two minutes, and a latency to onset rarely longer than 30 seconds, yet the anaphylactic response usually requires several minutes and sometimes longer before it manifests itself, and it reaches its peak a good deal later. *Reuse* (1950) found that antihistamines could prevent the rise in portal pressure in the dog after 48/80, but not after antigen. *Schachter* (1953) studying histamine release in the rabbit found a poor correlation between the effects of antigen in sensitized animals and those of 48/80. In general antigen was more effective than 48/80, particularly in the liver where 48/80 was completely inactive. He also noted, as had been shown for cat's skin, that horse serum could release histamine from a non-sensitized tissue, the rabbit's liver. Some other differences are implied in the interactions between a chemical liberator and an antigen reported by *Mongar* and *Schild* (1952). Thus they observed that although pretreatment with 48/80 prevented any important histamine release by subsequent administration of antigen on the lung, the aorta and on the uterus, they found that 48/80 treatment made antigen much more active on the intestine. They likewise observed that previous treatment with 48/80 did not prevent the muscular contraction of the uterus or of the intestine by antigen. A further important difference emerged from the work on intracellular particles. It has been generally shown that treatment of these particles with representative liberators will always succeed in releasing histamine. On the other hand, if the particles are prepared from a sensitized animal no histamine release is produced on addition of antigen; and even an attempt to induce passive sensitization by adding antibodies to the particles and following it with antigen fails (*Copenhaver* et al., 1953; *Mongar* and *Schild*, 1954; *McIntosh*, 1956). Finally, *Mongar* and *Schild* (1955) have found, that metabolic inhibitors, such as oxygen lack or iodoacetate, will depress histamine release by anaphylaxis induced in minced guinea-pig lung, although these procedures will increase histamine release by Compound 48/80 or Octylamine.

The result of these experiments must be that one cannot equate the process of histamine release and that of anaphylaxis. On the other hand, these experiments do not exclude the possibility that as a result of the antigen-antibody reaction there is formed locally a substance with the properties of a histamine liberator.

The chief objection to this is that several workers have tried to demonstrate the formation of a typical histamine liberator during the antigen-antibody reaction and none has succeeded in finding it. But to dismiss the analogy between the histamine liberators and anaphylaxis as a pharmacological coincidence is not satisfactory, since the analogies are so striking and since the property of the histamine liberation is so widely distributed. The most plausible hypothesis is probably the one just mentioned, that a histamine liberator is formed in some parts of anaphylactic process, but that it is formed in small amounts and in such close relationship to the site of the action that it is difficult to detect.

An important aspect of the relation between the two processes is the possiblity of mitigating allergic or anaphylactic responses by previous treatment with a histamine releasing agent. It might be suggested, of course, that a previous eliciting of an anaphylactic or allergic response would be the simplest way to protect against subsequent responses. But on the whole, the antigen-antibody reaction is a difficult one to graduate and control, whereas the intensity of reaction produced by histamine releasing substances is simply a matter of grading the dose. Some importance attaches, therefore, to the attempts to mitigate allergic responses by previous administration of a histamine liberator. This has sometimes been successful. *Feldberg* and *Talesnik* (1952) were able to reduce the intensity both of the anaphylactoid response to egg albumen and of the photosensitization after treatment with haematoporphyrin in the rat by previous treatment with 48/80. *Feinberg* et al. (1954) were able by repeated injections of 48/80 in human skin, to develop a local refractoriness to the allergic response to ragweed or grass pollen. The refractoriness developed, however, was about the same as could be obtained with the antigen. *Lecomte* et al. (1953) were also able to show that treatment of the skin with three liberators, diaminodecane, stilbamidine, or cystinamine, shortly before a tuberculin response was elicited, could greatly reduce it. These drugs had to be given before the tuberculin was injected. If they were given soon after the tuberculin was injected they were much less effective. Unpublished experiments have taken place trying to desensitize a dog to anaphylaxis by previous treatment with 48/80; although it is possible that the symptoms are somewhat mitigated the response is certainly not attenuated far enough to give a useful degree of protection. *Herxheimer* and *Paton* (unpublished) have tried

