HISTAMINE RELEASE BY COMPOUNDS OF SIMPLE CHEMICAL STRUCTURE

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I. INTRODUCTION

Several stages mark the development of our knowledge about histamine and its actions in the body. First came its isolation, and the description of its pharmacological effects, akin on the one hand to those produced by "shock", and on the other to certain anaphylactic reactions. Following this came the discovery that histamine was a constituent of normal tissues, that it was released in sensitization reactions, and that it, or something very like it, was concerned in one of the fundamental responses of the skin—the "triple response" of Thomas Lewis. Along with this went a steadily increasing knowledge of its distribution in the body, and an expanding study of possible roles in controlling the contraction of various smooth muscles or the secretion of gastric juice. A further impetus to this field of study came with the discovery and development of the antihistamines, which allowed, amongst their other uses, an approach to the physiology of histamine similar to that provided, for instance, by the use of atropine in unravelling autonomic physiology. But despite this accumulating experience, to which knowledge about histamine metabolism was also added, histamine still remained somewhat of a physiological puzzle—a highly active and widely distributed tissue constituent to which no physiological role could be attributed with certainty.

A new approach became possible when means became available for reducing the amounts of histamine in the tissues by the substances now known as histamine liberators or histamine releasers. It is true that substances capable of freeing histamine from its attachment in mammalian tissues have long been known. But these had been for the most part materials such as venoms, pro-

teolytic enzymes, lysolecithin, and bacterial toxins. Certain peptone preparations, whose effects have been studied over fifty years, can indeed be compared with the more recent histamine-releasing drugs, but they need to be given in massive dose, and are liable to contamination with histamine itself. The other substances just mentioned, though active in releasing histamine, were liable to produce gross tissue damage and were usually lethal in any histamine-releasing dose. But in 1939, Anrep and his colleagues (2), during work on blood flow through muscle, discovered that curarine could release histamine from dog muscle, showing further that repeated injections led to lessening histamine releases, and that the histamine content of the muscle was at the same time reduced. The ability of certain other curarizing compounds to release histamine from dog muscle was demonstrated by Schild and Gregory in 1947 (207), who showed that strychnine also had this power. In the same year, MacIntosh and Paton (134) showed that the ability to release histamine was far commoner than previously suspected, being exerted by a wide range of dibasic substances, some of them very simple chemically, as well as by some benzamidine derivatives and a number of other somewhat complicated molecules. At the same time it was found that these compounds could also release heparin in the dog, furnishing a provocative analogy between their action and that of anaphylaxis. Of recent years, the number of substances shown to release histamine more or less readily has rapidly multiplied. There are now a number of compounds of known structure available with which certain tissues at least can be depleted of their histamine, without gross damage or lethal action. But at the same time, the situation has become complicated by evidence of different mechanisms of action, and by the discovery that different species and different tissues may react differently. It is necessary first, therefore, to review the main methods used, and to describe the results obtained, before approaching the more general problem of what light this work on histamine release has thrown on the physiology of histamine.

The earlier work on histamine release is reviewed by Feldberg (68). The review by Rocha e Silva (193), and that by Parrot and Reuse (169) should also be consulted. An important account of current research in this field will be found in the proceedings of the Histamine Symposium, held in 1955 jointly by the British Physiological and Pharmacological Societies with the Ciba Foundation, and published in 1956 in the series of Ciba Foundation Symposia.

II. METHODS OF STUDY AND CRITERIA OF HISTAMINE RELEASE

1. Methods using the whole animal. a. Rise in histamine content of systemic blood. Perhaps the most decisive evidence that a compound can release histamine in the mammalian body is the demonstration of a rise in concentration of histamine in the plasma after the injection of the compound concerned. It is then established that release occurs when the tissues are in their natural environment receiving blood through their proper vessels, and the histamine can be pharmacologically identified as rigorously as occasion demands.

This method has been used by a number of investigators (2, 77, 113, 135, 142,

170, 180, 200, 201) but the method is not without complications. (a) It is known that histamine (given by slow infusion in man) can produce quite prominent effects in the body, such as circulatory actions or stimulation of flow of acid gastric juice, although its concentration in the plasma (less than 3 ng/ml) may be demonstrable only by highly refined techniques (*cf.* 1). To obtain plasma histamines of a level which can be demonstrated easily (for instance by testing a small quantity of plasma directly on guinea-pig ileum *in vitro*) an animal must be thrown into a state either of shock or bordering on shock. Under such conditions, other substances, such as catecholamines, also appearing in the blood, may complicate the assay; and quantitative comparisons of histamine-releasing potency are very difficult. (b) The assay of histamine in the plasma of whole blood is complicated in many species by the existence of histamine and hydroxytryptamine in the leucocytes or platelets (110). This necessitates either scrupulous care to avoid damage to these structures in preparing the plasma, or, if whole blood is used, a simultaneous verification that there has been no change in the proportion of these formed elements in the blood. Such a change is known to occur, for instance, in anaphylaxis in the rabbit, and led to the belief, for a time, that anaphylaxis did not cause systemic histamine release in this animal although this is now established (201).[1] (c) A rise in plasma histamine is not informative as to the origin of the histamine. It should be possible, however, to demonstrate the main sites of histamine release by sampling venous blood at various points after injection of a histamine liberator. Little work of this type in the whole animal has been done, although it is known that Compound 48/80 injected arterially into a dog's leg causes a rise in histamine content of femoral vein blood (79).

b. The delayed depressor response. The histamine-liberating activity of licheniformin, and of many other substances, first came to notice through the characteristic effect it exerts on the blood pressure, when injected intravenously into the cat anaesthetized with chloralose (134, 135). After the injection, the blood pressure is unaltered for 20 to 30 sec, and then falls sharply; with the appropriate dose, a rapid recovery follows. Often a pronounced tachycardia develops as recovery of the blood pressure occurs; and if blood pressure of the animal was initially low, the recovery may be followed by a pressor response. It is thus possible to obtain a depressor response closely resembling that seen (for instance) with histamine, save for the single difference that it occurs about 25 sec later. The latency in onset means that the drug does not have a rapid direct vascular action of its own, comparable to that of acetylcholine, histamine, nitrites, veratrum-like substances, or detergents. Yet, because the depressor response, when it comes, is abrupt, it is hard to suppose that the delay is due to an intrinsically slow direct action by the drug. Further, the interval to action is significant, for it agrees closely with what was subsequently shown to be

[1] The ability of plasma from certain species, when diluted with saline, to form slow-contracting substances, is an additional difficulty. (Schachter, M.: A delayed, slow contracting effect of plasma and serum due to the formation of a substance resembling kallidin and bradykinin. J. Physiol. **129:** 30P-31P, 1955.)

the circulation time in the cat for an injection of saline to pass from the femoral vein to its second appearance in the carotid blood (91). The conclusion follows that the drug does not itself directly influence the blood pressure, but releases a vaso-active material at the periphery, which reveals itself only after return to the heart and discharge into the systemic arteries. In view of the known instability of acetylcholine in blood, the existence of histamine in the tissues, and the similarity of the response to that due to histamine, the plausible inference could be made, from the character of the response alone, that histamine is being released.

The argument outlined was originally no more than suggestive, but it has been substantiated by direct evidence. It is striking that, at least within the writer's knowledge, only one exception exists to the rule that compounds which show this delayed, abrupt, depressor response are histamine liberators. The exception is that such a response may sometimes be seen with a large dose of an anticholinesterase (57). But the principle still operates. Here acetylcholine, continuously released and usually destroyed in the tissues, but suddenly preserved when the anti-esterase is injected, takes the place of histamine. The presence of the delayed depressor response, therefore, provides a simple and useful test for histamine-releasing activity. It offers the advantages that the test is performed on a whole animal, that small doses can be used, and that quantitative comparisons or estimates of threshold doses can be obtained. This method also allows an estimate of the speed of histamine release. Gray and Paton (91) found, for 2 ml injections into the femoral vein of a cat under chloralose, that the mean circulation time for the second appearance in the carotid artery was 18.2 sec, with S.D. about 4 sec. MacIntosh and Paton (135) obtained latencies in the delayed depressor response between 20 and 30 sec. If a few seconds are allowed for the transit time of blood from the carotid artery to the vessels on which histamine acts, and for the time required for the action of histamine (once started) to become manifest, the latency in the response is indistinguishable from the circulation time. This implies that the process of histamine release, when the drug is injected intravenously, can take at most a few seconds to begin, and possibly much less.

It is not true that, when the delayed depressor response can *not* be seen, histamine release cannot occur. We now know that substances, such as tolazoline, bile salts, and neoarsphenamine can release histamine (200); but these compounds produce other vascular actions, which obscure the characteristic histamine-liberating action. Sometimes, as with d-tubocurarine, when previous artificial respiration and ganglionic block have been established, the other vascular effects can be prevented and a typical response seen (135). In general, a lack of histamine-releasing activity can be inferred from this type of test only if the drug is devoid of any action whatever on the blood pressure.

The delayed depressor response does not, in itself, indicate the source of the histamine released. Some evidence of this may be obtained by testing whether the response can be seen after skinning the animal or after evisceration, by comparing the results of injection into the right and into the left auricle, or

by injecting retrograde into the inferior mesenteric artery so as to restrict the field of initial action of the drug to the skin and muscle of the hindquarters (135). From such results it was inferred that the histamine liberators acted principally on skin and muscle in the cat, rather than on the viscera, lungs, or heart, a suggestion which was established later by experiments on perfused organs.

c. Blood pressure responses in other animals. The delayed depressor response is generally seen only in the cat under chloralose. In the dog, a fall in blood pressure of shorter latency is usually obtained. Such a difference would be explained if, in the cat, the histamine release took place at a point in the tissues well down the chain of peripheral vascular resistances, but, in the dog, at a less distal point so that the vasodilatation in the region of release could reveal itself. This hypothesis is supported by the fact that, in the dog, the infusion of adrenaline (so as to increase the peripheral resistance at the arteriolar level) will convert the latency of action of a histamine liberator from 7 to 10 sec to 20 to 25 (135). In other animals, such as rabbit and rat, the effects on blood pressure are variable and not very informative. Occasionally pressor responses, not unlike those produced by histamine, can be elicited. But the sensitivity of these animals to histamine is in any case rather low.

d. Other responses in the whole animal: oedema, itching, gastric secretion, bronchospasm. The injection of a histamine liberator into an unanaesthetised dog produces a dramatic picture (119, 175). In a few minutes there is evidence of intense itching, apparently all over the body; the skin, particularly of the muzzle, around the eyes and the ears, but also in other regions, becomes red and oedematous. If a gastric cannula is present, a profuse secretion of acid gastric juice occurs. In the rat, a corresponding picture of oedema and erythema, particularly of snout and paws, is seen, similar to that produced by dextran or ovomucoid (56, 82, 202). In the cat, a large dose of compound 48/80 (213) produces signs of itching, salivation, tears, and a state of prostration with tachypnoea, vomiting, micturition, and defaecation. Later, as the animal recovers, oedema, especially of nose and eyelids, appears (213). In anaesthetized animals an increase in gastric secretion can be demonstrated (72, 135). In the guinea-pig, bronchospasm has been recorded with histamine liberators injected parenterally (56, 75, 135) but it is clear that it is less readily produced than with histamine itself. Given by aerosol, histamine liberators will regularly produce bronchospasm (56, 108) but this is not a very specific response, as it can be produced by parasympathomimetics and by hydroxytryptamine also (109).[2]

These records of incidence of oedema find a counterpart in experiments using dyes to test the effect of histamine liberators on capillary permeability.

[2] G. Asboe-Hansen and O. Wigelius (Histamine and mast cells. Studies on living connective tissue in the hamster check pouch. Acta physiol. scand. **37**: 350–358, 1956) describe the response of the hamster to histamine release (shock, erythema, cyanosis, oedema of muzzle and paws), and demonstrate mast cell degranulation in the connective tissue of the cheek pouch.

In guinea-pigs receiving 48/80, bluing becomes intense around the eyes, ears, nose and mouth, nipples and perineal region. The contrast with the faintly stained skin of trunk, ventral abdominal wall and skin of hind limbs is striking. In the cat, bluing is chiefly of eyelids, around the jaw, ears, nipples, and perineum. The rabbit resembles the guinea-pig, with staining chiefly of eyelids, ears, muzzle and lips, nipples, and perineum. In mice, less regular results occurred: in some, little difference from control animals was seen; in others, a generalized bluing occurred, deeper over head, shoulders, and limbs (75, 144).

Some other actions of histamine can also be recognized in the effects of histamine liberators in the whole animal: (a) rise in the haematocrit (135); (b) increased flow of lymph from the thoracic duct (142, 171); (c) increase of limb volume (135); (d) in the dog, swelling of the liver and rise in portal pressure (121, 135, 142, 170). With large doses, haemorrhages in the pericardial, epicardial, and endocardial surfaces of the heart, and in the mucosa of the upper part of the intestine occur, recalling the similar, though much more intense, lesions observed in anaphylaxis or after certain venoms (68).

A simple method of studying release, developed by Fawcett (66), is by intraperitoneal injection into the rat. A given dose of liberator, in a volume of 20 cm^3 Tyrode solution is injected, and the fluid sampled 30 min later. Release of histamine into the fluid is proportional to dose of liberator given, and disruption of mast cells can be tested.

e. Urinary excretion of histamine. The presence of free histamine in the body can be demonstrated more easily by its appearance in the urine than by assays of the histamine content of plasma (1). It is estimated, from experiments on the infusion of histamine in man, that about 1% of the dose infused can be recovered in the urine: although this is a small recovery, the fact that it is somewhat concentrated by the kidney, and that assay on urine is sometimes easier than on plasma, makes the study of the urinary output of histamine after histamine liberators a valuable tool. Two improvements in technique may help to produce more regular results, at least in rats. It is known that a large part, if not the bulk, of the normal urinary histamine in the rat depends on histamine formation and absorption from the intestine, so that it is liable to substantial variation with intestinal state. But intestinally active antibiotics can reduce the bacterial content considerably, including those organisms forming the histamine (203, 231), thus lowering the urinary histamine output to a much lower, and perhaps more constant level. Secondly, the output of histamine in the urine can be greatly increased by treating the animal with inhibitors of histaminase, thereby preventing destruction of free histamine in the body (202a, 204, 205). Thus the use of rats receiving oxytetracycline and aminoguanidine may allow a better estimate of histamine output to be obtained.

f. Intradermal injections. A very useful method, applicable both in man and in animals, is to study the results of intradermal injection of the drug. In man, the characteristic triple response described by Lewis (128) can be seen in all its stages. Indeed, he described such a response for morphine and atropine. The production of a triple response in itself indicates either the presence of a hista-

mine-like activity or histamine-releasing activity. The two can be differentiated by making a similar injection some hours later after the effects of the first have died down (135). Under such conditions histamine will again produce a triple response, whereas a histamine liberator produces no response or a reduced response, since the histamine available for release has been diminished or removed. In animals intradermal injection will increase the permeability of the capillaries to dyes (75, 144). Thus bluing of the area of an intradermal injection can be obtained in the skin of a rabbit or of a guinea-pig. In the rat, such bluing is not easy to see from the exterior, but if the animal is killed and the skin examined from the inside the same phenomenon can be very elegantly demonstrated (100). The production of bluing is not an entirely specific response, since there are a number of substances such as leukotaxin and other inflammatory polypeptides, which can cause it and which might be in some way mobilized by the drug. The triple response in human skin, however, is a characteristic phenomenon which has not so far been described as produced by any other compounds than histamine or histamine-releasing drugs, except that in occasional subjects acetylcholine and related substances may elicit it. But no difficulty arises in determining whether a particular drug or a particular individual are producing this particular syndrome.

g. The demonstration of histamine loss from a tissue. It has now been shown that, for every histamine liberator in which the point has been tested, reduction of the histamine content can be achieved in skin, and to a less extent in other organs (2, 77, 82, 135, 158, 187, 188, 191, 213). This offers an additional means of demonstrating histamine release by the relatively simple method of comparing the histamine contents of, *e.g.*, the skin of rats treated and untreated by a drug. There is, however, one complication that might lead to error. The intradermal injection of some liberators, or local skin damage, in some species, may give rise to a temporary *increase* in histamine content. This may be partly attributed to the trapping of leucocytes or platelets at the site of the reaction (52–54). Systemic administration of the drug should avoid this.

The work on histamine release has greatly expanded our knowledge of the histamine content of various tissues. Differences in content contribute significantly to differences in the effects of liberators in various species. The following references include most of the results so far recorded: 70, 75, 82, 96, 104, 110, 120, 140, 147, 172, 186, 191, 193, 197, 201, 213, 220.

2. Methods using perfused organs. Convincing quantitative evidence for the release of histamine by a drug can be obtained by the use of perfused cat, dog, or rat tissues. With histamine liberators, release takes place most readily from perfused skin preparations, and somewhat less easily from perfused muscle (77). The perfused skin preparation is a very satisfactory test object, since it combines the following advantages: 1) sensitivity to histamine liberators, in a manner correlating well with their activity in a whole animal, or in producing bluing or "triple response" when they are given intradermally; 2) a good output of histamine (rendering assay easy); 3) a low spontaneous histamine output and remarkable robustness, so that the injection of acid solution, or suspension

of the perfusion for a few minutes, do not themselves cause the output of histamine to rise. Histamine outputs of 100 μg from a single skin flap are easily obtained, and as much as 360 μg has been recorded. The method, however, offers some technical difficulty; and quantitative comparisons of potency are not easily made since the histamine released dwindles rapidly with successive injections. The perfused cat paw has also been used. This is simple to prepare, does not become so oedematous, and yields a good histamine output. A disadvantage is that it contains more than one tissue, but its reactions appear to be equivalent to those of a skin flap (223). Perfused liver (except in the dog), lung, or intestine seem, in general, to be less sensitive to histamine-releasing action.

The use of the perfused organ also allows a deeper analysis of the characteristics of the release process. With perfused muscle, indeed, under the action of histamine liberators, the output of histamine in the venous outflow follows accurately an exponential course, and behaves as though the histamine were released rather rapidly into a volume quantitatively the same as the extracellular space, from which it is then washed out by the perfusion fluid (77). This analysis, together with the promptness with which release in the whole animal takes place, judging by the character of the delayed depressor response, has led to the release being characterized as "explosive", and indicates that the main part of the action can only take a few seconds.

With the perfused organ, it is also convenient to be able to make up a balance sheet of histamine initially in the tissue, histamine released, and histamine remaining in the tissue after release. The fact that such balance sheets show that no new histamine is formed, but that release takes place entirely at the expense of preformed histamine, is important for understanding the mechanism of release (77).

The perfused rat hindquarters have also been employed (61, 76, 196). Here skin and muscle are present, so that it is not certain whence the histamine comes, or in what proportions. The preparation is liable to have a high spontaneous histamine output, and to become very oedematous. It also seems to be more vulnerable to non-specific influences. But it is a simple preparation, allowing the assay of histamine as such, and is reasonably sensitive.

Release of histamine has been recorded as a result of ischemia of the rabbit hindquarters (18).