the effect of aerosols of 48/80 and octylamine as a means of reducing the response to aerosols of an antigen in sensitized animals. A definite reduction in the intensity of the asthmatic response to the antigen could be obtained. But, interestingly, no reduction in the histamine content of the lung, or of any other tissue in the body, due to the treatment with histamine-releasers was found. This introduces the question of anaphylaxis involving, at least in the lung, some other factor than histamine. *Brocklehurst* (1953, 1955) has shown that if the antigen-antibody reaction is elicited in perfused lung, there may appear in the perfusate, in addition to histamine, one of the "slow-reacting substances" already mentioned in other connections. This substance will, when produced from human lung, contract human bronchi although not those of other species, and it is resistant to antihistamines. It is not at all impossible that the protection achieved with histamine liberators in *Herxheimer*'s and *Paton*'s experiments has something to to with such bronchoconstrictor substances other than histamine itself.

It seems probable that the protection that can be achieved with histamine liberators against anaphylactic or allergic responses is best with cutaneous reactions. But even with these the protection is limited. Thus *Brocklehurst*, *Humphrey* and *Perry* (1955) have found that skin allergic reactions in the rat are unaffected by depletion of skin histamine down to 10% of normal. They further showed that antihistamines do not block allergic responses in depleted rat's skin despite the demonstration of a powerful antagonism, under these conditions, to the local effects of histamine. They have concluded, therefore, that histamine is not a causative factor in the production of skin allergic responses in the rat or indeed in some other species. If this is the case, then clearly histamine-liberating agents should not be able to produce any useful desensitization to the allergic process.

A final difficulty must be mentioned in discussing the question of treatment with histamine liberators. Although with a single injection the effect is essentially that of histamine release, repeated administrations lead to more serious results. Thus *Smith* (1953) in his attempts to deplete the histamine in cat's tissues only succeeded with doses which severely damaged the health of the animal. *Gaitonde* and *Paton* (unpublished) have found that with at least some histamine liberators, particularly propamidine, there is an

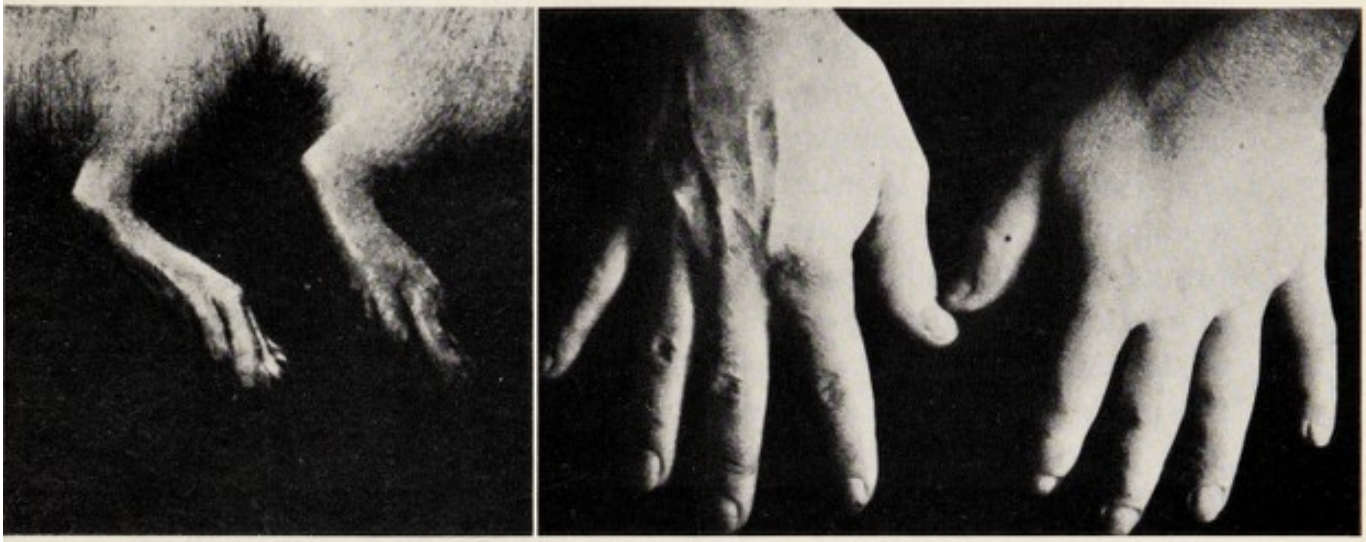


Fig. 20. Oedema produced by injection of dextran in the rat (left), and of d-tubocurarine in man (right). (From *Briot* and *Halpern*, 1952 and *Grob* et al., 1947.)

attack on the liver such that it loses its glycogen, with initial hyperglycaemia followed by longlasting and possibly fatal hypoglycaemia, together with an accumulation of amino acids in the blood. This interesting syndrome had been decribed previously for synthalin after it had been proposed as a possible synthetic substitute for insulin. This experience makes it probable that repeated injections of histamine liberators, or at least those at present available, may not be entirely safe.

Histamine Liberation in Man

The histamine liberators as a group have the same type of action in man as in other animals, although naturally deliberate experiment with them in man has been much more restricted. There is a good deal of clinical experience with the therapeutic diamidines, in which urticaria and intense widespread pruritus occurred, of a kind very like that seen in the lower animals. Clinical experience with morphine, and the tendency to exacerbate bronchial asthma, no doubt corresponds to the spasm of the bronchus which can sometimes be produced in the guinea-pig. Intra-arterial injection of d-tubocurarine (*Grob* et al., 1947) has been shown in man to produce an oedema of the treated arm which resembles closely that achieved by a local injection of dextran into a rat (Fig. 20). *Lecomte* has made a number of deliberate experiments

with known histamine liberators (*Feldberg* and *Lecomte*, 1955) injected into man and found that, provided they are injected fairly rapidly intravenously, they reproduce virtually the whole symptomatology seen in animals (*Lecomte*, 1955 and *Lecomte* and *Borensztajn*, 1953; *Lecomte*, 1956). The histamine release syndrome in man is commonly called the nitritoid crisis, although this is clearly an unsuitable name. There is in fact some analogy with the effect of nitrites, since they resemble histamine release in causing flushing of the skin, tachycardia, fall in blood pressure and headache. But the puffy oedema of face and extremities, the intense itching, the acid gastric secretion, the colic or diarrhoea and the occasional bronchospasm are not seen with nitrites. The name "nitritoid" could with value be abandoned and replaced entirely by a term such as "anaphylactoid".

The Antagonism of Histamine Releasing Processes

No really satisfactory way of preventing the process of histamine release has yet been discovered. In experiments on whole animals there are suggestions that anaesthesia is able to reduce the intensity of the histamine release; *McIntosh* and *Paton* (1947) found that the histamine liberators were somewhat less effective under ether anaesthesia. *Katz* (1940) has found urethane anaesthesia to lessen the release of histamine from guinea-pig tissues in response to antigen. *Junqueira* et al. (1955) found that mast cells were less readily disrupted by 48/80 if a rat received urethane. Finally the experience with dextran, in which certain preparations seem to produce anaphylactoid responses in normal subjects, quite commonly, while only rarely doing so in anaesthetised subjects, probably represents a similar phenomenon. How an anaesthetic process should interfere with histamine release is unknown; but the idea that the anaesthetic stabilizes some cellular membrane concerned in the process could serve as a provisional model. The induction of anaesthesia is of course not a general therapeutic weapon, but it is probably fair to be somewhat less apprehensive about histamine release when using substances which are capable of it, if the subjects are anaesthetised.