3. Diffusion techniques. Schild and his colleagues (147, 196) have developed in detail methods whereby a tissue is suspended *in vitro* in Tyrode solution and the histamine diffusing out of it, in response to histamine liberator in the bath diffusing into the tissue, is measured at various times after the application of the histamine-releasing agent. This is a simple method in which the estimate of histamine released can be made easily and as precisely as required, and in which accurate quantitative assays and comparisons can be made. Appreciable time must be allowed for the diffusion process to approach sufficiently near equilibrium. With large doses of releasing agent, ten minutes is sufficient, but with lower concentrations the "half-time" to equilibrium is about thirty min-

utes. The method has been applied particularly to tissues from rat and guinea-pig. A useful feature is that it can be used for tissues for which perfusion is difficult.

4. Fragmented tissues. It has been found that, if a tissue is minced by suitable means into pieces of approximately 2 mg weight, the particles retain most of their histamine content in a state from which release can still occur (148, 149). The mince is washed, suspended in Tyrode solution, and kept stirred by agitation with a glass ball. A spontaneous release of histamine takes place during the first twenty minutes, of about 20% of the total histamine content, but then a steady state is attained with a low constant rate of loss of about 0.1%/min. The application of a releasing agent causes the appearance of histamine in the suspending fluid. It takes up to an hour to reach full release, and longer with low concentrations of releasing agent, but with high concentrations, the bulk of the release may be completed in ten minutes. The speed of release is not accelerated by mincing the tissue eight times finer (in terms of particle weight) so that, presumably, the speed of release is not being limited by diffusion.

The method offers the advantages that a number of uniform samples of mince can be prepared for comparative tests, that all the histamine is released, and that, since concentration-action curves are steep and parallel, quantitative comparisons between drugs are possible.

In general, agents active in the whole animal are also active in this preparation. But it displays some important quantitative differences, to be discussed later, notably that 48/80 is less active than it is in the whole animal, in perfused skin, or on intradermal injection; and that the alkylmonoamines, particularly octylamine, are more active than in other tests.

5. Whole blood, leucocytes and platelets. Rabbit blood, in which relatively high concentrations of histamine occur both in the platelets and in the leucocytes, is most commonly used in this method for studying histamine release (99, 117, 137–139). The blood must be handled with circumspection, using siliconed glassware and avoiding mechanical agitation, to prevent breakdown of the cells concerned. The histamine is conveniently assayed biologically after a preliminary extraction from the plasma, or by chemical means.

The method provides a valuable supplement to other studies. Histamine release here, however, does not seem quite comparable to that obtained from other tissues, and these results are described separately below.

6. Intracellular particles. Finally, the analysis of release has been carried down below the cellular level by the use of differential centrifugation techniques (47, 98, 137, 150, 152, 157). It has been found that the histamine is chiefly localised in the larger granules of liver and lung cells. A suspension of such granules in Tyrode solution is itself pharmacologically inactive if injected, showing that the histamine is not in free diffusible form. But the application of distilled water, detergents, and histamine liberators will mobilize the histamine, so that it can become pharmacologically effective and identifiable.

7. Mast cells. Histamine release in mast cells has been studied, although in a more indirect way. The correlation of histamine content of a tissue with mast

cell content has been well established (41, 89, 90, 158, 186, 191), so far principally in the rat although discrepancies exist (84, 158, 189, 190). This allows one to use a tissue rich in mast cells, such as part of the mesentery, as a test object for histamine release by a drug. The disruption of individual cells and the discharge of granules, together with a subsequent failure of the cells to take up a stain such as toluidine blue, can be taken as signs of such histamine release. The method may be rendered quantitative and tests made comparing various concentrations of a releasing agent, the activity of different drugs, or the effectiveness of antagonists (160, 161).

Historically, it is interesting to note that the destruction of rat mast cells by egg white was observed by Webb twenty-five years ago (226), a finding still to be completely interpreted.

Critical assessment of evidence for histamine release

It is usually easy to establish that a particular drug or procedure can mobilize histamine from its cellular confinement. But claims that particular drugs exert their actions in the whole animal through histamine release, either in part or entirely, have met with a number of criticisms. The problem is important, from the practical point of view, because there is a large number of substances in clinical use for which histamine release has been demonstrated by one means or another. A discussion of some of the objections raised to characterising such substances as diaminodecane or 48/80 as histamine liberators may help to clarify the matter.

The failure of antihistamines to be as effective as expected against the depressor effects of 48/80 or diaminodecane, as well as other histamine liberators, has been commonly noted. A similar discrepancy has been observed for certain allergic and anaphylactic responses. As a possible explanation for the latter, Dale (48) has developed a distinction between "intrinsic" and "extrinsic" histamine: this concept has been so widely exploited that it seems worth while to quote his words in full:

"Consider the relationship between the cells from which histamine is released—the sensitized cells on which the antigen or haptene acts in the case of the allergic reactions—and those which react to the released histamine, producing the familiar syndrome. The cells which release histamine and those which respond to it may be identical, as they probably are when sensitized plain muscle encounters the antigen, and we may plausibly picture this as a reaction to histamine internally liberated and speak of it as a response to *intrinsic* histamine. On the other hand, when histamine is released in the liver it is obviously carried widely by the circulation to other histamine-sensitive tissues, causing a general vasomotor collapse and stimulating the plain muscle of remote organs. Even when the injury releases histamine from epidermis or nasopharyngeal epithelium it is not the epithelial cells which respond to it but the subjacent vascular plexus, which thus reacts to *extrinsic* histamine. It will be clear that the antagonism of an antihistamine, whatever the precise mechanism of its action, may be widely different against histamine from within and histamine from without. There might obviously be analogies with the action of atropine in suppressing readily certain effects of acetylcholine applied from without while leaving practically unchanged corresponding effects of its release from nerve endings. There is an even closer analogy in Schild's observation that the guinea-pig's sensitized plain muscle can be made

tolerant of excess of histamine in the surrounding fluid so that it gives no response to further additions of histamine to the bath but still responds to the specific antigen—presumably, therefore to *intrinsic* histamine. Such considerations may throw light on the relative effectiveness of antihistamines in urticaria and vasomotor rhinitis on the one hand and in spasmodic asthma on the other. The point is at least worth discussion in the light of clinical experience of the relative values of these drugs in different types of allergic reaction."

Such a distinction remains crucial, until we know more about the intimate details of histamine release and action. But our recent knowledge, that much of the histamine mobilized by histamine liberators comes from mast cells, forces us to assume that the histamine chiefly concerned in the depressor responses in animals is largely "extrinsic", *i.e.*, escaping from one cell, the mast cell, to act on another, the smooth muscle or capillary. Histamine so released should, therefore, be susceptible to antagonism by an antihistamine. But it may also be noted that the mast cells from which release takes place may be in very close relation to the blood vessels. Now the relationship between the distance from a point source of diffusible material and the concentration of the material at that point is a rather steep one. Assuming free diffusion, it is of the form $C = Q \cdot e^{-r^2/4kt}/8\sqrt{\pi(kt)^3}$ where c is the concentration at a distance r from the point source containing Q of the material with diffusion constant k, at a time t after the beginning of diffusion (40). For instance, after 10 sec, the concentration 300 μ from the centre is about one thousand times less than 30 μ from the centre. Accordingly, structures close to the mast cell might be exposed to extremely high concentrations, although those little more remote would be exposed to substantially lower ones. Now it is well known that antihistamines can only antagonise the effects of small doses of histamine on the blood pressure; thus the action of 0.1 μg intravenously may be abolished, whereas that of 10 μg may be hardly affected. It can be estimated, from Gray and Paton's (91) work on the circulation, that these doses will give rise to peak plasma histamine concentrations of 10^{-8} and 10^{-6} respectively. If, then, antihistamines can hardly show their antagonism to the vascular effects of histamine when its concentration in the body fluids is 10^{-6}, it is hardly likely that they will be effective with a concentration resulting from the release of histamine, initially at a concentration estimated at 10^{-2} (89), in the neighbourhood of a mast cell. Only when diffusion, or dispersal of histamine in the blood stream, has lowered the histamine concentration ten thousand times, could they be expected to show their action. One would expect, further, that a favourable time to demonstrate the effect of an antihistamine would be fairly late in the depressor effect, when histamine had been generally distributed; and, indeed, MacIntosh and Paton noticed this *restorative* effect of an antihistamine.

However, it must be added, that, although the relative failure of antihistamines to prevent the depressor effects of histamine liberators cannot be taken as an argument against those effects being due to the action of released histamine on the blood vessels, yet other reasons for this failure exist. In some species, of course, such as the mouse, antihistamines have little protective action against histamine itself (55). Further we know that other substances are also released, including vasoactive "slow-reacting substances" and (at least in the rat) hy-

droxytryptamine, against which antihistamines are inactive, and these may contribute to the depressor action, at least with large doses of liberator (15, 16, 113, 170). It is difficult to exclude, in addition, some alteration of the blood vessels in the neighbourhood of the cells releasing histamine. Riley (184) has described an oedema and change in staining properties of the mesenchyme of the tissues round the mast cells, which might extend to, or influence, the vessels carried by it. The circumstances of release, as well as the histamine itself, may well influence the picture observed.

A second doubt, as to whether a particular histamine liberator is properly so-called, has resulted from failure to detect histamine in the plasma after a significant depressor effect had been elicited. But abundant evidence has now been obtained that rises in plasma histamine occur, provided large doses of liberator are given. The difficulties of assay of histamine in plasma by simple methods are sufficient to account for reported failures to detect a rise after moderate doses of liberator, just as after moderate doses of histamine itself.

Thirdly, it has been noted that the toxicity of a compound such as 48/80 is roughly the same in all species, unlike that of histamine; that 48/80 produces bronchospasm in guinea-pigs only with difficulty (unlike histamine); that it is rarely pressor in the rabbit (unlike histamine); and that it falls far short of histamine in stimulating smooth muscle such as that of the guinea-pig ileum. But a failure of parallelism between the effects of injected histamine and that of a substance depending for its action on freeing histamine in the tissues would be anticipated. Although a dose of histamine may be held constant from one test to another, the effect of a liberator must vary with the histamine content of particular tissues in particular species, with the proportion of it which is releasable, and with the rate of access of the drug to the tissue, as well as the rate of release of histamine and transport to effector sites.

A different question, of practical importance, arises for those concerned to assess accurately the properties of drugs likely to come into therapeutic use, as to what test for histamine release should be employed. It is probably best that the tests should meet three requirements: (a) to include a test in the whole animal on a species with a sensitivity to histamine and to histamine liberators comparable with that of man; (b) to allow the demonstration of released histamine as such; (c) to include a simple test in man. The first may conveniently be met by a test on the cat blood pressure; several compounds may be tested quantitatively at one time, and there is also the great advantage that other actions by the drug may reveal themselves. Of the methods available for identifying histamine released, the assay of plasma histamine after a large dose in the cat, the release of histamine from the perfused hindquarters of the rat, or the release of histamine into the peritoneal cavity of the rat after intraperitoneal injection of a volume of fluid containing the test drug, offer themselves as relatively simple. Finally, it is useful to test the drugs intradermally in man, a test that can with due precaution be made quantitative (43, 44, 135); the test can be completed by repeating it the following day to verify that, in the skin partly rid of histamine, the liberator is less effective.

III. EXPERIMENTS ON LEUCOCYTES AND PLATELETS

It has long been known that the blood of some animals, especially the rat and the rabbit, is relatively rich in histamine. The cells containing the histamine vary from one species to another. In the rabbit both platelets and polymorphonuclear leucocytes contain substantial amounts of histamine; in the guinea-pig only the eosinophils contain much histamine, and in the rat, as in man, the bulk of the histamine is in the leucocytes. Other species have lower histamine contents, hardly significant compared to those mentioned (110). Graham *et al.* (89, 90) have also shown that basophils (*i.e.*, mast cells in the blood) have a high histamine content. In bloods with a high content of histamine it has become clear, as the technique for avoiding damage to formed elements of the blood has improved, that the amount of histamine circulating free in the plasma is very small; and that most, if not nearly all of it, is held in the cellular elements. This fact makes it possible to use blood for studies on histamine release, rabbit blood, in particular, being employed as a rule. Successful release has been achieved by various antigens acting on blood from sensitized animals (117, 138). But a number of simple chemical compounds have also been found to be effective (137, 139); including a number of long-chain aliphatic primary amines, for which activity was maximal for octadecylamine, a quaternary pyridinium derivative with a 16 carbon atom side chain, certain fatty acids, of which myristic acid was the best, and dodecyl sulphate. Activity disappeared from the aliphatic amines if the nitrogen group was substituted, and from the pyridinium compounds if the side chain was short or if a carbomethoxy group was replaced by bromine or a carbamine group. The activity of these compounds, despite their obvious surface activity, did not correlate simply with their haemolytic action. For instance, octadecylamine was not haemolytic, although the trimethyl substituted analogue, free of histamine-releasing action, was strongly so. A number of the histamine liberators found active in other test objects, such as d-tubocurarine, diaminodecane, diguanidinohexane, and codeine were all inactive. Only morphine was active in rabbit blood, as well as in whole mammalian tissues. Humphrey and Jacques (111), using isolated platelet suspensions, found, likewise, that release of histamine did not occur even after a large dose of 48/80 or thalassine.

Another series of compounds shown by Haining to release histamine in rabbit blood are the dextrans, and, somewhat more readily, the dextran sulphates. The activity of these compounds depended on molecular weight. Compounds of less than 10000 to 15000 molecular weight were virtually inactive. Maximal effect in the substances tested was obtained with molecular weights between 200000 and 1000000. Sulphonation increased activity roughly fivefold (99).

In both these investigations inhibitors of release could be found. In the experiments of McIntire and his colleagues, tertiary and quaternary derivatives of octadecylamine, a halogen derivative of the pyridinium compound and tertiary derivatives of dodecylamine were effective inhibitors. In Haining's experiments the inactive dextran sulphates were good antagonists to release, as were heparin and maltotriosesulphate. Sodium oxalate (0.021 M), provided it was added

before the releasing agent was administered to the blood, also abolished release by dextrans. Oxalate also prevents release by octodecylamine, pyridinium, and morphine. Haining draws an analogy between the active dextran sulphate as a histamine-releasing agent, and as a fibrinogen precipitant. He points out that (a) both these actions occur only when the molecular weight of a compound is above a certain limit, (b) excess dextran sulphate will inhibit both reactions, and (c) heparin or low-molecular dextran sulphate will inhibit both release and precipitation. He suggests that the release process may be preceded by a clumping of platelets in rabbit blood which then gives rise to the mobilization of histamine. This suggestion is reminiscent of the well known accumulation of platelets in the lungs of dogs and rabbits in anaphylactic shock (193).

Some interest has focussed on whether activation of proteolytic enzymes is responsible for, or contributes to, the release in rabbit blood. McIntire (137), however, has failed completely to demonstrate that soyabean trypsin inhibitor will depress the release by any of his agents, as it also fails to prevent release by an antigen. It seems doubtful, therefore, whether proteolysis is playing any part in these phenomena.

It will be clear from these results that histamine release in blood does not parallel that from other tissues, although it is possible to demonstrate with it release by antigens, by peptone, and by simple chemical substances. The main differences seem to be (a) that the most active substances are very surface-active (although not necessarily haemolytic), a property not specially associated with histamine release in the whole animal; (b) histamine liberators are generally inactive; (c) the release can be inhibited by a relatively large number of substances, including some bearing a relation so close to the releasing agent as to suggest a competitive antagonism of some sort. With blood cells, aggregation and interaction of the histamine-containing cells can occur in a way impossible with a fixed tissue: the responses described may well reflect a fundamentally different mode of mobilizing cellular histamine.

IV. EXPERIMENTS ON MITOCHONDRIAL PARTICLES

The existence of histamine in intracellular particles is now well-established. Hagen (98), by differential centrifugation of homogenized dog liver, was able to show that the bulk of the histamine was localized in the large granule fraction. From these particles octylamine 10^{-3} released histamine, although 48/80 (10^{-5}) was inactive. Copenhaver and others (47) have also shown that, with preparation from dog liver, histamine is mostly in the granules, but that in dispersions of guinea-pig lung the histamine is more evenly distributed between the supernatant, the large granule fraction and the small granule fraction. They also found that stilbamidine was not active in releasing histamine from these intracellular elements and that the antigen-antibody reaction could not be elicited in liver homogenates. Mongar and Schild (150, 152), using guinea-pig lung homogenates, found a similar distribution of histamine between the different cellular fractions. They were able to release the histamine almost equally readily by octylamine and by 48/80 (10^{-4}), but antigen was ineffective on the mitochondrial particles from a sensitized lung.

Grossberg and his colleagues (95) also studied the large granule fraction of dog liver homogenate and found, like other workers, that it contained 90 to 95% of the histamine in bound form. From this form it was released by distilled water, freezing, 90% acetone, saponin, bile salts, and lysolecithin. Histamine liberators such as diaminodecane, propamidine and 48/80 all mobilized the histamine to a degree depending on temperature, hydrogen ion concentration, and concentration of the liberator. They concluded from these analyses that the histamine was not bound chemically but was enclosed in some diffusible form in a mitochondrion-like particle from which either rupture of the limiting membrane or an increased permeability of the membrane would allow it to escape.

These experiments, on what appear, at first sight, to be mitochondrial particles, may require some re-interpretation in the light of the observation by Mota *et al.* (157) that the large granular fraction from dog liver is in fact full of mast cell granules, showing the characteristic metachromatic reaction. They were able to make similar granule preparations from rat subcutaneous tissue. This has been corroborated by West (228) who has obtained similar granule fractions from mast cells from mouse subcutaneous tissue. These granule fractions were rich in histamine and known to contain anticoagulant material. Since dog liver is known to be rich in mast cells, it is probable that the "mitochondria" studied previously were contaminated with, or even consisted largely of, mast cell granules, and that the properties of these granules should be considered with those of the mast cells themselves.

V. EXPERIMENTS ON MAST CELLS

Riley (185) recently reviewed the evidence then existing that mast cells contain both histamine and heparin, and that they are responsible for the bulk of the histamine content of a number of tissues. This generalisation rests on a number of mutually supporting observations (41, 158, 186, 187, 191). A satisfactory correlation of histamine content, heparin content or metachromatic reaction, and mast cell population has been observed 1) in ox lung and liver capsules; 2) in the skin of numerous species; 3) in the tissue of young and old animals in which histamine, as well as the mast cell population, increased with age; 4) in animals treated with histamine liberators in which the mast cells are disrupted at the same time as the histamine content decreased; 5) in spontaneous mast cell tumours in which some of the highest histamine contents ever described have been recorded; and 6) in artificially induced mast cell aggregates produced by painting carcinogens on mouse skin. The mast cells themselves do not seem to be a completely uniform population but vary in size, staining reaction, and sensitivity to drugs (160, 184, 188). Often they are concentrated round the blood vessels but possibly move out into tissue spaces as they mature (183).

This correlation of histamine content and mast cell count was also found by Graham and colleagues (89, 90). Here the previous finding that the blood basophils contained a large amount of histamine led them to test whether the corresponding cell in the tissues was also rich in histamine. Using a technique of serial sections from frozen tissue, such a correlation was found to exist. On the basis of assay of histamine content of sections combined with counts of the mast

cells contained in them, and assuming that all the histamine was contained in the mast cells, they concluded that the histamine might be present in a concentration of 1 % in the mast cell and that each cell contained roughly 6 $\mu\mu$g of histamine.