The most important therapeutic weapons against histamine release are antihistamines, and adrenaline or a longer acting sympathomimetic amine. The antihistamines as a whole seem to

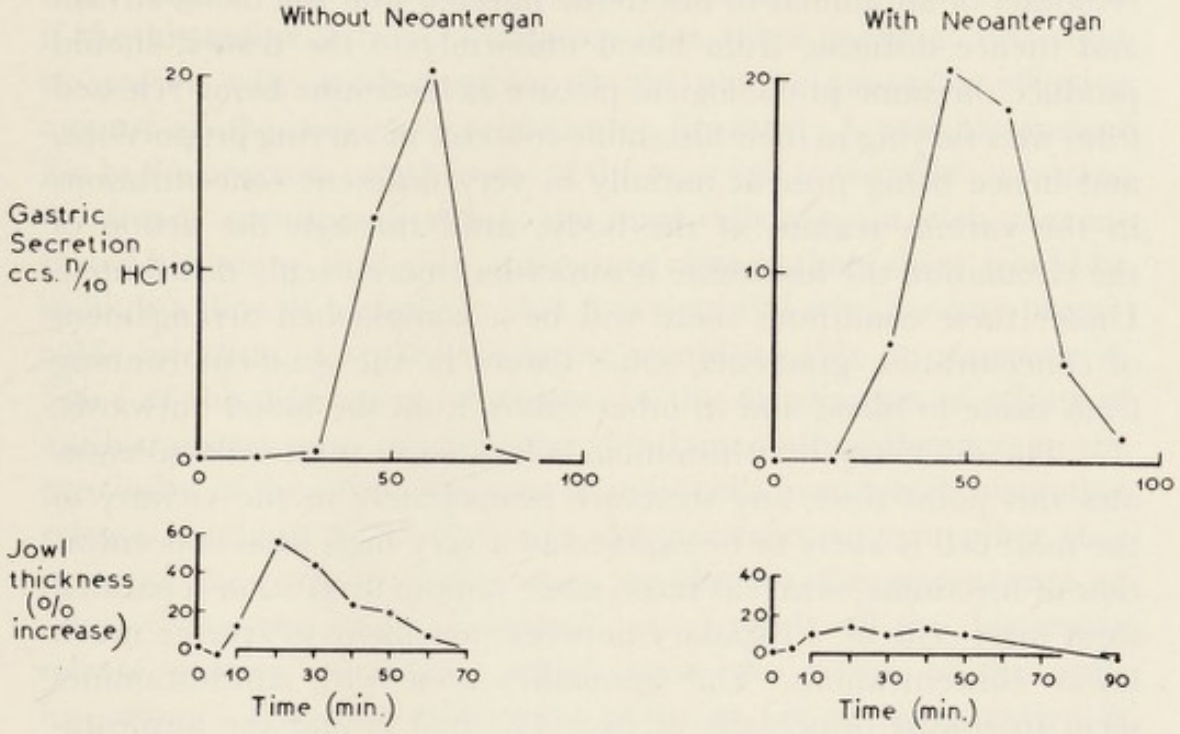


Fig. 21. Effect of an antihistamine (mepyramine 2 mg./kg.) on the gastric secretion (above) and on thickness of the upper lip (below) produced by 10 mg. compound 48/80. (From *Paton* and *Schachter*, 1951.)

be unable to influence the histamine releasing process (despite some reports to the contrary; cf. *Burstein* and *Parrot*, 1949), since the gastric secretion produced by histamine liberator proceeds unmodified or possibly even increased in the presence of representative antihistamines (*Paton* and *Schachter*, 1951; *Halpern* et al., 1953) (Fig. 21). A corresponding experiment has not been done for adrenaline, and indeed would not yield a useful result, since adrenaline is known itself to depress a histamine gastric secretion, probably by interfering with gastric blood flow; but there is no reason at present to suspect adrenaline of interfering with histamine release process, particularly as some of its related amines are actually cabable of mobilizing histamine. The use of antihistamines has, however, brought to light some anomalies which have sometimes led workers to believe that a histamine releasing drug is producing its effects in some other way than by histamine release (*Alles* et al., 1953; *Ellis* et al., 1954). This criticism is usually founded on a failure of parallelism between the effects of injected histamine and of the postulated histamine liberator. Reflection makes one realize, however, that it would be remarkable if the

responses of an animal to histamine injected into the blood stream and thence diffusing from blood uniformly to the tissues, should produce the same physiological picture as histamine being released from sites varying in their histamine content, in varying proportions, and hence being present initially in very different concentrations in the various regions of the body, until through the action of the circulation the histamine is somewhat more evenly distributed. Under these conditions there will be a complicated arrangement of concentration gradients, some tissues in the gradient running from tissue to blood and in other tissues from the blood outwards.

The discovery that histamine is located in mast cells accentuates this point since any structure immediately in the vicinity of the mast cell is likely to be exposed to a very high local concentration of histamine, whereas those more remote from it, or separated from mast cells by a capillary network, are likely to receive much lower concentrations. The anomalies seen with antihistamines seem to consist principally of two. The first is that the antihistamines are more effective in reducing the urticarial, oedematous effects of histamine liberators than their effects on blood pressure, the bronchi, or their general lethal action. Secondly the antihistamines seem in general to be more effective against injected histamine than against injected histamine liberators.

One possible reason for this would be the ability of antihistamines to produce capillary constriction, which *Haley* et al. (1949) claimed to have demonstrated. Such an action would, of course, mitigate considerably the effects of histamine liberator in subcutaneous tissues while it would leave less affected the effects on for instance the bronchi. But this work has not been confirmed (*Conard*, 1951) so that it is not justifiable to explain the distribution of action of antihistamines yet on this basis. An alternative explanation, which is owed to *Sir Henry Dale* (1948), rests on a distinction between "extrinsic" and "intrinsic" histamine; the idea here is that where histamine is released from the same structure as that of which it acts, it is likely to have a less readily antagonised effect than when the histamine is released from one structure and then has to diffuse or to be carried from the blood some distance to act on another structure. This concept has been very widely exploited but it needs to be reinterpreted in the light of the new knowledge that histamine is located in mast cells to a large extent; for this would imply that the bulk of histamine is always extrinsic,

since nobody postulates an effector action for the mast cells. But if the histamine is largely extrinsic then there seems at first sight no reason why antihistamines should not be generally effective against all the actions of a histamine liberator. A possible answer lies in the nearness or otherwise of the mast cell to the effector organ; as already mentioned earlier, the mast cells contain such concentrated histamine that with a structure close to them there would be so high a dose of histamine, that it is doubtful whether any practicable injection of an antihistamine would be able to overcome it. Some of the differences, therefore, in the distribution of effects of antihistamines, may be, therefore, fundamentally differences in the proximity of the effector organs to mast cells from which histamine release is taking place. But it is also necessary to remember that some of these discrepancies may be due to the appearance of other "slow-reacting substances" as a result of the histamine release process, substances which resist the action of the antihistamines. The most important point so far as investigation is concerned is that it is not valid to assume, that because an antihistamine fails to mitigate a response, therefore histamine release plays no part in it. On the other hand if an antihistamine can be shown to have an effect, in doses not so high as to introduce the other actions of antihistamines (such as local anaesthetic or atropinic effects) then useful evidence of the participation of histamine is obtained. But the only decisive way of proving the liberation of histamine is the identification of histamine as such in the tissues or a demonstration of loss of histamine from the tissues after the reaction is over and the released histamine has been dissipated. From the therapeutic point of view it seems clear that antihistamines are the drugs of choice, combined with adrenaline for emergencies, for any of the anaphylactoid phenomena. But it is unfortunately also clear that they may themselves sometimes give rise to histamine releasing effects (*Arunlakshana*, 1953) although possibly, in human practice, only in susceptible subjects.

The Mechanism of Histamine Release

Since the property of histamine release is relatively widely diffused the mechanism by which it occurs is of some interest in its own right. But it deserves further attention for its bearing on the mechanism of histamine release in anaphylaxis. It must be

stated at once that there is no generally accepted theory for the release of histamine by any chemical substance or by the anaphylactic process. But there are four main types of theory each having something to commend them which will be briefly outlined. For a further discussion of the problem the reader should consult the relevant papers in the Ciba Foundation-Histamine Symposium-Volume and elsewhere.