Numerous investigators have studied the alterations in mast cells produced by histamine-releasing agents. Fawcett (66) has used a simple method injecting material intraperitoneally into rats. If distilled water is so injected there is a rapid release of histamine into the water accompanied by a disruption of cells. A corresponding injection of Tyrode solution was ineffective. Injection of a low concentration of 48/80 produced a large release. This release could only be produced once effectively and a state of refractoriness lasting several days followed it. Fawcett also noted that whereas injection of distilled water damaged the mast cells irreversibly, the injection of a liberator had a different action, leaving recognizable mast cells and principally causing a discharge of the granules. Leitch and Haley (127), giving 48/80 intraperitoneally, found that it disrupted the mesenteric mast cells, and, in female rats, led to an increase in urinary histamine; this histamine output was not found in male rats on an ordinary diet but required a calcium-deficient regime. X-irradiation led to similar results, but was less effective than 48/80. Disruption of mast cells by ovomucoid in the rat has been shown by Benditt *et al.* (11, 12) for the mast cells in the region of the feet and snout. In isolated skin strips both ovomucoid and 48/80 could be shown to release histamine, as well as disrupting of the cells. Mota *et al.* (156, 159) injected 48/80 and stilbamidine intravenously and found that it always led to disruption of mesenteric mast cells with a preference for the perivascular site. If the histamine liberator was applied to portions of mesentery *in vitro*, this perivascular preference did not occur.[3]

Riley (184) has shown that disruption can be produced with a number of histamine liberators including propamidine, pentamidine, stilbamidine, diaminoheptane, Witte peptone, and d-tubocurarine. His experiments with stilbamidine are of particular interest, as they took advantage of its fluorescent property described by Hawking and Smiles (106). It was possible to show that the liberator was concentrated in mast cells, especially those of the peritoneum and to a less degree in those of subcutaneous tissue. Histamine did not cause disruption except to a slight degree where there was acute vasodilatation and tissue oedema. As expected, heating and scalding of the skin produced disruption. Anaphylatoxin was also effective.

Junqueira and Wedelman (114) have also recorded the ability of compound 48/80, *in vitro*, to lead to the extrusion of granules by rat mast cells. They state that "no disruption of the cells occurred, and the cell cytoplasm was apparently maintained." This extrusion of granules could be prevented if the temperature was less than 25°C., if the pH was low, or by a number of metabolic inhibitors.

[3] B. Larsson (Effects of Compound 48/80 on blood pressure and plasma histamine level of normal dogs and dogs with mastocytoma. Acta physiol. scand. **39:** 12–21, 1957) has shown that dogs with mastocytoma are more vulnerable than normal animals to compound 48/80, indicating that tumour mast cells react like normal ones.

Dinitrophenol, sulphydryl-blocking compounds, arsenite, and urethane were all effective. Sulphydryl-blocking compounds could be reversed by reduced glutathione but the inhibition produced by the others was not reversed by adenosinetriphosphate (ATP).

A careful study of mast cell disruption by compound 48/80 has been made by Norton (160) using rat mesentery isolated in Locke solution. After treatment with histamine liberator the mesentery is fixed and mounted and histological examination made after staining with toluidine blue. In unfixed tissues treated with neutral red, the mast cell exposed to 48/80 was seen to swell and become balloon-like, sometimes twisting and turning as it did so, no doubt because of attachments to the connective tissue, and then to extrude its granules. The cells did not appear to be in fragments and granules were often found outside intact cells. The percentage of cells which became disrupted was graded, according to concentration of the liberator, so that an assay of activity of the liberator by the mast cell disruption was possible. One important observation was made that hypotonicity of the suspending medium, such that it was half or a quarter of tonicity of normal Locke solution, did not increase the activity of 48/80 but in fact considerably reduced it. Norton used this as a critical experiment to distinguish between three theories of disruption of the mast cell by the liberator: (a) that it was due to concentration of 48/80 inside it; (b) that it was due to release by the liberator of osmotically active material within the cell; (c) that the cell became more permeable to extracellular ions. If (c) was correct, then hypotonicity should reduce the effect of 48/80, whereas if (a) and (b) were correct, it should make 48/80 more effective by increasing the osmotic load on the membrane. The fact that hypotonicity actually reduced the disruption was taken to indicate that the membrane became more permeable to extracellular ions in the presence of 48/80. It is possible that this conclusion is not valid, if one supposes that the hypotonicity itself increases the volume of the cell; for then, the entry of a given amount of 48/80 into it would lead to a lower intracellular concentration of 48/80 than normal. But this experiment is most valuable in indicating that an action by which 48/80 weakens the cellular membrane is hardly probable, for the osmotic swelling must surely have placed a burden on the membrane itself. It was also interesting that, in the hypotonic solution, 48/80 in higher concentrations lessened the disruption produced by the solution itself.

Finally, Riley and West (187, 188) have extended these observations on mast cells in two directions. First, they have tested the action of histamine liberators in the mouse and have found that, although histamine here is likewise associated with the mast cells, yet the disruption of mast cells by known histamine liberators, as well as the reduction of skin histamine content, is much less readily produced. In the mouse, even with prolonged treatment of 48/80, reductions of histamine content of subcutaneous connective tissue or of the ears below 50% could not be achieved. After the injection of compound 48/80 ceased, there was a striking recovery of mast cell distribution in these areas in the mouse, together with a rapid return of histamine content. Secondly, in the rat, study of the ef-

fects of prolonged administration of 48/80 revealed the interesting result that during its continued administration both, histamine and mast cells, begin to return to the subcutaneous tissue (although not to the ear). The restoration of mast cells was brought about by the appearance of rather small cells close to the blood vessels, and sometimes by the refilling of old degranulated cells with metachromatic material.

VI. GENERAL SURVEY OF HISTAMINE-RELEASING SUBSTANCES

Before discussing briefly what can be said about structure-action relationships, some further comments are called for on some of the members of Table I. With unsubstituted α,ω-aliphatic straight-chain *diamines*, activity is maximal in the cat at about C10 (3, 135). Substitution of the nitrogen atoms may increase activity (3). In addition to evidence for histamine release, these substances have some antihistamine and antihistaminase action. *Cystamine* is closely related, obtained by the oxidation of two molecules of cysteamine. The parent compound is well known for its protective action against x-irradiation, as well as for a modest neuromuscular and ganglion-blocking action; cystamine is devoid of anti-radiation activity, and is also less active in the latter tests (see 88 for references).

The active *diamidines* include aliphatic and aromatic derivatives of considerable variety, including the trypanocidal diamidines. There is an extensive literature about the latter, including striking descriptions of manifest histamine release among their side actions (118, 131, 208). Stilbamidine has a useful antihistaminase action (129a). Their chronic toxicity has also been studied in detail, and deserves attention as indicating the types of toxic action likely to be met if histamine liberators were used clinically (230). Parenchymatous lesions of kidney and liver occur. Stilbamidine can also cause delayed nerve lesions, but this is probably related to its stilbene link (208, 209).

Among the active *diguanidines* is Synthalin® (decamethylenediguanidine dihydrochloride), well-known as a possible insulin substitute, based on its ability to produce hypoglycaemia. This was shown, however, to depend not on a true insulin action, but on an action upon the liver (19, 24).

Among *monoamines*, the aliphatic monoamines (particularly octylamine) and the sympathomimetic amines are discussed below. The length of chain leading to maximum activity varies with the test. On guinea-pig minced lung, the maximum was at C 10, and C 18 was inactive (148); on the histamine excretion test in the rat, C 8 was much more effective than C 10 (232); on rabbit blood, there was little activity at C 12 and a maximum at C 18 (136). *Ammonia* is a substance of interest, for which Schild (206) showed, in 1949, that as an agent releasing histamine from rat muscle it acted in the unionised form. Unfortunately, it is of little use as an analytic weapon in the whole animal in view of its central actions, its ability to produce pulmonary oedema, and other effects (*e.g.*, 39).

Among certain carbamino-quinolinium derivatives histamine-liberating activity has been identified (115), the compound referred to as McN 259-15 being the most active.

TABLE I

Substances able to release histamine

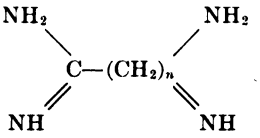
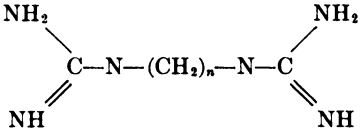
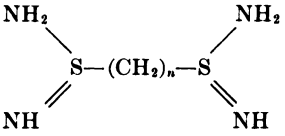
Compound	Test Objects Used	Reference
Diamines		
$NH_2 \cdot (CH_2)_n \cdot NH_2$.	Cat and dog blood pressure	62, 135
n = 2 to 16	Intradermal injection	124, 135
	Minced lung	149
	Mast cell disruption	184
	Intracellular particles	95
$R_1R_2N \cdot (CH_2)_n \cdot NR_1R_2$.	Dog blood pressure	3
Cystinamine = Cystamine = 1591L.	Intradermal injection	124
$NH_2 \cdot (CH_2)_2 \cdot S—S \cdot (CH_2)_2 \cdot NH_2$.	Rat hindquarters	123, 123a
Diamidines		
	Cat and dog blood pressure	135
	Intradermal injection	135
n = 8, 16		
(Also certain aromatic diamidines below)		
Diguanidines		
	Cat blood pressure	135
	Perfused skin	174
n = 5, 10, 18		
Diisothioureas		
	Cat blood pressure	135
n = 6, 16		
Diquaternary compounds		
$(CH_3)_3N—(CH_2)_n—N(CH_3)_3$.	Cat blood pressure	135
n = 12		
(see also muscle relaxants, below)		
Monoamines		
$NH_2 \cdot (CH_2)_n \cdot CH_3$	Minced lung	148
n = 2 to 12	Excretion of histamine	232
	Intracellular particles	98, 152
	Perfused muscle (cat, rat, guinea-pig)	76
	Perfused lung (cat, rat, guinea-pig)	76
	Perfused skin (cat, rat, guinea-pig)	76, 173
Ammonia	Rat diaphragm	206
Phenylethylamine	Perfused skin	174
Tyramine	Perfused skin	174

TABLE I—*Continued*

Compound	Test Objects Used	Reference
Monoamidines		
Aliphatic and substituted benzamidines	Cat blood pressure	135
Aminoacids		
Arginine	Perfused skin	60
Lysine		
Carnosine		
Ornithine		
SPECIFIC DRUGS		
Histamine liberators		
Compound 48/80	Cat and dog blood pressure	8, 56, 170, 182
Polymers of 2, 3, and 4 units of (I)[1]	Plasma histamine. Gastric secretion	56, 170, 175, 212
	Perfused organs. Isolated organs	16, 17, 71, 76, 77, 79, 81, 87, 147, 170, 201
	Minced tissues	148
	Mast cell disruption	66, 114, 156, 158, 159, 160, 165, 166, 183, 184, 186, 187, 188
	Bluing reaction	75, 144
	Intradermal injection	170
	Excretion of histamine	127, 203, 203a, 205, 230
	Histamine depletion	67, 158, 213,
	Aerosol (guinea-pig)	56, 108
	Intracellular particles	95, 98, 137, 152
Compound 1935L (II)[1]	Blood pressure	73, 122
	Perfused organs	73
	Intradermal injection	124
Antibiotics and chemotherapeutic agents.		
Antrycide	Perfused skin	77
Chlortetracycline	Mast cell disruption	161
Licheniformin	Blood pressure; plasma histamine; intradermal injection	135
Neoarsphenamine	Perfused skin; plasma histaamine	200
Polymyxin	Blood pressure; intradermal reactions; mast cell disruption; histamine depletion	37, 161, 167
Propamidine	Blood pressure; intradermal reactions; perfused organs; mast cell disruption; histamine depletion; plasma histamine	77, 82, 95, 135, 184, 229
Quinine	Perfused skin	200

Although *thiamine* cannot be a powerful liberator (135), the evidence is suggestive that bronchospasm produced by large doses in the guinea-pig is mediated by histamine release (215).

Sinomenine, a convulsant drug related to morphine, has been shown to produce, in dogs, shock with portal hypertension, incoagulability of the blood, increased lymph flow and a rise of plasma histamine, and a triple response in human skin. The histamine released derives chiefly from skin and muscle. Cross-refractoriness with peptone has also been shown, although with the latter the proportionate release of histamine from the liver was greater (142, 143).

Tween 20, a dispersing agent free of nitrogen, produces histamine release in dogs with a rise in plasma histamine, the anaphylactoid syndrome, and refractoriness to a second injection. This appears to be specific for the dog; no wheal is produced by intradermal injection in the skin of guinea-pigs, rats, or man (119).

1. Structure-action relationship. The first main conclusion from surveying the known histamine-releasing agents is that basicity predisposes to it. Only the 'large molecule' group, the detergents, Tween 20 and bile salts, neoarsphenamine, and chlortetracycline provide exceptions; and with all of these we may well be dealing with different processes. The fact that within the fairly homogeneous group of amino acids it is only the strongly basic ones which release histamine (60) furnishes important support.

But it is obvious that basicity *per se* does not confer activity. The inactivity of ethylamine, hexamethonium, and benzamidine illustrates this (135). But the replication of basic groups seems invariably to induce histamine release, provided their spatial separation is sufficient. Reviewing the work on diamines, diamidines, diguanidines, diisothioureas, diquaternary salts, polylysine, and 48/80, it seems to follow that any compound containing basic groups separated by more than about 5 or 6 atoms, preferably 8 to 14, has a strong chance of being highly active. The situation is curiously analogous to the requirements for neuromuscular block (all the agents in clinical use conforming to this pattern) as well as for some antibacterial actions, amine oxidase inhibition and diamine oxidase inhibition (21, 22, 85). It cannot be supposed, however, that polybasicity is essential for action. The butylamine derivative L 1935, trimetaphan, morphine and the substituted benzamidines, all monobasic, are all active; and L 1935 is among the most active compounds we possess. It is noticeable, however, that of the monobasic compounds, the active ones consist of a basic group attached to substituted aromatic rings, and no example exists of a monobasic compound, lacking any other polar group, capable of the specific histamine-liberating activity.

The nature of the basic group is also important. Comparing similar series, such as aliphatic bistrimethylammonium compounds with homologous diamines, the former are very much less active. This points to histamine liberation being mediated by the unionised radical. But it is not true that all quaternary salts are equally though weakly active, as might be supposed from their permanently charged state. d-Tubocurarine, for instance, is considerably more active than decamethonium. If, therefore, quaternary salts tend to be less active by virtue

TABLE I—*Continued*

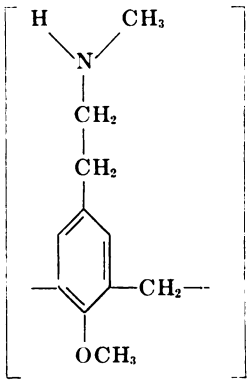
Compound	Test Objects Used	Reference
Antibiotics and chemotherapeutic agents. (Continued)		
Stilbamidine	Blood pressure; intradermal reactions; mast cell disruption	124, 135, 156, 184
Centrally active substances		
Apomorphine	Perfused muscle	77
Codeine	Perfused skin and muscle	77
Morphine	Cat blood pressure. Perfused skin and muscle. Intradermal injection	64, 77, 128
Nalorphine	Perfused skin. Intradermal injection	174
Papaverine	Perfused muscle	77
Pethidine	Perfused skin	200
Strychnine	Perfused muscle and skin	174, 207
Thebaine	Perfused muscle	77
Muscle relaxants		
Curarine	Plasma histamine. Perfused muscle	2
Decamethonium	Cat blood pressure	135
Dimethyltubocurarine	Cat blood pressure: intradermal injection	43, 44
Laudexium	Cat blood pressure; intradermal injection	23, 43, 44
Succinylcholine	Perfused skin; intradermal injection	174
d-Tubocurarine	Perfused skin, muscle, lung and liver, cat and dog blood pressure. Isolated tissues. Human arm. Mast cell disruption	45, 77, 93, 121, 135, 147, 153 180, 184, 207, 217
Drugs active on the circulation.		
Amphetamine	Perfused skin. Intradermal injection	174
Antazoline	Minced lung	6
Atropine	Perfused skin. Dog blood pressure. Intradermal infection	34, 35, 128, 200
Diphenhydramine	Minced lung	6
Tolazoline	Perfused skin	200
Trimetaphan (Arfonad)	Cat and dog blood pressure. Perfused skin	136, 174, 179
Large molecule compounds		
Dextran	(Rat) Bluing and oedema. Isolated skin. Mast cell disruption	26, 27, 58, 99, 102, 154, 155, 218, 225
Horse serum	(Cat) Perfused skin	79
Ovomucoid	(Rat and cat)	11, 42, 94, 101, 125, 126, 202
Polyvidone	(Dog) Blood pressure. Bluing reaction.	100, 100a, 103

TABLE I—*Concluded*

Compound	Test Objects Used	Reference
Miscellaneous		
Ammonia	Rat diaphragm	206
Bile salts	Perfused skin. Plasma histamine.	5, 135, 200
Sinomenine		142, 143
Thalassine		113
Thiamine		135, 215
Tween 20		119
McN. 259-15 (III)[1]		115

[1]

(I)



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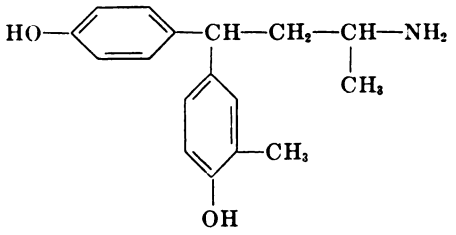
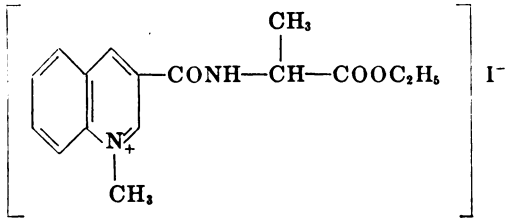


(III)



of their complete ionization, other features of their molecules (possibly simple increase of hydrocarbon bulk) can restore activity again.

Histamine liberation, then, appears when basic groups in a molecule are multiplied at sufficiently spaced distances, or when single groups are attached to substituted aromatic rings; too low or too high a basicity depress activity, but bulk and complexity of a molecule favour it. These statements are extremely general; but this is not inappropriate to so generally diffused a property as the ability to mobilize histamine.

2. Centrally active substances. The histamine-releasing action of the opium alkaloids and related compounds provides a satisfactory reason for some of the side effects described (78): lethality in bronchial asthma, urticarial responses, and anaphylactoid reactions after intravenous injection. Sensitivity to morphine is reviewed by Salter and White (199); it is clear that by no means all the side actions can be due to histamine liberation. The significance of itching is discussed

later. Nalorphine, like morphine, can produce a triple response in human skin and release of histamine from perfused skin of the cat (174).