The first theory is that the histamine releasing substances have a general lytic action on histamine containing cells. This was certainly true for some of the venoms and toxins studied earlier. But it has been one of the major, and most useful differences, of histamine liberators from the earlier substances that they can produce a release of histamine approaching the specific, while apparently leaving the skin or other tissue otherwise not greatly affected. The recent work on mast cells led at first to the idea that histamine liberators could disrupt or virtually destroy mast cells. But with closer examination it appears that the action of the liberators is fundamentally to degranulate the cells so that the granules pass out through the limiting membrane and then go into solution in the interstitial fluid, but that the cell itself retains its integrity and may indeed subsequently be recharged with granules. This implies therefore that the liberators do not have any general cellular destructive action. This is confirmed by the observation that during the action of a liberator such as Compound 48/80 on a piece of perfused skin, no potassium escapes, despite an effective release of histamine (*Paton*, 1956) (Fig. 22). Compound 48/80 may here be contrasted with a substance like octylamine. This simple monobasic amine, prototype of a series of monobasic alkylamines studied by *Mongar* and *Schild*, is rather active on minced guinea-pig tissues although on perfused tissues the effective concentration approaches 1 mg./ml., a good deal higher than on minced preparations. In this series of compounds the activity appears to increase as the surface activity of the compounds increases until the aliphatic chain is 10 or 11 carbon atoms long when it falls away again. This point of fall-away corresponds approximately to the point at which micelle formation by the increasingly soapy material occurs, a process which effectively prevents a substance going fully into solution thus reducing any effects it may have. With a compound like this, which is both basic and also rather strongly surface active, the possibility of a

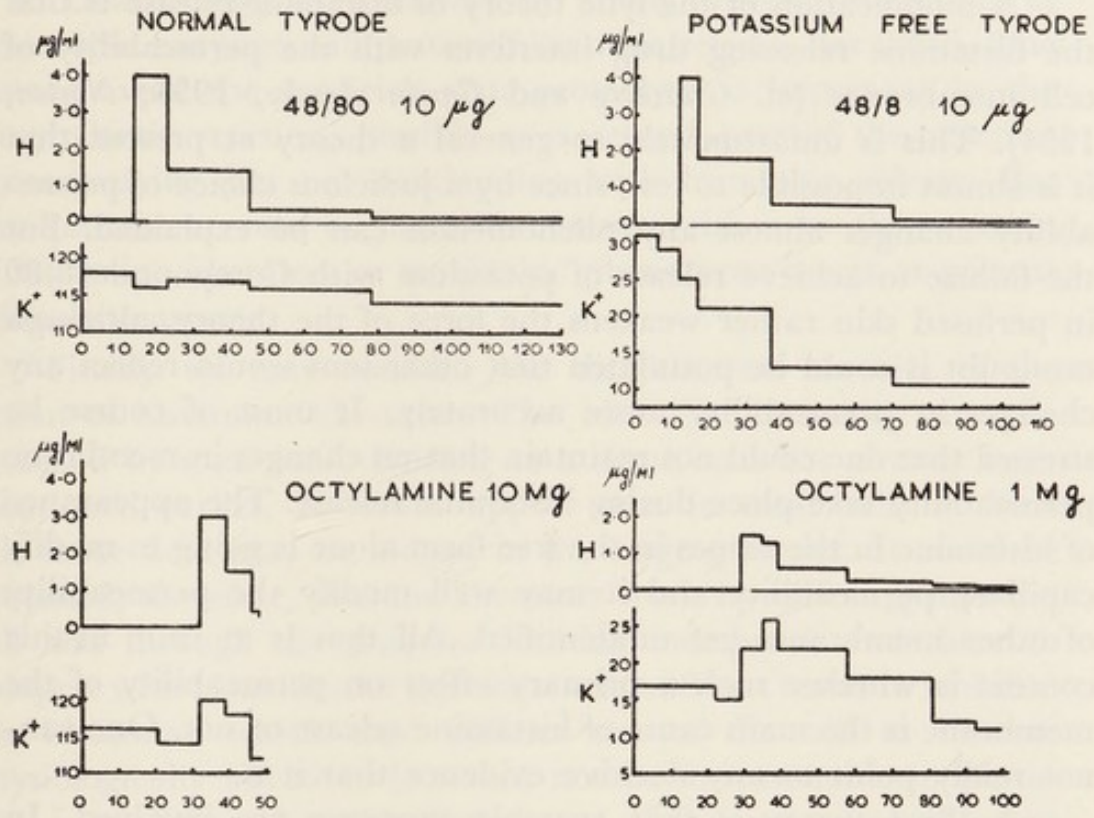


Fig. 22. Comparison between the ability of compound 48/80 and octylamine to release potassium as well as histamine from perfused skin. In the left hand tracings normal tyrode was used, in the right hand tracings, potassium-free Tyrode. Compound 48/80 did not release potassium, despite a good histamine release; octylamine released about 10 times as much potassium as histamine. (From *Paton*, 1956: by permission of Ciba Foundation.)

lytic action is more real; and it is indeed found that on the perfused skin octylamine will release potassium in considerably larger amounts than histamine. It seems, therefore, that in the action of 48/80 and drugs like it, which are capable of producing the typical depressor response in the cat and which are relatively uniformly active on perfused organs as well as in vivo, there is a specific histamine releasing action which octylamine lacks. On the other hand octylamine is more active than a compound which simply has a great activity in lowering surface tension. It would seem, therefore, that the fact that octylamine is basic does give it some histamine releasing action and that in octylamine we have a less specific histamine releasing action compounded partly of a rather modest ability to act as a base, combined with an ability to lyse cell membranes which probably shows itself more readily when a tissue has already been partly damaged by fragmentation.

A modification of the lytic theory of histamine release is that the histamine releasing drug interferes with the permeability of cell membranes (cf. *Grossberg* and *Garcia Arocha*, 1954; *Norton*, 1954). This is unfortunately so general a theory at present that it is almost impossible to test, since by a judicious choice of permeability changes almost any phenomenon can be explained. But the failure to achieve release of potassium with Compound 48/80 in perfused skin rather weakens the force of the theory, although no doubt it could be postulated that other ions would reflect any changes in permeability more accurately. It must of course be stressed that one could not maintain that no changes in membrane permeability take place during histamine release. The appearance of histamine in the tissues in the free form alone is going to modify capillary permeability and it may well modify the permeability of other membranes yet unidentified. All that is at issue in this context is whether such a primary effect on permeability of the membrane is the main cause of histamine release or not. One cannot really point to any decisive evidence that it is.

A third theory is that enzymic processes are involved. In general two types of interaction might be postulated; first that histamine releasing agents activate some enzyme leading to the splitting off of histamine from some attachment, second, that these agents inhibit some enzyme thus allowing some histamine to be preserved or mobilized in an unusual way. The enzymic theory originated partly many years ago when the proteolytic activity of the antigen antibody reaction was being analysed, partly later when *Rocha é Silva* was able to demonstrate that certain proteolytic enzymes could reproduce some of the phenomena of anaphylaxis. It has received additional interest from study of enzymes such as fibrinolysin, which offered a circulating proteolytic enzyme complete with a system of inhibitors and activators, all prepared, as it were, for participation in allergic responses. But just as the idea of histamine being attached to the binding site in the tissues by a bond corresponding to the peptide bond has become less acceptable in view of what is now known about histamine mobilization, so has the idea that the histamine release is achieved by proteolysis. There is, indeed, some suggestive evidence in various fields (see *Ungar*, 1956) that when the histamine release takes place proteolysis also may take place. But the evidence has never been more than suggestively circumstantial, and every attempt to put

it to critical test, for instance, by seeing whether inhibitors of proteolytic enzymes will prevent histamine release, has failed. One cannot avoid the suspicion that proteolysis is important in the anaphylactic reaction, possibly as a second part of the reaction accompanying but not overlapping with histamine release. But any prominent role in histamine release either to the reaction of histamine liberators or in allergic phenomena seems at present difficult to assume.