3. Chemotherapeutic agents. There is a remarkable association between histamine-releasing and chemotherapeutic activity, appreciably wider than indicated simply by the list in Table I. Such activity exists for many other diamidines, diguanidines, and diisothioureas (85) which are of no present chemotherapeutic importance, and is recorded for 48/80 (161) also. There may, indeed, be a deeper relationship. Ormerod (162) has shown, for instance, that for the polybasic trypanocidal drug antrycide (itself a histamine liberator) the therapeutic action appears to depend on an ion exchange type of reaction, probably displacing protein from its combination with nucleic acids, thus forming inclusion bodies within the cytoplasm of the trypanosome. Snapper *et al.* (216) have demonstrated similar inclusion bodies in myeloma cells after treatment with stilbamidine. It is argued below that a similar type of action may lie at the root of histamine release in the body.

4. Large molecule substances. Considerable interest has been taken in a number of histamine-releasing substances of quite different nature, being of high molecular weight and colloid nature. These will not be reviewed in detail here, but some general accounts of them have appeared (169, 173, 193) and particular papers of interest as follows: egg-white and ovomucoid (11, 42, 94, 101, 125, 126, 202, 210); dextran (26, 27, 58, 99, 102, 154, 155, 218, 225); polyvidone (100a, 103); horse serum (79); anaphylotoxin (192–195).[4]

5. Muscle relaxants. Curarine is one of the first compounds for which evidence for histamine release was obtained (2), and it was the first for which gastric secretion was used as a sign of histamine release (72). The use of such compounds in anaesthesia has maintained an interest in their ability to release histamine, particularly because they may be given fairly rapidly intravenously, and because bronchospasm or circulatory effects are of particular importance in anaesthesia. All the muscle relaxants in clinical use so far studied appear to be capable of some degree of histamine release; the order of activity (from most to least active) appears to be roughly as follows: d-tubocurarine and its methyl ether, laudexium, mytolon, decamethonium, succinylcholine and gallamine: (23, 43, 44, 135, 217). But an estimate of the chance of release in practice must take account of the normal dosage used. On the basis of the ratio of releasing activity to total dose given, perhaps only d-tubocurarine and possibly succinylcholine would lead to occasional release. But with the latter, the full dose is usually only given slowly by infusion over a considerable time, a condition making release much less likely. It is with d-tubocurarine, therefore, that evidence for release in human practice would be expected. Such evidence, however, is rather scanty. When given arterially into the human forearm, both an acute urticarial reaction (45, 93, 153) and the appearance of histamine in the brachial venous blood have been demonstrated. But unequivocal 'anaphylactoid' responses in clinical use are rare. This is partly because changes in blood pressure or in respiratory func-

[4] A provocative observation is that by V. W. Adamkiewicz and Y. Langlois (Canad. J. Biochem. Physiol. **35**: 251–256, 1957) that insulin will sensitize rats to the dextran "anaphylactoid" reaction.

tion can be influenced by so many other factors in the process of anaesthesia that unambiguous records are hard to obtain. It may partly be, however, that surgical anaesthesia *per se* reduces the intensity of the histamine-releasing process.

d-Tubocurarine has been studied particularly closely. It is known to release histamine from dog muscle and liver, from perfused tongue, gastrocnemius, and the skin of the cat, from many isolated organs, from minced tissues, from intracellular granules (2, 5, 77, 148, 196, 207). It produces typical histamine shock in the dog (121, 180), and the delayed depressor response in the cat (135). It causes an increased flow of lymph (171) and depression of the clotting power of the blood (121, 180). After an effective dose, an animal is refractory to circulatory effects of later dosage. This detailed, and self-consistent, knowledge makes it a standard drug for the study of histamine release in conditions where its neuromuscular, ganglionic, and other actions are not displayed.

β-Erythroidine has either little or no histamine-releasing action (45, 93, 121).

6. Sympathomimetic amines. Since the first World War, it has been known that adrenaline can produce a shock-like state both in animals and man (63). Bainbridge and Trevan (7) showed that, given intravenously or intraportally in the dog, it could produce a rise of portal pressure with engorgement of the liver and increased flow of lymph. It is among the compounds which produce the alarm reaction in rats (211). Sustained infusions in the dog lead to increased capillary permeability, a fall in plasma volume, shock, and haemorrhages in the endocardium and duodenal mucosa (83). In the rabbit, continued infusion of adrenaline in increasing dose, so as to produce a maintained hypertension, led to a shock-like state, although if renin was used a given degree of hypertension could be maintained for days without harmful effects (20). In man, the abrupt cessation of a continued infusion of adrenaline leads to a considerable hypotension (*e.g.*, 92).

To account for the phenomenon of "adrenaline shock", two explanations may be advanced. First, it might be attributable to a paralysis of sympathetic ganglia by adrenaline, an action which is now well-recognized (33, 132, 141, 176), although the conditions of its appearance are not completely defined. But some features of adrenaline shock (such as portal hypertension, flow of lymph, capillary damage, and endocardial and duodenal haemorrhages) are not produced by ganglion paralysis. They are far more like the syndrome of haemorrhagic, traumatic, or histamine shock; and the first of these, indeed, is mitigated by sympathetic paralysis. The possibility that some of the hypotension after ceasing the administration of adrenaline may be due to ganglion paralysis is hard to prove or to exclude; but knowing that adrenaline becomes more effective as a pressor agent in the presence of ganglion paralysis, one must hesitate to postulate the presence, for a considerable time, after the end of an infusion, of an amount of circulating adrenaline sufficient to paralyse autonomic ganglia yet insufficient to exert its usual pressor effect.[5]

[5] H. Dunér and U. S. v. Euler (Secondary fall in blood pressure following noradrenaline infusion in the cat. Acta physiol. scand. **38**: 364–372, 1956) have concluded, however, from

Secondly, it might be that adrenaline can release histamine. This possibility was first explored, and finally rejected, by Burn and Dale (49), and Dale and Richards (50) in a study of the vasodilator action of adrenaline. Interest in it was renewed by the claims of Staub (219) and of Eichler and Barfuss (59) that, in man, adrenaline will raise blood histamine levels. But these experiments need re-examination in the light of our knowledge concerning histamine in the white cells and platelets, its release, and the effects of adrenaline on white cell counts. Wilson (232) has shown, in rats, that adrenaline will increase the histamine output in urine. The most rigorous attempt to demonstrate release in man was that of Mongar and Whelan (153), who used arterial infusions and assayed brachial venous blood. They were unable to demonstrate any histamine mobilization by adrenaline in doses up to 10 μg infused over 5 min, although d-tubocurarine (10 mg) was effective. Histamine release by other sympathomimetic amines can, however, be easily demonstrated. Phenylethylamine, tyramine, and amphetamine, but not ephedrine, applied to isolated skin preparation of the cat in doses of 10 mg will release 40–120 μg histamine and produce a triple response when injected intradermally (174). This suggests, although it would be hard to establish, that there may be, in man, a rather slight histamine-releasing action hidden by the more prominent effects normally exerted by adrenaline, revealed only by continued administration, and exerted perhaps chiefly on mast cells in close vicinity to the blood vessels in such a way that a small release has a large local vascular action. Therapeutically, adrenaline "shock" need present no problem. Noradrenaline is less liable to produce it; and if the infusion is terminated gradually rather than abruptly, no hypotension need be observed.

7. Vasoactive compounds. Of the compounds listed in this section, only trimetaphan and possibly hydrallazine, have an important histamine-liberating action. With the others (amphetamine, antazoline, atropine, diphenhydrazine, tolazoline) it is doubtful whether doses ordinarily used could normally release histamine, although they may do so in cases of special sensitivity. Hydrallazine is of low activity, but the doses given clinically may be large: and it is interesting that headache, urticaria, and flushing are among the side actions. But the other toxic action (arthritis, lupus erythematosus, and skin reactions) are more serious.

With trimetaphan, histamine release is more important. It produces circulatory shock and liver engorgement in dogs, with a rise in plasma histamine (136, 179). On cat skin, doses down to 10 μg are active (174). In the skin of man, dog, and guinea-pig, it produces a triple response or its animal equivalent (146); and such a response is known round the course of the vein even with ordinary venous infusion. In surgical practice, it can be shown to produce a secretion of acid gastric juice, under conditions where the stomach does not normally secrete (177). Judged by its action in the cat, its activity in producing ganglionic block and in eliciting the "delayed depressor response" are about equal. The relevance

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experiments in the cat, using buffer nerve section, ganglion block and ergotamine, that the fall in blood pressure after noradrenaline is partly due to a blocking effect on transmission within the vasomotor system.

of histamine release to its clinical use has yet to be fully explored. Trimetaphan is usually believed to be a drug of brief action; but no evidence has been obtained for its rapid destruction; and, in the cat, ganglionic action is comparable in duration with that of hexamethonium (174). With a histamine liberator, however, a transient effect is normally produced (by the explosive release and removal of histamine) whether the liberator concerned is slowly or rapidly destroyed; and a sustained depressor action is only obtained by repeating the dose soon after, or by giving a very large dose. It seems possible that the characteristic action of trimetaphan represents the effect of repeated histamine releases, presumably on a background of modest ganglionic block.

VII. THE PROPERTIES OF COMPOUND 48/80

Fassett and Hjort (65), in a study of the actions of certain N-methyltetrahydroisoquinolines, synthesised by Ide and Buck, found one preparation to be exceptional in being strongly depressor. This activity disappeared when it was synthesised in another way, so that possible contaminants were next investigated. This study by Baltzly *et al.* (8) showed that, in general, the condensation of ortho- or paramethoxyphenethylmethylamines with formaldehyde led to compounds possessing strong depressor activity in dogs, although the mechanism of the depressor activity was unknown. It was then found to produce the typical delayed depressor response in the cat, a rise in plasma histamine in cat and dog, and a weal when injected intradermally (170), and to release histamine from perfused muscle and perfused skin of the cat (77), and of the dog (79). Its general pharmacology has been fully studied by Dews *et al.* (56). Its high and relatively specific activity in these tests was notable and has led to its widespread use for studying problems of histamine release. Despite this satisfactory aspect of the substance, it is unfortunate that it has no definite chemical structure. It is believed to consist of a mixture of low polymers of p-methoxy-N-methylphenylethylamine nuclei, chiefly dimer, trimer, and tetramer. But it is suspected that the methoxy groups and the methylene bridges linking the nuclei may be in varying positions, so that the preparation may actually contain a mixture of more than three chemical substances. This is borne out by the difficulty of obtaining any separation of its constituents even by chromatographic methods. It is possible, therefore, that there exists, in the preparation of 48/80 now used, a principle more active than the parent mixture.

Although histamine release can account for all the effects of 48/80 ordinarily seen, it has some other actions. Given intra-arterially into the perfused superior cervical ganglion of the cat, in a dose of 25 μg, it will begin to block ganglionic transmission, and 100 μg produces complete block. The block appears to be competitive in type, and acetylcholine output is not reduced (87). Its activity in this respect is roughly $\frac{1}{25}$ that of hexamethonium. In the whole cat, 0.1–1 mg/kg hexamethonium is required for a useful (but not maximal) action; one may estimate, then, that 2.5–25 mg/kg of 48/80 would be needed for a similar action, 250 to 2500 times more than its threshold histamine-releasing dose. Dews *et al.* (56) found that 0.25 mg/kg in the cat failed to produce ganglionic block.

It seems improbable that the ganglion-blocking action plays much part in the results of 48/80 administration, except with doses producing profound circulatory shock and its accompanying reactions.

On the small intestine, 48/80 stimulates at 40 μg/ml (81), and displays a modest antagonism not only to histamine and to acetylcholine, but also to bradykinin, Substance P, the SRS (slow-reacting substance) of cat plasma after 48/80 shock, and the SRS of ground up intestine of cats (170). On frog gastrocnemius, 0.4 μg/ml will reduce the effect of acetylcholine. No doubt it can also display other antagonisms that any amine with a sufficiently large molecule commonly exerts. On the frog rectus in concentrations of 3 μg/ml or more, it can, in addition to antagonizing acetylcholine, produce an unusual contracture of slow development and passing off. By close arterial injection into the cat tibialis muscle, neuromuscular block of a complex type occurs; but 48/80 cannot have more than about $\frac{1}{50}$ the activity of decamethonium (174). Compound 48/80 also possesses a moderate antibacterial action on both Gram-positive and Gram-negative organisms (161). None of these actions modify significantly the view that in moderate doses, the action of 48/80 is for practical purposes simply that of histamine release. It is inactive by mouth. A dose of 0.5 mg/kg does not produce hyperglycaemia, but a slight hypoglycaemia: this contrasts with the effects of histamine and of peptone shock (163).

Comparison with octylamine. Mongar and Schild (148) found that primary aliphatic monoamines (especially octylamine and decylamine) could release histamine readily from guinea-pig minced lung suspensions, although MacIntosh and Paton (135) had found such compounds (as well as aliphatic monoamidines, monoisothioureas, and monoguanidines) inactive or pressor in the whole animal. Barger and Dale (9) comment only on the pressor, cardiac depressant, and convulsant actions. This has led to detailed comparisons of the relative potencies of the two drugs; these are summarized in Table II, showing potency ratios (48/80: octylamine) ranging from 0.1 to 1000, on different test objects. The calculation of these ratios, however, assumes similar modes of action of the two drugs, and possibly obscures an important point. Taking the octylamine results, it will be seen that its activity is roughly constant on intact tissues, requiring a concentration of about 1 mg/ml to produce a moderate effect. Only with preparations involving the fragmentation of tissue does its activity increase. Compound 48/80 on the other hand, on intact tissues, is sometimes far more active (skin or muscle) but if not, approaches the lower potency of octylamine (lung, rabbit liver, intestine). The impression is gained that 48/80 has some specific releasing power which is lacked by octylamine and which is demonstrable particularly on skin and muscle. With an unsusceptible tissue, this power no longer appears to be exerted, but another, shared by octylamine, becomes apparent.

There is other evidence that the action of 48/80, when it is exerting its specific action, differs from that of octylamine. The time course of histamine release in perfused skin is slower with octylamine than with 48/80 (76) and oedema formation is greater, recalling the slow release and oedema formation seen with bile salts (200). When the output of urinary histamine is compared, after intraperi

TABLE II

Comparison of histamine-releasing powers of compound 48/80 and octylamine by various tests[1]

	Species	Compound 48/80	Octylamine
1. Tests *in vivo*.			
Delayed depressor response (threshold dose)	cat	10 μg/kg	—
Weal (concentration required)	man	1 μg/ml	1 mg/ml
2. Perfusion experiments.			
(Dose for moderate effect)			
Skin	cat	1 μg	200 μg
Muscle	cat	5 μg	1000 μg
Lung	cat	100 μg	2000 μg
Hindquarters	rat	1 μg	1000 μg
Lung	rat	250 μg	1000 μg
Hindquarters	guinea-pig	30 μg	2000 μg
Lung	guinea-pig	1000 μg	1000 μg
3. Minced lung.			
Concentration for 50% release	guinea-pig	500 μg	100 μg
4. Mast cell disruption.	rat		
Concentration for 50% disruption		0.3 μg/ml	—
5. Intracellular lung particles.			
Concentration for histamine release	guinea-pig	100 μg/ml	100 μg/ml

[1] Data taken from 76, 148, 150, 160, 170, 173.

toneal injection in the rat, 48/80 gives a high rate of output which lasts only 1 to 2 hours, whereas octylamine leads to a lower rate of output, sustained for many hours (232). Finally, using the perfused skin, octylamine causes a release of potassium, as well as histamine, whereas 48/80, in a dose releasing as much or more histamine, does not do so (173).

It might be suggested that the action of octylamine depends simply on its lytic action on cell membranes, due to its surface activity. But this seems improbable, in view of the relative inactivity of bile salts on the perfused skin. On the other hand, this surface activity may well *contribute* to its action, in view of the tendencies to oedema, potassium release, and prolonged action, and of its increased potency after tissue fragmentation; compatible with this, too, is the fact that Mongar and Schild found the activity of the alkylmonoamines to increase up to decylamine and to decline thereafter, a peak corresponding to the point at which micelle formation becomes important.

It may be provisionally supposed, therefore, that even among the relatively simple compounds of the type discussed in this review, two modes of histamine release exist: (a) that exemplified by 48/80 and exhibited notably by dibasic or multibasic compounds, for which activity is high *in vivo* and in perfused skin or muscle, and with which the delayed depressor response can be demonstrated; (b) that exemplified by octylamine, where activity is lower except in fragmented tissues, and is displayed perhaps by any substance of sufficient basic strength and surface activity.

VIII. HISTAMINE-RELEASING AGENTS OF NATURAL ORIGIN

The existence of any potent drug inevitably suggests that a similar substance may play some part in normal physiology. Hence the interest which attaches to the existence of histamine liberators of natural origin, of which a number are known to exist, in addition to the well-known peptone preparations.

1. Amino acids. The ability of peptone to imitate anaphylaxis has long been familiar; and it is known that it will release histamine into the blood of the whole animal, from perfused liver, and from rabbit blood cells. But it is relatively inactive, and doses of the order of 100 mg/kg are necessary to obtain useful effects. A failure to obtain any highly active fraction from one of the more active preparations, Difco Proteose Peptone, led Eldridge and Paton (60) to suspect that histamine-releasing activity of a low order might be diffused throughout its constituents, and that the aminoacids themselves might be active. This proved to be the case; aminoacids with an isoelectric point greater than 8 (arginine and lysine) together with their derivatives ornithine and carnosine were active. Potency was very low (about 1/10,000 that of 48/80), but comparable with that of peptone.

A polylysine, with fifteen residues, has been shown (86) to be active, about $\frac{1}{3}$ the potency of diaminodecane. Reduction of clotting power of the blood in the dog, and the appearance in the blood of "slow-reacting substance" were also found. Polylysine will form a complex with heparin. Protamine was also active, about $\frac{1}{200}$ the potency of diaminodecane.

2. Antibiotics. Licheniformin, an antibiotic polypeptide containing arginine and lysine (38) has been shown to produce the delayed depressor response, to raise the plasma histamine in the cat and to produce a number of histamine-mediated reactions, including a weal by intradermal injection. It has about $\frac{1}{60}$ the activity of 48/80 (139).

Polymyxins B and E, polypeptides containing α,γ-diaminobutyric acid (105) are also effective histamine liberators (37, 161). In cats, 2 mg/kg will elicit the typical blood pressure response. In dogs, 2 to 10 mg/kg are needed to reduce the blood pressure. Repeated doses become ineffective. Antihistamines delay the fall in blood pressure, without altering the ultimate level attained. Polymyxin B, injected under the dorsal skin of a rat, will cause depletion of histamine content of ventral skin; Polymyxin E is less effective. Given intraperitoneally in rats, mast cell disruption was produced, with histamine release into the peritoneal fluid. Polymyxin is known to produce a triple response in human skin, and it causes bluing in guinea-pig skin. Compared to 48/80, the polymyxins are about $\frac{1}{200}$ as active on cat or dog blood pressure, and $\frac{1}{2}$ as active by the guinea-pig "bluing" test.[6]

Norton and de Beer (161) have studied quantitatively the degranulation of mast cells *in vitro* by a number of antibiotics. Polymyxin, at 0.4 μg/ml, and Aureomycin, at 77 μg/ml, produced 50% fragmentation. Bacitracin, penicillin, achro-

[6] It has been shown that polymyxin releases histamine almost selectively from rat skin, little 5-HT being mobilised. Compound 48/80 releases both amines, reserpine only 5-HT. (J. R. Parratt and G. B. West: Action of polymyxin B on tissue mast cells of the rat. J. Physiol. **135**: 24P, 1957.)

mycin, and sulphanilamide (500 μg/ml) produced no significant effect. Cyclizine reduced the degranulation somewhat.

3. Gastrin. Smith (212, 214) has examined in detail the possibility that gastrin may induce gastric secretion, not directly, but by release of histamine in the stomach. Gastrin will mobilize histamine from perfused muscle or perfused skin, and a little from perfused stomach. Its secretagogue action is greater when injected into the coeliac artery than when given intravenously or intraportally, but the amount of histamine released by it with coeliac injections is very small. With 48/80, it was concluded that, although intravenous doses caused gastric secretion by systemic histamine release, injections into the coeliac artery did so by an action on the gastric mucosa.

4. Hydroxytryptamine and tryptamine. Feldberg and Smith (80) have made the interesting observation that tryptamine and 5-hydroxytryptamine both release histamine, as judged by tests on cat skin, cat muscle, and depletion of histamine in rat skin and muscle. The two compounds were about equiactive, and were estimated to have about $\frac{1}{100}$ the potency of 48/80. Reid (181) recorded a slight erythema but nothing more at the site of an intradermal injection of tryptamine 5×10^{-3}, which is compatible with it not being very active; the diamidines produce weals at 10^{-4}, and 48/80 at 10^{-6}.

5. Leucotaxin. The possibility that leucotaxin can release histamine has been suggested by Rocha e Silva and Dragstedt (194a) and greatly strengthened by Miles and Miles (144), from their studies on the bluing reaction in guinea-pigs. Eldridge and Paton (unpublished) found that it could, in fact, do so from cat skin and rat perfused hindquarters, and that it caused a weal in human skin, but its activity was low.

6. Lymphagogues. The description by Heidenhain (107) of the effects of injecting a decoction of crayfish muscle (one of his lymphagogues of the first order) into a dog closely parallels the effects seen with 48/80. It was found that representative lymphagogues prepared by Heidenhain's method (from lobster, rock lobster, and mussels) were, in fact, histamine liberators, and that histamine liberators, such as 48/80, propamidine, morphine, and stilbamidine, could provoke a flow of lymph. This direct histamine-releasing action of crustacean muscle may well contribute to the urticarial results of dietary indiscretion, but sensitization reactions are probably also important (171).

7. Thalassine and cowhage. To the long list of substances mobilizing histamine with which plants and animals are equipped, seemingly for self-defence, are now to be added thalassine and cowhage. The former, one of the fractions extractable from sea anemone tentacles, has been shown to release histamine from cat skin, to produce the delayed depressor response in the cat under chloralose, and to raise the concentration of histamine in the plasma (113). Thalassine also causes the appearance of a slow-reacting substance in the plasma. Its activity is about the same as that of the diamidines.

A study of cowhage, or "itching powder", the name given to the trichomes which cover the pod of the tropical plant *Mucuna pruriens*, has indicated that its action is due not to a content of histamine but to an ability to release histamine

(28). The active principle could be extracted from the hairs, rendering them inactive. It has subsequently been shown that the hairs contain 5-hydroxytryptamine (25).[7]

IX. VARIATION OF HISTAMINE RELEASE WITH TISSUE AND SPECIES

Feldberg and Paton (77) found that with perfused skin from the leg of a cat the histamine content could be reduced to undetectable levels leaving less than 1% of the original amount of acid-extractable histamine, a state to which the term histamine depletion is appropriate. With muscle, on the other hand, only two-thirds of the histamine in the muscle at most could be freed. Other perfused tissues, such as lung and intestine in the cat, could hardly be attacked at all. With tissues from the guinea-pig, using the diffusion technique *in vitro*, Mongar and Schild (147) found that skin and muscle released histamine readily, and arranged tissues tested in diminishing order of proportion of histamine content released as follows: diaphragm, aorta, uterus, heart, bladder, skin, trachea, oesophagus, lung, stomach, spleen, large intestine, small intestine. Recently, Feldberg and Greengaard (71) have found that only about 50% of the histamine in nerve can be released by 48/80.

In experiments on the whole animal, substantial reduction in histamine content can be readily produced only in skin. With repeated injections the content of the skin can be brought down to a few μg/g from values as high as 50 or 100 μg/g, both in cats and in rats. Muscle histamine can be reduced by perhaps 30% of its original content and that in heart slightly less. The intestinal tract and liver, in general, are little changed, although in the cat the mucosa of the corpus of the stomach can have its histamine content lowered (82, 213).

There appear to be appreciable differences even within one tissue. For instance, Feldberg and Miles (75) found that the intensity of the bluing reactions produced by 48/80 differed considerably over a guinea-pig skin, and was most intense on the paws, nipples, ears, and snout. This they related to the histamine content of the tissues, but did not exclude other factors such as differences in blood flow, resulting in a higher concentration of liberator in certain regions. A similar result is seen in the effects of any histamine liberator in the rat, the dog, or in man when the development of oedema is centered, for instance, on the snout, muzzle, or face respectively.

In addition to this variation with the tissue concerned, there are signs of important species variations. Some of these species variations, of course, reflect differences in histamine content from one animal to another; and a useful result of this interest in histamine release has been a considerable addition to the data on histamine content in the organs of different animals (70, 74, 96, 104, 120, 140, 147, 172, 201, 213, 220). But variations in sensitivity not to be explained in this way occur. The skin of man, cat, dog, rat, and guinea-pig are all sensitive to low concentrations of 48/80, that of rabbit is somewhat less sensitive, and the

[7] See also B. Högberg, B. Thufvesson and B. Uvnäs: Histamine liberation produced in the perfused paw of the cat by 48/80 and extracts from jelly fish (*Cyanea capillata*) and eel worm (*Ascaris lumbricoides*) from swine. Acta physiol. scand. **38**: 135–144, 1956).

mouse somewhat resistant. In perfused preparations, 48/80 in fairly high dose will release histamine from cat lung, but more is needed for rat lung, and more still for guinea-pig lung. Small doses of 48/80 are effective on the perfused rat hindquarters, but 10 to 20 times bigger doses are required for effective release from guinea-pig hindquarters (76).

A curious observation is that, despite variations of the type just mentioned, the lethality of compound 48/80 is rather constant. For intravenous injection, Loew and Papacostas (130) obtained LD_{50} values for 48/80 (in mg/kg) of 1.29 in guinea-pigs, 1.5 in rabbits, 1.95 in mice, and 2.4 in rats, against corresponding figures for histamine diphosphate of 0.5, 4.0, 200, and 400. Experiments in dog and cat suggest that intravenous doses of about 1 mg/kg of 48/80 would also approximate to the LD_{50}. This constancy of lethality might mean, of course, that 48/80 exerts some additional lethal action not related to histamine, and not species-dependent. But no action of sufficient importance for this has yet been demonstrated. On the other hand, the constancy of lethal dose may reflect an important fact about histamine in the whole body; that the amount that can be mobilized in a whole animal (*i.e.*, the product of histamine content and histamine lability) is inverse to the sensitivity to histamine of some vital organ in the animal. Thus, comparing the rat and the guinea-pig, it is noticeable that guinea-pig tissues in general neither release histamine very readily nor contain much histamine, while the animal is amongst the most sensitive to histamine; on the other hand, the rat, with plenty of histamine in its skin and muscle and other tissues, from which it can be rather easily mobilized, is perhaps the most resistant of all to histamine.

X. OTHER SUBSTANCES RELEASED BY HISTAMINE LIBERATORS

1. Heparin. Besides histamine, it has already been noted that heparin can be mobilized, at least in the dog. This conclusion rested originally on the facts that the blood became incoagulable, that this coagulation defect could be overcome by toluidine blue, and that the plasma displayed, with toluidine blue, some measure of the metachromatic reaction (135). A complicating factor, of course, is the presence of the liberator itself in the plasma. Since histamine liberators, in general, form complexes with heparin (133, 135, 156) the amount of free heparin will be reduced, according to the solubility of the complex. Incoagulability of the blood in the dog is more striking with diaminodecane or peptone than with 48/80 (135, 170) and indeed with the latter it may be difficult to demonstrate (178). This may be related to the lower solubility of the 48/80-heparin complex compared to that with diaminodecane, so that after 48/80 less heparin circulates in the blood in active form than after diaminodecane. It may be mentioned here that complex formation between a liberator and a large molecular weight acid forms the basis of the antagonism by suramin to the liberating action of the therapeutic diamidines (97).

The discovery that histamine liberators act on mast cells provided an elegant explanation for the joint release of histamine and heparin. But it has also rendered more acute the problem of why no signs of systemic heparin release occur in

species other than the dog. We do not yet know whether the heparin in mast cells in the cat, for instance, is not mobilised, or does not escape into the circulation, or is freed in an inactive form.

2. Slow-reacting substance. Release of histamine and heparin does not complete the picture. It was also found that 48/80 caused the appearance in the plasma, usually after both histamine and heparin had reached their peak concentration, of one of the so-called "slow-reacting substances" (S.R.S.) identifiable by its producing a contraction of guinea-pig ileum in the presence of mepyramine, the contraction being of a characteristically sluggish onset and showing tachyphylaxis (170). It was interesting that 48/80 could antagonize the action of this S.R.S. on the gut, as well as the action of a number of other similar substances (170). S.R.S. has also been found in blood of animals shocked by anaphylaxis (15), polylysine (86), or thalassine, and appears after induction of the anaphylactic reaction even in saline-perfused lungs (29, 30). A substance of this kind is known to occur in the intestine (Substance P), and it will be remembered that 48/80 and other liberators in large doses, as well as anaphylaxis, cause haemorrhages in the upper intestine. It is possible that the appearance of S.R.S. in the plasma is associated with these haemorrhages, either being released by the accompanying tissue damage, or actually contributing in some way to their development.

3. 5-Hydroxytryptamine. It is now known that hydroxytryptamine (HT) is released after the injection of 48/80 into the perfused hindquarters of the rat (16). After the action of 48/80, the extent of reduction of HT in the skin parallels the degree of mast cell disruption and histamine depletion (165). It is suggested, therefore, that HT may also be present in the rat mast cells (although possibly elsewhere in addition). Reserpine is now known selectively to mobilize the HT from gut, brain, the blood platelets (32), and from the skin (17), without freeing the histamine and without disrupting mast cells. If a histamine liberator is applied to a reserpine-treated animal, histamine release occurs normally (17). If, now, an animal whose skin is low in HT is treated with 48/80 or egg white, histamine release occurs, but the typical oedema does not (166). Rowley and Benditt (13, 198) go further, and suggest that HT is the more important for oedema production. If HT and histamine are compared with respect to their ability to produce oedema of the skin of the rat foot by cutaneous injection, HT is roughly 200 times the more active; so that, although there is about $\frac{1}{25}$ as much HT in the skin as there is histamine, HT is present in more effective amounts. Further, if specific antagonists to oedema production by histamine and HT (mepyramine and dibenamine, respectively) are used in rats treated with 48/80, dextran, or ovomucoid, dibenamine has the more prominent effect. The best antagonism, however, which is virtually complete, is with a combination of the two antagonists. Parratt and West (167) have reached a similar conclusion, and state also that the oedema fluid in the rat contains detectable amounts of HT but not of histamine.

This work necessitates a re-examination of the actions of HT and tryptamine. It was noted earlier that HT was itself a histamine liberator. The fact that it can be released itself by the action of a liberator opens up the intriguing possibility

of an autocatalytic process being concerned. But this is probably not the case, since HT is not very active, since in concentrations producing oedema in rat skin no mast cell degranulation occurs (198) and since antihistamines have no antagonistic action to such oedema production by HT (198). Although tryptamine and HT were found about equally active as histamine-releasing agents on cat skin (80), HT is about ten times as active in producing oedema by local injection (198). It is probable, therefore, that, in the rat, anaphylactoid phenomena are mediated by the release of HT from mast cells, with histamine playing a supporting role. But this seems to be true only for the rat. Other species have much lower HT content in skin, and there is no evidence that HT is so strongly oedema-forming in other species. These new results furnish some insight into why the rat may have a peculiar reaction to substances such as dextran or ovomucoid; but they leave unexplained why the rat should have so much histamine in the skin, why the dog should also have a species-specific anaphylactoid response (to Tween 20 and polyvidone), and why different mechanisms of oedema production should occur in different species. An interesting problem, too, is the nature of the binding of HT in the mast cell; it is such that it may be released by reserpine, apparently so slowly that no local effects are produced, and without mast cell damage.

NOMENCLATURE

It is convenient at this point to comment on various names, carrying somewhat different connotations, which have been applied to drugs capable of mobilizing tissue histamine. The term "histamine liberator" was first applied to a wide group of bases which, administered into the blood stream of an animal, or injected intradermally, produced the reaction characteristic of histamine free in the tissues. Then, following an intensive study of further compounds on fragmented tissues *in vitro*, the term "histamine releaser" was coined. Most recently, it has been suggested (16) that those compounds able to act on mast cells should be called "mast cell depleters". It is usually sufficiently obvious what is intended when any one of these terms is used. There is, however, a distinction which it may be valuable to preserve, indicated in the earlier discussion of the difference between 48/80 and octylamine. There is, on the one hand, the group of bases, of which the original histamine liberators are typical, with which histamine release can be achieved, with roughly the same concentration of the drug, from the skin and muscle of a wide variety of species, either with isolated tissues, or by eliciting the characteristic syndrome of histamine liberation in the whole animal. To this group belong compound 48/80, stilbamidine, propamidine, d-tubocurarine, morphine, trimetaphan, compound L 1935, and many dibasic compounds. On the other hand, there is a more varied group, where activity may depend strongly on species (*e.g.*, Tween 20, dextran, polyvidone) or on the intactness or otherwise of the tissue (*e.g.*, octylamine), or where the other effects produced make it impossible to study the histamine-releasing process in the whole animal (*e.g.*, ammonia). It would be unfortunate if the nomenclature used were to imply that, for instance, 48/80, dextran, ammonia, and octylamine had equiva-

lent effects in the animal body, quite apart from the danger of implying a single histamine-releasing process where several different ones may well be operative. In this review, therefore, the term histamine release is used to refer to the activity of the whole range of drugs which can mobilize histamine, somehow, in some tissue; and within the group of histamine releasers the histamine liberators are distinguished for their more specific action. The term mast cell depleter has not been used, despite the attractiveness of a term which conveys some further information, partly because our knowledge of the subject is growing so rapidly that additional christenings are premature, partly because the term might convey the misleading ideas that these drugs will rid all mast cells not only of all their histamine but also of any other active substance within them; in fact, many mast cells seem refractory to the drugs and there are signs that in sensitive cells differential depletion of one, but not the other, amine can be achieved.

XI. ANTAGONISTS TO HISTAMINE RELEASE

Three main types of antagonism to the effects of histamine releasing drugs exist; physiological antagonism to histamine, competitive antagonism to histamine, and the prevention of the release itself. Of these the first two are undoubtedly the most effective, and remain the sheet-anchor of any therapy where histamine mobilization contributes to the pathological process concerned. But both methods are severely limited, either by the unwanted effects of sympathomimetic amines when administered for long periods, or by the failure of antihistamines to control all the effects of histamine-releasing agents. Considerable importance attaches, therefore, to any hint that an attack on the release process itself might be successful, an attack which would, of course, be theoretically the most satisfactory, by preventing the appearance of the peccant local hormone. Such an antagonism would provide, in addition, useful evidence about the nature of the release process. It must be admitted, however, that no general antagonist has emerged, and it is only possible to survey the substances for which a restricted effectiveness has been described.

1. Antihistamines. Antihistamines are only moderately effective against the cardiovascular effects of histamine liberators and rather inactive against their lethality (3, 56, 62, 130, 133, 139). Such a result speaks against the antihistamines used reducing the intensity of the release process. The urticarial symptoms may, of course, be considerably abated. Oedema in rats, oedema and itching in dogs, and the triple response or bluing response in man or guinea-pig can be nearly abolished by antihistamines (82, 101, 135, 144, 175); but these benefits must be attributed for the time being to an antagonism of the circulating histamine, rather than of the release process. There still appears to be uncertainty as to whether a capillary-constrictor action by antihistamine drugs contributes to their effects (46). Further, it is possible, in rats and cats, to deplete the tissues of histamine while protecting them from the severest effects of release with an antihistamine (82, 213). In addition, in a deliberate attempt to test this point, it was found that the gastric secretion, in the dog, produced by injection of a histamine liberator was not reduced but actually increased, by previous ad-

ministration of mepyramine (175). In experiments on the rat, histamine release and mast cell degranulation was not prevented by antihistamines (66, 198). Finally, it is worth noting that antihistamines, *in vitro*, although probably not *in vivo*, may release histamine themselves (6). There is, therefore, a substantial body of evidence that antihistamines in general cannot reduce the release process.

On the other hand, it has been stated that promethazine will reduce the intensity of the shock and the rise in plasma histamine and heparin due to peptone or atropine (35, but *cf.* 168); that mepyramine and chlorcyclazine will prevent the extrusion of granules by rat mast cells under the influence of 48/80 *in vitro* (161), and that mepyramine can prevent degranulation of rat mast cells by stilbamidine *in vivo*, without interfering with the uptake of stilbamidine by the mast cell (183). Promethazine is an exceptional compound in its ability to restore the permeability of capillaries, and is very effective, for instance, against the pulmonary oedema produced in rats by adrenaline. It is thus possible, bearing in mind that it is also a strong antagonist to many drugs other than histamine, that it possesses some activity depressing the release mechanism, and it deserves further attention. As regards the protection *in vitro* against mast cell disruption by mepyramine and cyclizine, it is possible that the membrane of the mast cell *in vitro* no longer bathed by plasma, but by Tyrode solution, changes its properties so that mepyramine can now protect it. But further experiments are needed to clear up some contradictions in this field.

2. Heparin. MacIntosh and Paton (135) could demonstrate for heparin only a modest antagonism to the action of histamine liberators in the whole animal. But an ability to interfere with the release of histamine from rabbit blood by dextran sulphate of high molecular weight was shown for heparin, and also for maltosetriosephosphate and low molecular weight dextran sulphate (99). A similar antagonism has been shown to the antigen-antibody reaction in rabbit blood (138). Heparin might, of course, exert an antagonism by forming an inactive complex with the releasing agent, as it is known to do with many liberators, but the concentrations concerned are probably too low for this to occur. Antagonism by means of complex formation has been shown, however, by suramin against the action of the diamidines (97).

3. Anaesthesia. MacIntosh and Paton (135) found that ether anaesthesia rendered the activity of propamidine and similar histamine liberators more easily antagonised by calcium. Wien had earlier described the latter antagonism (229). Systematic study of the influence of different anaesthetics on histamine release has not been made; but it is of interest that urethane and ether reduce the release of histamine from a variety of guinea-pig tissues sensitized to egg albumen (116), and urethane lessens the disruption of mast cells of the rat by 48/80 *in vitro* (114). In contrast, chloralose increases the defect in capillary permeability due to polyvinylpyrrolidone in the dog (103).