Finally we may consider the displacement theories of the action of histamine liberators. The idea that histamine may be attached by some ionic binding to a tissue acid has already been mentioned earlier. It would appear that in mast cells at least this tissue acid may be lodged in the large granules. It could be proposed, therefore, that the liberators work by diffusing into the cell and through the granular limiting membrane and there to compete with histamine for this tissue acid and as a result to mobilize the histamine which then diffuses out of the cell to become physiologically effective. It would not be unreasonable to postulate, in addition, that this disturbance of the normal equilibrium between histamine and the tissue acid might serve to free some of the tissue acid itself, and possibly even to bring into solution, or into osmotic activity at least, histamine base and tissue acid which were previously osmotically inactive. This would give rise to a swelling of the granules as well as the freeing of their constituents to diffuse, and so an increase in osmotic pressure of the cell; this should in turn swell, and hence, as an end result, discharge its granules, with a final return of the cell to approximate osmotic equilibrium. Such a model will account for the general tendency for bases to release histamine, for the appearance of heparin after their administration and for the degranulation preceded by a slight swelling of mast cells which occurs during the action of histamine liberators.

Conclusions

The recent work on histamine release has had as one of its consequences a considerable expansion in our knowledge of the distribution of histamine. This new knowledge deserves some emphasis because if no histamine is present in a tissue, or only

trivial amounts, then clearly histamine release within that tissue must be unimportant, and sometimes the mediation of histamine in a pathological process can be simply excluded by showing that histamine is not present. Further the detailed dissection of histamine distribution has shown that it is at least to a large extent located outside effector organs, in the mast cells, and that in these cells it occupies a site corresponding to that of mitochondria in other cells. To an important extent, therefore, the physiology of histamine is now the physiology of the granules of mast cells. The general picture of this distribution is, as has been argued elsewhere (*Paton*, 1955), one along the interfaces between the organism and the body; that is, the histamine is concentrated in skin, lung and intestine, and tends to be in higher concentrations in those parts of the skin which come most frequently into contact with the outside world. This does not point directly to any function of histamine, but it does suggest that whatever function it has is related to these contacts with the outside world rather than to some process concerned with the ordinary physiological regulation of the internal physiology of the animal.

The results of depletion have been disappointing in one sense in that so little change in the physiology of the animal takes place. Thus depleted skin looks like normal skin, has normal sensation (*Benjamin*, 1954) and normal growth of hair. This can only mean that histamine is not important for ordinary everyday functioning of this tissue. Equally the depletion furnishes some protection at least to certain reactions such as the photosensitization in the presence of haematoporphyrin, or to some allergic responses, confirming that histamine is important in these. One's impression is undoubtedly that histamine is not concerned so much with normal physiology as in the ordinary everyday minor pathology of the tissues.

An important outcome of the work has been the distinction between the states in which histamine can be present in the body. The distinction between tissue free and physiologically active and that bound in the tissues is an old one, recognized by *Trethewie*. But to it must now added the idea of histamine being bound in two ways, one readily mobilized the other resistant. The significance of this is obscure but it may well be a situation which holds for other physiologically active substances in the body for which knowledge of distribution is not so far advanced.

Among the physiological roles which histamine has been given an active part have been the continuous control of capillary tone, of gastric secretion, of uterine or intestinal activity. Our recent knowledge on the whole fails to justify any of the proposals. Thus as techniques for handling blood avoiding release of histamine from white cells and platelets have improved, the plasma level of histamine normally occurring has fallen lower and lower, and it is perhaps doubtful whether there are significant amounts of histamine in the plasma under normal conditions. Again so far as gastric secretion is concerned, the histamine in the stomach proves curiously refractory to mobilization by any known procedure, so as to raise doubts as to whether in fact it is a viable constituent of the stomach wall. It is disappointing that gastrin does not appear to act as a histamine liberator primarily, although there may be still an element in its action to be discovered (*Smith*, 1953, 1954). The idea that histamine might contribute to parturition is excluded by the observation on the human uterus that, although the non-pregnant uterus contains significant amounts of histamine, as pregnancy advances this falls to vanishing point (*Gunther* and *Paton*, unpublished). Finally the possibility that histamine release may be involved in controlling intestinal movements (*Feldberg* and *Smith*, 1954) loses its attractiveness in view of the relative resistance of histamine in the small intestine to mobilization; it will be necessary to find some rather active histamine liberator in the wall of the intestine, for which there is no evidence at present, before one can confidently believe that histamine is playing a major part in the modulation of intestinal movement.

It is obvious that the physiology of histamine still presents a major riddle, possibly because its importance is not physiological at all but pathological.

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