4. Metabolic inhibitors. Mongar and Schild (151) found that iodoacetate and similar substances did not prevent histamine release by octylamine or 48/80 from minced guinea-pig lung, but even increased it, although these agents prevented release in the anaphylactic reaction. They have used this contrast to

differentiate the two processes. On the other hand, Junqueira *et al.* (114) found that a number of metabolic inhibitors (SH-blocking compounds, dinitrophenol, arsenite) could prevent the disruption of rat mast cells by 48/80. Since different preparations and inhibitors were used by the two groups of workers, the apparent discrepancy may be resolvable. It is possible that cells undamaged by fragmentation of tissue release their histamine with the aid of metabolic processes, but that this link with metabolism is broken down by fragmentation.[8]

XII. REFRACTORINESS AND HISTAMINE DEPLETION OF TISSUES

The ineffectiveness of histamine liberators or releasing agents, when given again after a first fully effective dose, has been repeatedly recorded. The depressor effect, the rise in portal pressure, the increase in lymph flow, the flow of gastric juice, the signs of oedema, the bluing of the skin, the triple response, the systemic signs of alimentary disturbance, respiratory distress, or general prostration, have all been shown to be mitigated or abolished as administration is repeated.

An important element in the development of refractoriness to histamine release is the exhaustion of the releasable histamine. We owe the fullest study of this to Feldberg and Talesnik (82) who showed, in the rat, that very substantial depletion of skin, and distinct depletion of muscle, heart and uterus could be achieved, together with a progressive insensitivity to 48/80. The animal became at the same time resistant to egg white and to sensitization to light by haematoporphyrin. It is interesting, however, that, in the whole animal, it seems to be impossible to rid the skin of histamine entirely (31). This is in contrast to what can be achieved with isolated perfused skin, in which the histamine content can be reduced virtually to nil (77). The difference may point either to a continual replenishment, in the whole animal, of the depleted skin from histamine stores elsewhere in the body, or to a tissue response not seen in the isolated preparation. Riley and West (187, 188) have made the interesting observation that with long-continued administration of 48/80, the histamine content of rat subcutaneous tissue begins to rise again slowly, a process accompanied by the appearance of small dense new mast cells in close relation to the blood vessels, cells which now resist degranulation by 48/80. Such results seem to make it less likely that a full and sustained histamine depletion of any tissue will be achieved by means available at present.

A detailed study of depletion has also been described in the cat (213). In these experiments, it could be shown that skin (especially that of ears and face) could be deeply depleted, muscle somewhat less so, lungs, heart, and liver detectably,

[8] B. Högberg, G. Südow, I.-L. Thon and B. Uvnäs (The inhibitory action of a compound obtained from hip seeds (HSC) on the release of histamine and the disruption of mast cells produced by Compound 48/80 and extracts from jelly fish (*Cyanea capillata*) and eel worm of swine (*Ascaris lumbricoides*). Acta physiol. scand. **38**: 265–274, 1957) have shown that a polysaccharide material extracted from hip seeds could prevent both histamine release and mast cell disruption by several different procedures. This important observation has led the authors to consider the possibility that enzymic processes are involved in histamine release.

but the alimentary canal hardly at all, save for the mucosa of the corpus of the stomach.

But exhaustion of supplies of histamine hardly accounts for all the phenomena of refractoriness recorded. The major reduction of histamine content of the tissues is only achieved by repeated treatment with doses initially strongly active. Now, in the dog, it is found that a single injection of 48/80, which produces itching, oedema, and vigorous gastric secretion, can induce a refractory state lasting 1 to 2 days, such that the dose repeated 6 to 12 hours later is totally ineffective (175). It is improbable, judging from the rat experiments, that a reduction of histamine content of the tissues would be large enough to account either for this total insensitivity or for the rate of recovery from it. A contributory factor may be a simultaneous release of heparin; if heparin persists in the tissues for some time, it is possible that it takes up the histamine liberator, so reducing the amount available for histamine release. Alternatively, desensitization to histamine may occur. This is an important possibility, already demonstrated by Lewis in certain skin reactions, and recently described for the bluing reaction in the guinea-pig (144); this is improbable in the dog experiment quoted, since gastric secretion to an injection of 48/80 is depressed but not that to histamine. It is difficult, finally, to exclude other compensatory reactions of a local or general hormonal nature. It must be recognized, in short, that the administration of a histamine liberator to an animal leads not only to a reduction of histamine in particular tissues, but also to a sequence of poorly understood protective mechanisms.

XIII. EFFECTS OF HISTAMINE LIBERATORS IN MAN

The use of the therapeutic diamidines in the treatment of trypanosomiasis provided the first evidence, on any scale, of the effects of histamine release in man by substances of this type (118, 131, 208). Among the side actions of these drugs (stilbamidine, propamidine, pentamidine) were colic, fall in blood pressure, and severe itching generalized over the whole body. In some cases oedema of the face was noted. The reaction was progressively less intense with repeated doses. These compounds have now been replaced by other, less toxic ones.

Lecomte (122, 123) has studied in detail the reactions, in man, produced by two histamine liberators, cystamine and the substituted butylamine L 1935, with special reference to the analogies with so-called "nitritoid crises". With L 1935, 0.1 mg/kg led in a few minutes to severe itching in palms and face, flushing and tachycardia; the blood pressure was unaltered. A dose of 0.3 mg/kg produced more intense itching, intense warmth of the skin, first of face, then of trunk, with erythema of head and thorax. The blood pressure fell 20–40 mm, the heart accelerated and there was some headache. The highest dose used, 0.5 mg/kg, led to immediate intense general itching, generalised feeling of heat, and congestion of the face. The blood pressure fell 40–60 mm, with a firm but accelerated pulse, and severe headache. Five minutes later the blood pressure rose above normal, oedema of face and eyes appeared, the skin of the thorax and abdomen showed giant urticaria, and there was colic, nausea and acid vomit. These effects are only

seen if the injection is made rapidly by the intravenous route, and the response diminishes with repetition. Cystamine (200–400 mg) produced similar effects.

There is a curious analogy with the response to nitrites, which likewise can produce flushing of face and upper thorax, fall in blood pressure with tachycardia, and headache. But histamine release may lead also to itching, oedema, diarrhea, colic, bronchospasm, and acid secretion in the stomach. The term "nitritoid" does not do justice to the phenomena of histamine release in man, and is misleading as to aetiology and treatment. It should be replaced by a term such as "anaphylactoid". The response cannot be fully imitated by histamine infusion, for this does not produce either itching or oedema of the type seen after histamine release. Ability to release histamine, giving rise to reactions of this type, which resemble anaphylaxis but occur on the first exposure to a drug, has now been recognized for many substances in clinical use. The chance of histamine release occurring in practice depends, of course, on the dose of the drug given and is greatly increased if it is given rapidly by vein (122, 123). Of the substances given in Table I which are used clinically, the therapeutic amidines, d-tubocurarine, and trimetaphan are active in the whole animal in doses of about 0.5 mg/kg and produce weals or histamine release from skin in a concentration of about 0.1 mg/ml or a little less. Except for the other quaternary salts, the remaining compounds require concentrations of about 1 mg/ml to act, and a typical delayed depressor response has been shown only for morphine. The remaining quaternary salts, excluding d-tubocurarine, require about 10 mg/ml to produce a good weal, although detectable effects are seen with lower concentrations. (It must be emphasized that these estimates of potency are necessarily approximate, being summarized from a number of different workers.) It seems probable that direct histamine release is not likely to be a common complication of giving most of these drugs; but it also seems likely that in particular sensitive individuals many of these drugs may produce unwanted anaphylactoid reactions. Unfortunately it is often difficult to find unambiguous records of such reactions. The possibility that d-tubocurarine may cause bronchospasm by histamine release, during surgical anaesthesia, is a case in point; there are a number of suggestive reports, but the difficulty of distinguishing this situation from bronchospasm due to other causes has so far prevented any confident assessment. Sensitivity to morphine has been studied, and its lethal action in bronchial asthma may well be due to a conjunction of histamine-releasing action and a susceptible individual (78).

The correlation of animal with human experience makes it clear that man corresponds fairly closely to an animal such as the cat, not only in the general reaction to histamine release, but also in dosage required to produce it. Minor discrepancies exist, such as the observation that one of the analogues of laudexium is less active than d-tubocurarine on the cat blood pressure, but more active in producing a weal in human skin (43). But in general, experience in man with the amidines, cystamine, and L 1935 given systemically and with 48/80, the amidines, many muscle relaxants, and octylamine given intradermally, shows that the response of the cat blood pressure or of the perfused skin of the cat gives a reasonable quantitative guide to the likelihood of histamine release in the human.

Significance of itching after histamine release

Among the more striking results of the injection of histamine into human skin is the sensation of itching. All the histamine liberators tested in this way also cause itching. Although histamine given parenterally fails to have this action (227), liberators still produce itching after intravenous or intramuscular injection, with an intensity proportional to the dose given and to the other actions produced. There is no established explanation why parenterally injected histamine should not be pruritogenic. Since histamine so administered can cause the skin to flush, it can presumably reach the nerve endings concerned. The explanation may lie in the fact that after intradermal injection of histamine or after histamine release from the tissues there will be high *local* concentrations of histamine, whereas after parenterally injected histamine the nervous tissue concerned is exposed to a more or less uniform concentration of the drug. In a recent important analysis of the action of veratrine, Burns *et al.* (36) have shown that the ability of veratrine to produce repetitive discharge in striated muscle to a single shock depends on obtaining a concentration gradient of veratrine along the muscle surface; and that, as soon as the whole muscle is exposed to the same veratrine concentration, repetitive firing to single shocks ceases. They believe that the repetitive firing of veratrinized muscle, like that of the cerebral cortex, depends on the juxtaposition of excitable membranes one of which repolarizes more slowly than the other. The contrast between the effects of histamine systemically administered, on the one hand, and histamine intradermally injected or released by a liberator, on the other, suggests the operation of a similar mechanism, in which the response of sensory nerve endings to casual stimulation might be made repetitive (and hence pruritic) by the existence of histamine concentration gradients. If this were the case, it would, indeed, explain how the itching is usually a more transient phenomenon than the erythema and oedema accompanying local injection or release of histamine; for with lapse of time, local diffusion and carriage of histamine in the blood would steadily reduce the initial local concentration gradients, so that the whole of the sensitive area of particular nerve fibres would become exposed to histamine instead of only a limited part as at the start.

The ability of liberators to cause itching has prompted the suggestion that drugs or pathological states in which itching is prominent may involve histamine release; the itching of morphine addicts and of obstructive jaundice are cases in point. It has been shown that bile salts have detectable, though slight histamine-releasing action, best from the liver in dogs after portal or arterial injection (5, 200). It is also stated that, in experimental obstruction of the common bile duct, a rise in blood histamine occurs (4). On the other hand, in a group of patients with liver disease, no correlation between plasma histamine and itching could be found; and the observation was made that cortisone could lower the plasma histamine, although the itching persisted, and that testosterone could abolish the itching without changing the plasma histamine (145). As regards the action of morphine, Feldberg (69) has pointed out that it has a pruritogenic action of central origin. The appearance of itching after the administration of a drug which may release histamine is, therefore, not necessarily due to histamine re-

lease; but the likelihood of this is much increased if other signs of histamine release are also present.

XIV. THE MECHANISM OF HISTAMINE RELEASE

Until recent years theories of histamine release were focussed chiefly on the possibilities of lysis (by lysolecithin-like materials) or digestion (by protease) of cells or cell constituents. These ideas developed naturally from studies on the mode of action of venoms, proteases, and toxins. But with the appearance of a large number of simple bases, all capable of mobilizing histamine without causing gross tissue damage, some other theory, resting on a more specific chemical basis, became necessary. It must be remarked, of course, that by suggesting that histamine liberators do not involve gross tissue damage, it is not implied that therefore they produce no morphological change at all. In fact, it is now clear that they do have such an action. Degranulation of mast cells, together with a change in the adjacent mesenchyme, has been described (184); and although a few intradermal injections of 48/80 into the skin have no long-lasting effect, necrosis of skin after frequently repeated intradermal injection has been reported (14). But the effects of a histamine liberator, in general, can be regarded as being non-pathological in the same sense as, for instance, stroking of the skin so as to produce Lewis' triple response can be regarded as not resulting in gross tissue damage.

Two theories of histamine release may be fairly readily dismissed. The first is that histamine liberators act by antagonizing the histaminase of the body thus allowing histamine, involved in some rapid production and destruction, to accumulate. It is known that a number of liberators, especially the diamidines and diguanidines, are fairly effective antihistamines (21, 22, 129a, 233). Apart from some intrinsically unlikely features in this (such as the rapidity of the release process), there is a very poor correlation between the ability of histamine liberators to inhibit diamine oxidase and their histamine-releasing action. The discrepancy became striking with the appearance of the isonicotinic acid hydrazide derivatives, and aminoguanidine, capable of antagonising powerfully not only histaminase but also "histamine-metabolising enzyme No. 2" (202a, 204) yet lacking the actions of histamine liberators.

The second hypothesis is that the compounds in some way caused, or accelerated, decarboxylation of histidine in the body forming new histamine. MacIntosh and Paton (135) did not produce any direct evidence against this, although the speed of release, and the fact that the liberator became less effective with repeated dosage, made it improbable. Feldberg and Paton's observation (77) that the amount of histamine released corresponds to the amount lost from the tissues satisfactorily met this point.

A more serious theory is that histamine liberators activate proteolytic enzymes which then, in some way, mobilize histamine from the tissue. This theory was originally discussed by MacIntosh and Paton (135) in general terms; it was later put forward in more exact form in the suggestion (86) that the basic liberators might combine with heparin (which was assumed to be attached to a proteolytic

enzyme), thus freeing the enzyme to exert its normal action. In favour of such a theory were the analogies between peptone shock, trypsin shock, and that produced by histamine liberators, bearing in mind that peptone is known to activate fibrinolysin (222) and the fact that liberators also are now stated to be able to activate fibrinolysin (222). Perhaps the fact that a "slow reacting substance" (which might be of polypeptide nature) also appears in the plasma may be adduced as slight supporting evidence. However, there is no general agreement about the activation of proteolytic enzymes in histamine-releasing processes. The ineffectiveness of soybeantrypsin inhibitor in preventing histamine release in rabbit blood has already been mentioned (134). It is uncertain whether a process of proteolysis would give the requisite speed of action. Histamine liberators work well in Tyrode-perfused organs, so that any proteolytic system concerned would, of course, have to be located in the tissues, and not in the blood for which the protease activity is best established. That proteases exist in skin is known, but activation of them has only, so far, been described by procedures such as burning (10). Fibrinolysis in the plasma of cats treated with histamine liberators is not increased. Fibrinolysin is not an active histamine-releasing agent (134). When the proteolytic theory first appeared, in connection with anaphylactic reaction, it was believed possible that histamine might be bound to protein by some linkage similar to a peptide bond (193). But this must be reconsidered in view of our new information about the association of histamine and heparin in the mast cells. If histamine is not coupled in some way to proteins or peptides, the proteolytic theory of histamine release by organic bases becomes very complicated and unattractive.

The other main theory, canvassed by MacIntosh and Paton (135), was that histamine liberation involved a process of ionic exchange. This theory arises from the striking circumstance that all the liberators identified then consisted of simple basic substances. It remains generally true that the vast majority of liberators are of this type. This fact, together with the appearance in the blood of a tissue acid, heparin, as a result of histamine liberator action, made plausible the idea that histamine might be normally attached to some tissue acid and then freed by ionic exchange with the liberator, the freeing being accompanied by some disruption of the binding of the acid so that it too was available for diffusion into the circulation. The subsequent discovery by Riley and West (186) that histamine and heparin were, in fact, associated in the tissues, increases considerably the interest in this approach.

One would imagine, on this theory, that among bases it would be only those of a strength comparable to the strength of histamine, or stronger, which would be active. This was in fact the case. The point was strengthened by the observation that among the amino acids it was only those of marked basicity which could produce histamine release from cat perfused skin; amino acids of corresponding acidity were devoid of histamine-releasing action (60). The characteristics of the bases which are effective in producing release *in vivo* readily seem to be, in general, twofold: either they are dibasic (or polybasic) compounds where the two basic groups are separated by a predominantly hydrocarbon

moiety, or else they are benzamidine or phenylethylamine derivatives with a polar ring substituent remote from the basic group. In each case some structural analogy exists with the structure of histamine (viewing it either as a dibasic compound, as an amidine, or as an aromatic ethylamine). The idea that the histamine-releasing action should increase with increasing basicity of the compound must be modified, of course, when quaternary substances are concerned. Here the problem of entry of the compound into the cell becomes of importance, since, in general, quaternary salts only slowly penetrate cellular membranes. It was found, in fact, that decamethonium, the quaternary analogue of decamethylenediamine, was only slightly active, a result indicating that the activity of the compound depends not only on basicity but also on its ability to enter the cell. On the other hand, not all quaternary salts are of equal activity, and d-tubocurarine has proved considerably more effective as a histamine liberator than decamethonium or gallamine. The principal structural difference between the two drugs is the presence of a relatively complicated aromatic structure in d-tubocurarine against a simple aliphatic chain in decamethonium. One might suppose that the abundance of lipophilic material in d-tubocurarine goes some way to overcome the quaternary nature of two of its polar groups, enabling it to enter the cell somewhat better than a more or less purely quaternary molecule such as decamethonium. But these considerations of structure and action are at present suggestive only.

Additional evidence for the theory that liberators compete with histamine for a tissue acid has come from the observation that the histamine liberators are able to form complexes with various substances which might play the role of tissue acid (as well as with many carboxylic acids, *e.g.*, suramin, (97) and indeed any acid of large molecular weight). It is known that the therapeutic diamidines, 48/80, d-tubocurarine, and diaminodecane will all form precipitates with heparin and antagonize its anticoagulant action (133, 135, 156). Indeed, it is possible to use heparin to assay, for instance, the amount of 48/80 in a solution, by means of a turbidimetric method. Heparin is not the only naturally occurring substance with which such complexes can be formed. They can be formed even more readily with nucleic acids; and it was found that the concentration of liberators required to form a visible precipitate with the appropriate concentration of ribose nucleic acid (RNA) corresponded very closely with their relative histamine-liberating action (173). Further, ATP and some other tissue phosphates have recently been shown to have the ability to form a precipitate with histamine liberators (51). Accordingly, there are several possible types of tissue acid (heparin, nucleic acids, and ATP and glucose phosphates) which may serve as the "ion exchange resins" with which histamine is bound and from which release might take place. Binding of histamine by lecithin and cephalin has also been reported (129) but how this occurs is still obscure.

But there are a number of difficulties to be mentioned in connection with the simple theory in this form. In the first place, it is now clear that with the most active liberators, notably 48/80, more histamine can be released than liberator applied. Thus in an isolated perfused skin 1 μg 48/80 may give rise to 10 or 20 μg of histamine base (77, 79, 170). But this does not necessarily make an ionic

exchange theory impossible. Even in a system in which the histamine and 48/80 and resin came into steady equilibrium, it would be possible, given the appropriate dynamic constants of association and dissociation, for 48/80 to compete with more than its own weight of histamine. In addition, it must be remembered that such dynamic equilibrium does not exist in any of the conditions which we have been considering. Rather there are considerable diffusion gradients, opposite in direction for the two substances, liberator and histamine. Under such conditions it could happen, for instance, that histamine released was washed away while the liberator acted at another site.

A second difficulty has been proposed, that, if the activity of histamine liberators depends on their ability to compete with histamine for a histamine receptor, there should be a close parallelism between the histamine-liberating action and the activity on other histamine receptors. This neglects the well-known fact that the receptors involved in different actions of a drug often have different properties; thus the structural requirements for antagonizing acetylcholine at ganglionic, neuromuscular, and muscarinic sites are strikingly different. MacIntosh and Paton (135) remarked on the ability of some of the liberators to antagonize histaminase and also on their antihistamine action on the guinea-pig ileum (although this action is a non-specific one). Mongar and Schild (149) have extended this considerably and studied also the ability of these compounds to potentiate histamine, a reflection of their ability to antagonize histaminase. They found that there was a poor correlation between the antihistaminase action and histamine-releasing potency on minced lung, although there was a better correlation between histamine release and antihistamine action. They concluded provisionally from this work that histamine-releasing agents may work by a "toxic" action, and were able to show that the release produced on minced guinea-pig lung ran parallel to the ability to interfere with the motility of the paramaecium. However, their estimate of histamine-releasing action was obtained with the *in vitro* method already discussed, which yields different results from that in the whole animal; it may well be that their conclusion with this preparation is right, *viz.* that they were measuring a toxic action on cells. This explanation can not, however, be directly applied to the whole animal.

Finally, some difficulties arise when one enquires more exactly what would be the state of the histamine when linked with a tissue acid. Although the liberators form insoluble complexes with heparin, nucleic acids, or ATP quite readily, histamine does not do this so easily, and a gross precipitate is quite difficult to produce. This makes somewhat improbable the simplest model, a tissue acid carrying histamine precipitated on it. But the possibility remains of a complex in 'micellar' form, not precipitated. In such a case the histamine-acid complex would have to be sought in a watery phase rather than in solid granular material. The additional problem exists as to why the histamine is not rapidly eluted from the complex by the other cations in the tissues. It is easy to absorb histamine on to some cation-exchange resin, and to displace it with a histamine liberator; and to demonstrate a competition for the resin between histamine and the liberator (61). But the histamine under these conditions is also rapidly released by any sodium or potassium salt. If, therefore, ionic binding of histamine in the tissues

occurs, it must take place behind some cellular or intracellular barrier which either excludes other cations (so that histamine, and perhaps similar amines, form the bulk of the cationic content) or allows histamine entry and prevents its exit. An intracellular particle carrying a tissue acid within a lipoid membrane might indeed furnish automatically a mechanism of the last kind, if histamine entry depended on lipoid solubility; for, while histamine in the fluid surrounding the particle might exist as 0.1 % unionised, and enter the particle accordingly, the concentration of intra-particular unionised histamine, on which exit rate would depend, would be less by a factor of 10 for each unit by which the pH was lower than that of the surrounding tissue fluid. But such a differential between entry and exit rates would not exist for permanently charged particles (such as sodium or potassium ions). Accordingly, partly ionized bases, such as histamine, would accumulate.

Theoretical considerations of this sort illustrate the importance attaching to the evidence that mast cells or granules within mast cells are important histamine carriers. The ionic theory of histamine binding and release must, in fact, be considered chiefly in relation to mast cells rather than in relation to some hypothetical tissue ion exchange resin. The permeability of the limiting membrane of the mast cells will clearly be important for histamine release, as well as that of any membrane limiting intracellular histamine-containing particles. This means that to mobilize histamine, a drug injected into the blood stream may have to overcome two barriers, that limiting the cell, and that limiting the particle. In fractionated preparations, the barrier membranes of the intracellular particles are susceptible to lytic agents; saponin, bile salts, and other strongly detergent materials have all proved successful in breaking them down, and the surface activity of octylamine may also be significant in this direction. These facts tend to indicate a lytic theory of histamine release. But it is worth noting that in the whole animal such surface-active materials are much less effective; and amounts of detergent far more surface-active than typical histamine liberators, if injected into the whole animal, are almost inactive in releasing histamine. Bile salts were shown by Schachter (200) to release histamine from perfused skin, but 50 mg/kg intravenously in the whole animal actually lowered the plasma histamine. This general activity of detergents *in vitro* but not *in vivo* raises a suspicion that the conditions of a cell suspended in a saline solution, and no longer bathed in normal plasma, are radically different from those in the whole animal. It is, in fact, not unreasonable to suppose that the plasma should normally exert some protective influence, comparable to its ability to prevent sphering of red cells in saline; and that cells suspended in Tyrode solution, particularly if in their preparation maceration of the tissue has taken place, should have their permeability characteristics altered or should become more vulnerable to some lytic process. It is true that minced tissues, or fractionated cell constituents still appear to be able to retain the larger part of their histamine; but this by no means excludes the possibility that the properties of cell or particle membranes are altered. The difference between the results *in vitro* and in the whole animal would then provide an indication of the nature of this change.

A purely lytic theory faces three other difficulties. The first is that careful scrutiny of the mast cell under the influence of histamine liberators shows that it does not lose its cellular integrity. The cells remain intact, and may become the site of granular regeneration. Some cells are not affected at all. It is indeed puzzling to consider how such large masses of granules may be extruded from the cell, but major lysis of the membrane does not appear to be the cause. Secondly must be mentioned Norton's observation (160) that hypotonicity does not assist the action of 48/80. This is difficult to reconcile with any theory postulating a weakening of the mast cell membrane by the liberator. Finally, 48/80 does not release potassium from perfused cat skin, although histamine is released, as might be anticipated on a lytic theory; octylaminine, however, releases more potassium than histamine (173).

The principal facts to be explained may be summarized as follows: (a) that a large number of bases can release histamine in many species, and heparin in the dog, especially if they contain more than one basic group suitably spaced; they become more active with increasing basicity, but quaternization reduces activity in homologous series; (b) these bases can form complexes with tissue acids, and (for nucleic acid at least) many do so in proportion to histamine-liberating power; (c) surface-active materials may also release histamine, particularly with fragmented tissues; (d) compound 48/80 does not release potassium from skin, although octylamine does; and hypotonicity does not increase the effectiveness of 48/80 in degranulating mast cells; (e) in many tests the amount of histamine released is greater than the amount of 48/80 administered; (f) mast cells under the influence of a liberator concentrate the liberator, swell, and then discharge their granules without obvious damage to the cell membrane. An attractive means of explaining these facts is to suppose that liberators act as competing bases mobilizing histamine from a bound to diffusible and osmotically active form inside the cell, that the cell becomes, in consequence, swollen, and when sufficiently distended discharges its contents, including undissolved granules. Such a theory fits with the evidence for entry of liberators into the mast cell, and for action by ionic exchange action; it also leaves room for the possibility that surface-active materials might favour such a release by modifying the properties of the membrane. But our knowledge of the intimate physiology of the mast cell does not yet permit the formulation of a completely satisfactory hypothesis.

XV. PHYSIOLOGICAL CONCLUSIONS

1. The new-won ability to mobilize histamine selectively, and even to reduce the histamine content of some tissues over prolonged periods, has brought with it a certain amount of new physiological knowledge. It seems clear, for instance, that histamine is not essential for normal functioning of the skin. After recovery from the initial effects of the histamine mobilization, the nutrition, vascularity, and condition of the skin surface and hair appear normal. Further, sensation is normal, both in animals and man (14), under conditions when the histamine content must have been very greatly reduced. Whatever the purpose of histaminin the skin, therefore, it is not one concerned with its physiological maintenance or

required for normal sensation. A similar argument applies to striated muscle, to autonomic ganglia, and to nervous tissue, although with less force, since the proportion of mobilizable histamine is smaller in these structures than in skin. But with all of them significant falls in content may occur without detectable abnormality in their function. The same may be true for the histamine associated with the blood vessels, chiefly no doubt in the mast cells associated with them; for there is no feature of the depleted animal which points to any vascular defect attributable to the depletion *per se*.

2. We know, also, that we must distinguish various states of histamine in the body. A small amount is circulating free in the plasma and tissue spaces, although refinements of technique bring the upper limit of normal levels in the plasma lower and lower. There is also a reserve of labile histamine, mobilized by manipulation of the skin or by histamine-liberating agents. It seems clear that much, possibly most, of this labile histamine overlaps with that contained in mast cells; and that, within the mast cells, the histamine is not free in the cytoplasm but concentrated in mitochondrial granules, as foreshadowed by Trethewie (221). Not all the histamine in mast cells, however, can be freed by liberators; and it is still possible that there is histamine in the epidermis (not in mast cells) which liberators can mobilize. But, provisionally at least, one can assume that the physiology of labile histamine is closely linked to the physiology of the mast cells. We have, then, a new channel by which such influence as nutrition or endocrine activity may influence and control histamine-mediated reactions in the body. Finally, there is histamine in a firmly bound state, such as the condition in which it exists in the intestine, where its presence can be demonstrated only by maceration and extraction of the tissue.

Not the least important aspect of the studies on how histamine is bound in the tissues is the reawakening of interest in the possibility that physiology of the storage and mobilization of substances active in the body may depend primarily on a rather simple acid-base reaction between these substances and tissue acids. There is abundant, though relatively unexplored, scope for individual differentiation by the nature of the acid and base concerned and by the properties of the membrane retaining the reacting substances. Now that these specific chemical notions can be plausibly applied not only to histamine in mast cells, but also to adrenaline in the granules of the adrenal medulla (112), and to the action of chemotherapeutic drugs (162, 216), older work, such as Ehrlich's classical studies (58a) on dyes, their distribution and actions, assume a remarkable and extraordinarily modern freshness.

3. The study of tissues depleted of histamine throws some light on processes of histamine metabolism. The recovery of histamine content by such tissues is quite slow; implying that the daily formation of new histamine is a small fraction of the histamine content of the tissues. Recent experiments indicate that, in normal rat skin, the turnover of histamine is slow. Although no increased rate of histamine formation and binding could be demonstrated in depleted animals when histamine or histidine was injected intracutaneously or given by mouth, evidence of increased rate of binding by skin *incubated* with C^{14} histidine has now been

obtained (203a). If depleted tissues do, in the whole animal, make and bind histamine more quickly than normal tissues, then the normal rate of turnover must be strikingly slow. This is in strong contrast with, *e.g.*, the synthesis of acetylcholine in a sympathetic ganglion (from which an output equal to its total acetylcholine content can be obtained repeatedly at intervals of 30 min or less), or the suprarenal cortex (the turnover time of whose hormone content is a matter of seconds). The evidence, from this quarter, points to histamine representing, not a regular participant in the physiology of the body, but a strategic reserve, built up in preparation for responding to occasionally recurrent stress.

The ability to exhaust the histamine depots offers hope of developing a new therapeutic approach to the allergies. This approach is still blocked by the difficulty of producing, in the whole animal, a depletion so great as completely to prevent histamine participating in allergic responses, as well as by the other harmful actions of the histamine-releasing drugs, and by the development of resistance (at least in rats and mice) to their action. But this principle of depletion, whether of histamine or of other local hormones, is an important one calling for much more study.

4. The new experience with histamine release has made intelligible the large collection of clinical phenomena, best called anaphylactoid but often classified as nitritoid. Many of them can now be attributed to a simple histamine-liberating action by a drug, independent of previous sensitization; their treatment is correspondingly obvious, antihistamines and adrenaline or other sympathomimetic amines. It also follows, both from a comparison of clinical experience and that with animals, and from the tests with known histamine liberators in man, that histamine release in man corresponds well with that in the lower animals.

5. Finally, the method of histamine release has been exploited to study the role of histamine in the mediation of certain physiological effects.

(a) *Gastric secretion.* It is now known that gastrin, as normally prepared, can release histamine from skin, muscle, and the mucosa of the corpus of the stomach. But its releasing action is relatively slight and probably insufficient to account for the full secretagogue effect of gastrin on the stomach.

(b) *Intestinal motility.* Although histamine is not very readily mobilized in large quantities from the intestine, small amounts may be fairly easily released. This makes it possible that locally formed histamine liberators might be important in controlling intestinal activity. There is, as yet, no clear evidence, however, of such liberators occurring naturally at this site, although some of the products of digestion of proteins deserve consideration for such a role.

(c) *The relation of histamine liberators to anaphylaxis.* It is still a striking and important fact that, by the injection of so simple a compound as diaminodecane (for example) into a dog, the salient features of anaphylaxis can be reproduced; these include a fall in blood pressure with peripheral circulatory failure, engorgement of the liver with rise in portal pressure and the appearance of histamine and heparin in the blood. With 48/80, the appearance of a slow-reacting substance, known to appear in anaphylaxis, can also be shown. Further, in work on a number of sensitized tissues *in vitro*, Mongar and Schild were able to show that the pro-

portion of histamine released, which varied from 2% to 40% according to the tissue, was much the same whether antigen, 48/80, or d-tubocurarine was applied. It is known that mast cell degranulation can be produced both by anaphylaxis and by histamine liberators. It is also established that treatment of liver or lung from a sensitized animal with the specific antigen reduces the histamine content of the intracellular particles of the tissues concerned; since the histamine in these particles is accessible to chemical liberators, it can be presumed that the histamine mobilized by the antigen-antibody reaction and by liberators is, at least in part, common. Finally, treatment with histamine liberators can induce desensitization to allergic reactions, just as can the previous eliciting of the allergic reaction itself (67, 124).

But there are many differences between the two processes. The effect of a histamine liberator in a species like the guinea-pig, where bronchospasm is hard to produce, cannot be regarded as resembling the anaphylactic reaction in this animal, for which bronchospasm is the most prominent and regular component. In the rabbit, too, antigen is more effective than 48/80, especially on the liver where 48/80 is ineffective (201). In the dog, antihistamines can prevent the portal hypertension produced by 48/80 but not that due to antigen (182). If the course of histamine release in perfused skin after 48/80 is compared to that after antigen given to skin from a sensitized animal, it is much less sluggish than with the latter, and successive doses of antigen produce increasing effects in a way not seen with 48/80. On tissues such as the intestine, *in vitro*, it has been noted that although 48/80 pretreatment of lung, aorta, or uterus prevented further histamine release by specific antigen, on the intestine this pretreatment made antigen more effective; and that previous treatment with 48/80 did not prevent the *contraction* of the uterus, or gut in response to antigen. Further, histamine liberators in sufficient dose will release histamine from intracellular particles, although antigen will not, and passive sensitization cannot be conferred on the particles. Finally, a number of metabolic inhibitors are now known to be able to depress the antigen-antibody reaction, although they do not reduce, and may intensify, the action of histamine-releasing agents.

The analogies mentioned have naturally prompted the idea that anaphylaxis may involve the formation of a histamine liberator in the tissues, to which the subsequent histamine and heparin release would be due. Such a liberator would be expected to be of basic nature, perhaps a basic polypeptide. From what has been said about the differences between the two processes, the formation of a liberator could only be supposed to occur after the antigen-antibody reaction had taken place. This brings back the old theory of the antigen-antibody reaction, that it gave rise to a proteolysis in the blood, and the formation there of an active substance responsible for the anaphylactic phenomena. In this form, the theory is now discredited. But it is still possible that the proteolytic product is formed at the cellular level, and acts in, or close to, the cell concerned, without passing into the blood stream. Certainly, it has not yet been possible to demonstrate the appearance, after an antigen-antibody reaction, of any histamine-liberating substance, so that a participation of proteolytic events in this way is purely specu-

lative. But the writer has found that some of the products of protein hydrolysis, prepared by the method of the Vaughans (224) (the so-called "protein poison") are, in fact, far more active in releasing histamine than ordinary peptones. The possibility, therefore, of interpreting the imitation by simple bases of anaphylaxis in some such way ought not yet to be abandoned.

If we review the evidence we now have about histamine and its release: the manner, discussed elsewhere (172), in which it is concentrated particularly in those tissues which represent an interface with the outside world; the fact that reduction of its content in the tissues appears to interfere, *per se*, very little with normal physiological processes; the indication that its formation in the body points to a "reserve" function rather than continuous participation in bodily processes; and the way that the phenomena accompanying its release carry us immediately into the field of pathology: the suspicion arises that to search for a normal *physiological* function for histamine may be an error, and that its true role remains for pathological physiology to unravel.

REFERENCES

1. Adam, H. M., Card, W. I., Riddell, M. J., Roberts, M. and Strong, J. A.: The effect of intravenous infusions of histamine on the urinary histamine and on gastric secretion in man. Brit. J. Pharmacol. **9:** 62–67, 1954.
2. Alam, M., Anrep, G. V., Barsoum, G. S., Talaat, M. and Wieninger, E.: Liberation of histamine from the skeletal muscle by curare. J. Physiol. **95:** 148–158, 1939.
3. Alles, G. A., Ellis, C. H. and Redemann, M. A.: Comparative physiological actions of some alkane-diamines. J. Pharmacol. **107:** 332–343, 1953.
4. Anrep, G. V. and Barsoum, G. S.: Blood histamine in experimental obstruction of the common bile duct. J. Physiol. **120:** 427–430, 1953.
5. Anrep, G. V., Barsoum, G. S. and Talaat, M.: Release of histamine by the liver. J. Physiol. **120:** 419–426, 1953.
6. Arunlakshana, O.: Histamine release by antihistamines. J. Physiol. **119:** 47P–48P, 1953.
7. Bainbridge, F. A. and Trevan, J. W.: Some actions of adrenaline upon the liver. J. Physiol. **51:** 460–468, 1917.
8. Baltzly, R., Buck, J. S., de Beer, E. J. and Webb, F. J.: A family of long-acting depressors. J. Amer. chem. Soc. **71:** 1301–1305, 1949.
9. Barger, G. and Dale, H. H.: Chemical structure and sympathomimetic action of amines. J. Physiol. **41:** 19–59, 1910.
10. Beloff, A. and Peters, R. A.: Observations upon thermal burns: the influence of moderate temperature burns upon proteinase of skin. J. Physiol. **103:** 461–476, 1945.
11. Benditt, E. P., Bader, S., Arase, M., Corley, C. and Lam, K. B.: Relationship of mast cells and histamine to mechanism of edema production by ovomucoid in rats. Fed. Proc. **13:** 422–423, 1954.
12. Benditt, E. P., Wong, R. L., Arase, M. and Roeper, E.: 5-Hydroxytryptamine in mast cells. Proc. Soc. exp. Biol., N. Y. **90:** 303–304, 1955.
13. Benditt, E. P. and Rowley, D. A.: Antagonism of 5-hydroxytryptamine by chlorpromazine. Science **123:** 24, 1956.
14. Benjamin, F. B.: Effect of histamine depletion on cutaneous pain threshold in man. Fed. Proc. **13:** 11, 1954.
15. Beraldo, W. T.: Formation of bradykinin in anaphylactic and peptone shock. Amer. J. Physiol. **163:** 283–289, 1950.
16. Bhattacharya, B. K. and Lewis, G. P.: The release of 5-hydroxytryptamine by histamine-liberators. Brit. J. Pharmacol. **11:** 202–208, 1956.

17. Bhattacharya, B. K. and Lewis, G. P.: Comparison of the effect of reserpine and 48/80 on the histamine and 5-hydroxytryptamine in mast cells of rats. J. Physiol. **133:** 10P–11P, 1956.
18. Billings, F. T. and Maegraith, B. G.: Chemical changes in tissues following obstruction of the blood supply. A. J. exp. Physiol. **27:** 249–269, 1937.
19. Bischoff, F., Sahyun, M. and Long, M. L.: Guanidine structure and hypoglycemia. J. biol. Chem. **81:** 325–349, 1929.
20. Blacket, R. B., Pickering, G. W. and Wilson, G.: The effects of prolonged infusions of noradrenaline and adrenaline on the arterial pressure of the rabbit. Clin. Sci. **9:** 247–257, 1950.
21. Blaschko, H. and Duthie, R.: Diamidines as inhibitors of enzyme action. Biochem. J. **38:** xxv, 1944.
22. Blaschko, H. and Duthie, R.: The inhibition of amine oxidase by amidines. Biochem. J. **39:** 347–350, 1945.
23. Bodman, R. I.: Evaluation of two synthetic curarizing agents in conscious volunteers. Brit. J. Pharmacol. **7:** 409–416, 1952.
24. Bodo, R. and Marks, H. P.: The relation of synthalin to carbohydrate metabolism. J. Physiol. **65:** 83–99, 1928.
25. Bowden, K., Brown, B. G. and Batty, J. E.: 5-Hydroxytryptamine: its occurrence in cowhage. Nature, Lond. **174:** 925–926, 1954.
26. Briot, M. and Halpern, B. N.: Symptomatologie et pathogénie du syndrome oedémateux produit par le dextran chez le rat. C. R. Soc. Biol., Paris **146:** 15–20, 1952.
27. Briot, M. and Halpern, B. N.: Action inhibitrice de la prométhazine (phénergan) et de l'adrénaline sur l'oedème du rat par le dextran. C. R. Soc. Biol., Paris **146:** 21–24, 1952.
28. Broadbent, J. L.: Observations on itching produced by cowhage, and on the part played by histamine as a mediator of the itch sensation. Brit. J. Pharmacol. **8:** 263–270, 1953.
29. Brocklehurst, W. E.: Occurrence of an unidentified substance during anaphylactic shock in cavy lung. J. Physiol. **120:** 16P–17P, 1953.
30. Brocklehurst, W. E.: Response of the cavy ileum to "SRS-A" from lung of man and of cavy. J. Physiol. **128:** 1P, 1955.
31. Brocklehurst, W. E., Humphrey, J. H. and Perry, W. L. M.: The role of histamine in cutaneous antigen-antibody reactions in the rat. J. Physiol. **129:** 205–224, 1955.
32. Brodie, B. B., Pletscher, A. and Shore, P. A.: Evidence that serotonin has a role in brain function. Science **122:** 968, 1955.
33. Bülbring, E. and Burn, J. H.: An action of adrenalin on transmission in sympathetic ganglia, which may play a part in shock. J. Physiol. **101:** 289–303, 1942.
34. Burstein, M. and Parrot, J. L.: Élévation de l'histaminémie chez le chien à la suite de l'injection d'une forte dose d'atropine. C. R. Soc. Biol., Paris **143:** 251–255, 1949.
35. Burstein, M. and Parrot, J. L.: Action du N-(β-diméthylamino-β-méthyl-éthyl)-phénothiazine (3277 R.P.) sur la libération d'héparine au cours du choc atropinique et du choc peptonique. C. R. Soc. Biol., Paris **143:** 1556–1559, 1949.
36. Burns, B. D., Frank, G. B. and Salmoiraghi, G.: The mechanism of after-discharges caused by veratrine in frog's skeletal muscles. Brit. J. Pharmacol. **10:** 363–370, 1955.
37. Bushby, S. R. M. and Green, A. F.: The release of histamine by polymyxin B and polymyxin E. Brit. J. Pharmacol. **10:** 215–219, 1955.
38. Callow, R. K. and Work, T. S.: Antibiotic peptides from Bacillus licheniformis, Licheniformins A, B, and C. Biochem. J. **51:** 558–569, 1952.
39. Cameron, G. R. and Sheikh, A. H.: The experimental production of pulmonary oedema with ammonium salts, together with a classification of lung oedemas. J. Path. Bact. **63:** 609–617, 1951.
40. Carslaw, H. S. and Jaeger, J. C.: Operational methods in applied mathematics. Oxford University Press, London 1947.

41. CASS, R., HEAD, K. W., RILEY, J. F., STROUD, S. W. AND WEST, G. B.: Heparin and histamine in mast cell tumors. J. Physiol. **125:** 47P, 1954.
42. CLARK, W. G. AND MACKAY, E. M.: Effect of l-epinephrine and l-arterenol on egg white edema in the rat. Proc. Soc. exp. Biol., N. Y. **71:** 86–87, 1949.
43. COLLIER, H. O. J.: Release of histamine—Clinical considerations. In: Ciba Foundation Symposium on Histamine, pp. 155–159. Churchill, London, 1956.
44. COLLIER, H. O. J. AND MACAULEY, B.: The pharmacological properties of "Laudolissin"—a long-acting curarizing agent. Brit. J. Pharmacol. **7:** 398–408, 1952.
45. COMROE, J. H. AND DRIPPS, R. D.: The histamine-like action of curare and tubocurarine injected intracutaneously and intra-arterially in man. Anesthesiology **7:** 260–262, 1946.
46. CONARD, V.: Action des antihistaminiques de synthèse sur la circulation capillaire au niveau de péritoine chez le rat. C. R. Soc. Biol., Paris **145:** 1875–1876, 1951.
47. COPENHAVER, J. H., JR., NAGLER, M. E. AND GOTH, A.: Intracellular distribution of histamine. J. Pharmacol. **109:** 401–406, 1953.
48. DALE, H. H.: Antihistamine substances. Brit. med. J. **2:** 281–283, 1948.
49. BURN, J. H. AND DALE, H. H.: The vaso-dilator action of histamine, and its physiological significance. J. Physiol. **61:** 185–214, 1926.
50. DALE, H. H. AND RICHARDS, A. N.: The depressor (vaso-dilator) action of adrenaline. J. Physiol. **63:** 201–210, 1927.
51. DARLOW, G.: Reaction between histamine liberators and phosphates. J. Physiol. **131:** 13P–14P, 1955.
52. DEKANSKI, J.: The effect of cutaneous burns on histamine in mice. J. Physiol. **104:** 151–160, 1945.
53. DEKANSKI, J.: The effect of severe burns and some protein-precipitants on skin-histamine in cats. J. Physiol. **106:** 33–41, 1947.
54. DEKANSKI, J.: The effect of protein hydrolysates (Leucotaxine) on skin-histamine in cats. J. Physiol. **108:** 233–245, 1948.
55. DEUTSCH, S. AND LOEW, E. R.: Failure of antihistamine drugs to antagonize lethal effects of histamine in mice. Fed. Proc. **13:** 348, 1954.
56. DEWS, P. B., WNUCK, A. L., FANELLI, R. V., LIGHT, A. E., TALLABEN, J. A., NORTON, S., ELLIS, C. H. AND DE BEER, E. J.: The pharmacology of No. 48–80, a long-acting vasodepressor drug. J. Pharmacol. **107:** 1–11, 1953.
D'IORIO, A. AND EADE, N. R.: See 112.
57. DOUGLAS, W. W. AND PATON, W. D. M.: The mechanisms of motor end-plate depolarization due to a cholinesterase-inhibiting drug. J. Physiol. **124:** 325–344, 1954.
58. EDLUND, T., LOFGREN, B. AND VÄLI, L.: Toxicity of dextran in rats. Nature, Lond. **170:** 125, 1952.
58a. EHRLICH, P.: The Collected Papers of Paul Ehrlich (compiled by F. Himmelweit and M. Marquardt, under the editorial direction of Sir Henry Dale). Pergamon Press, London and New York 1956.
59. EICHLER, O. AND BARFUSS, F.: Untersuchungen über den Histamingehalt des Blutes bei Infusion von Adrenalin und Histamin. Überlegungen nach dem Le Chatelierschen Prinzip. Arch. exp. Path. Pharmak. **195:** 245–257, 1940.
60. ELDRIDGE, E. AND PATON, W. D. M.: The release of histamine from cat's isolated perfused skin by amino-acids. J. Physiol. **124:** 27P–28P, 1954.
61. ELDRIDGE, E. AND PATON, W. D. M.: Unpublished observations.
62. ELLIS, C. H., VAN HARREVELD, A. AND WOLFGRAM, F. J.: A study of the haemodynamic actions of histamine and 1,10-diaminodecane in cats. Arch. int. Pharmacodyn. **97:** 287–298, 1954.
63. ERLANGER, J. AND GASSER, H. S.: Studies in secondary traumatic shock. III. Circulatory failure due to adrenaline. Amer. J. Physiol. **49:** 345–376, 1919.
64. EVANS, A. G. J., NASMYTH, P. A. AND STEWART, H. C.: The fall of blood pressure caused by intravenous morphine in the rat and the cat. Brit. J. Pharmacol. 7: 542–552, 1952.

65. FASSETT, D. W. AND HJORT, A. M.: Some tetrahydroisoquinolines. II. Their action on blood pressure, respiration and smooth muscle. J. Pharmacol. **63:** 253–271, 1938.
66. FAWCETT, D. W.: Cytological and pharmacological observations on the release of histamine by mast cells. J. exp. Med. **100:** 217–224, 1954.
67. FEINBERG, S. M., FEINBERG, A. R., REBHUN, J. AND MALKIEL, S.: Liberation and depletion of histamine from human skin. Comparison of effects of specific antigens and a new histamine liberator, compound 48/80. Quart. Bull. North W. Univ. med. Sch. **28:** 246–251, 1954.
68. FELDBERG, W.: Histamine and anaphylaxis. Annu. Rev. Physiol. **3:** 671–694, 1941.
69. FELDBERG, W.: On some physiological aspects of histamine. J. Pharm. Lond. **6:** 281–301, 1954.
70. FELDBERG, W.: Distribution of histamine in the body. In: Ciba Foundation Symposium on Histamine, pp. 4–13. Churchill, London 1956.
71. FELDBERG, W. AND GREENGAARD, P.: Release of histamine from the perfused sciatic nerve by 48/80. J. Physiol. **133:** 63P–64P, 1956.
72. FELDBERG, W. AND HOLMES, B.: The effect of muscular contractions and of curarin on acid gastric secretion in cats. J. Physiol. **99:** 3P–4P, 1941.
73. FELDBERG, W. AND LECOMTE, J.: Release of histamine by a substituted butylamine (L 1935): comparison with compound 48/80. Brit. J. Pharmacol. **10:** 254–259, 1955.
74. FELDBERG, W. AND LOESER, A. A.: Histamine content of human skin in different clinical disorders. J. Physiol. **126:** 286–292, 1954.
75. FELDBERG, W. AND MILES, A. A.: Regional variations of increased permeability of skin capillaries induced by histamine liberator and their relation to the histamine content of the skin. J. Physiol. **120:** 205–213, 1953.
76. FELDBERG, W. AND MONGAR, J. L.: Comparison of histamine release by compound 48/80 and octylamine in perfused tissues. Brit. J. Pharmacol. **9:** 197–201, 1954.
77. FELDBERG, W. AND PATON, W. D. M.: Release of histamine from skin and muscle in the cat by opium alkaloids and other histamine liberators. J. Physiol. **114:** 490–509, 1951.
78. FELDBERG, W., PATON, W. D. M., NASMYTH, P. A. AND STEWART, H. C.: Morphine in acute chest infections. Brit. med. J. **1:** 1199, 1950.
79. FELDBERG, W. AND SCHACHTER, M.: Histamine release by horse serum from skin of the sensitized dog and the non-sensitized cat. J. Physiol. **118:** 124–134, 1952.
80. FELDBERG, W. AND SMITH, A. N.: Release of histamine by tryptamine and 5-hydroxytryptamine. Brit. J. Pharmacol. **8:** 406–411, 1953.
81. FELDBERG, W. AND SMITH, A. N.: The role of histamine release for the motor effects of histamine liberators on the guinea-pig's ileum preparation. J. Physiol. **124:** 219–233, 1954.
82. FELDBERG, W. AND TALESNIK, J.: Reduction of tissue histamine by compound 48/80. J. Physiol. **120:** 550–568, 1953.
83. FREEMAN, N. E., FREEDMAN, H. AND MILLER, C. C.: The production of shock by the prolonged continuous injection of adrenalin in unanaesthetized dogs. Amer. J. Physiol. **131:** 545–553, 1941.
84. FREEMAN, L., MARX, L. AND MARX, W.: Tissue heparin and mast cell in rats and rabbits. Fed. Proc. **15:** 255–256, 1956.
85. FULLER, A. T.: Antibacterial action and chemical constitution in long-chain aliphatic bases. Biochem. J. **36:** 548–558, 1942.
86. GARCIA AROCHA, H., ASHWIN, J. G. AND GROSSBERG, A. L.: Release of histamine by protamine and polylysine. Abstr. Comm. XIX Internat. Physiol. Congress, Montreal 1953, pp. 378–379.
87. GERTNER, S. B.: The effect of compound 48/80 on ganglionic transmission. Brit. J. Pharmacol. **10:** 103–109, 1955.
88. GOFFART, M. AND DELLA BELLA, D.: Action de la cystéamine et de la cystamine sur la jonction neuro-musculaire. Arch. int. Physiol. **62:** 455–475, 1954.

89. GRAHAM, H. T., LOWRY, O. H., WAHL, N. AND PRIEBAT, M. K.: Localization of tissue histamine in mast cells. Abstr. Comm. XIX Internat. Physiol. Congress, Montreal 1953, pp. 404–405.
90. GRAHAM, H. T., LOWRY, O. H., WAHL, N. AND PRIEBAT, M. K.: Mast cells as sources of tissue histamine. J. exp. Med. **102:** 307–318, 1955.
91. GRAY, J. A. B. AND PATON, W. D. M.: The circulation time in the cat, studied by a conductivity method. J. Physiol. **110:** 173–193, 1949.
92. GREEN, D. M., JOHNSON, D., LOBB, A. AND CUSICK, G.: The effects of adrenalin in normal and hypertensive patients in relation to the mechanism of sustained pressure elevations. J. Lab. clin. Med. **33:** 332–346, 1948.
93. GROB, D., LILIENTHAL, J. L. AND HARVEY, A. M.: On certain vascular effects of curare in man: the "histamine" reaction. Johns Hopk. Hosp. Bull. **80:** 299–322, 1947.
94. GROSS, F.: Zur Auslösung und Beeinflussung der Oedembildung nach Injektion von Hühnereiweiss an der Ratte. Arch. exp. Path. Pharmak. **211:** 421–426, 1950.
95. GROSSBERG, A. L. AND GARCIA AROCHA, H.: Histamine liberation in vitro and mode of binding of histamine in tissues. Science **120:** 762–763, 1954.
96. GUGGENHEIM, M.: Die biogenen Amine. Karger, Basel 1951, 4th ed.
97. GUIMARAES, J. L. AND LOURIE, E. M.: The inhibition of some pharmacological actions of pentamidine by suramine. Brit. J. Pharmacol. **6:** 514–530, 1951.
98. HAGEN, P.: The intracellular distribution of histamine in dog's liver. Brit. J. Pharmacol. **9:** 100–102, 1954.
99. HAINING, C. G.: Histamine release in rabbit blood by dextran and dextran sulphate. Brit. J. Pharmacol. **10:** 87–94, 1955.
100. HALPERN, B. N.: Histamine release by long chain molecules. In: Ciba Foundation Symposium on Histamine, pp. 92–123. Churchill, London 1956.
100a. HALPERN, B. N. AND BRIOT, M.: Méchanisme histaminique de l'action de la polyvinylpyrrolidone chez le chien. C. R. Soc. Biol., Paris **147:** 643–647, 1953.
101. HALPERN, B. N. AND BRIOT, M.: Étude pathogénique et thérapeutique du syndrome oedèmateux provoqué chez le rat par l'ovalbumine. Arch. int. Pharmacodyn. **82:** 247–296, 1950.
102. HALPERN, B. N. AND BRIOT, M.: Libération d'histamine par la peau de rat sous l'effet du contact avec le dextran, *in vitro*. C. R. Soc. Biol., Paris **146:** 1552–1555, 1952.
103. HALPERN, B. N., MUSSO, E. AND NEVEU, T.: Action of the histamine-releaser polyvinylpyrrolidone on capillary permeability in dogs. Brit. J. Pharmacol. **10:** 223–229, 1955.
104. HARRIS, K. E.: Observations upon a histamine-like substance in skin extracts. Heart **14:** 161–176, 1927.
105. HAUSMANN, W. AND CRAIG, L. C.: Polymyxin B_1. Fractionation, molecular weight determination, and amino acid and fatty acid composition. J. Amer. chem. Soc. **76:** 4892–4896, 1954.
106. HAWKING, F. AND SMILES, J.: The distribution of 4:4'-diamidino stilbene in trypanosomes and mice as shown by fluorescence. Ann. trop. Med. Parasit. **35:** 45–52, 1941.
107. HEIDENHAIN, R.: Versuche und Fragen zur Lehre von der Lymphbildung. Pflüg. Arch. ges. Physiol. **49:** 209–301, 1891.
108. HERXHEIMER, H. AND PATON, W. D. M.: Unpublished.
109. HERXHEIMER, H.: The bronchial reaction of guinea-pigs to 5-hydroxytryptamine (serotonin). J. Physiol. **120:** 65P–66P, 1953.
110. HUMPHREY, J. H. AND JAQUES, R.: The histamine and serotonin content of the platelets and polymorphonuclear leucocytes of various species. J. Physiol. **124:** 305–310, 1954.
111. HUMPHREY, J. H. AND JAQUES, R.: The release of histamine and 5-hydroxytryptamine (serotonin) from platelets by antigen-antibody reactions (*in vitro*). J. Physiol. **128:** 9–27, 1955.

112. D'Iorio, A. and Eade, N. R.: Catechol amines and adenosinetriphosphate (ATP) in the suprarenal gland of the rabbit. J. Physiol. **133:** 17P–19P, 1956.
113. Jaques, R. and Schachter, M.: A sea anemone extract (thalassine) which liberates histamine and a slow contracting substance. Brit. J. Pharmacol. **9:** 49–52, 1954.
114. Junqueira, L. C. U. and Beiguelman, B.: Studies on the *in vitro* action of compound 48–80, a formaldehyde condensation product, on rat mast-cells. Tex. Rep. Biol. Med. **13:** 69–75, 1955.
115. Kamijo, K. and Koelle, G. B.: Cardiovascular and oxytocic actions of a series of quinoline derivatives. J. Pharmacol. **112:** 444–461, 1954.
116. Katz, G.: The action of anesthesia on the histamine release in anaphylactic shock. Amer. J. Physiol. **129:** 735–743, 1940.
117. Katz, G.: Histamine release from blood cells in anaphylaxis in vitro. Science **91:** 221, 1940.
118. Kirk, R. and Henry, A. J.: Observations on the toxicity of stilbamidine. Ann. trop. Med. Parasit. **38:** 99–118, 1944.
119. Krantz, J. C., Carr, C. J., Bird, J. G. and Cook, S.: Sugar alcohols. XXVI. Pharmacodynamic studies of polyoxyalkylene derivatives of hexitol anhydride partial fatty acid esters. J. Pharmacol. **93:** 188–195, 1948.
120. Kwiatkowski, H.: Histamine in nervous tissue. J. Physiol. **102:** 32–41, 1943.
121. Landmesser, C. M.: A study of the bronchoconstrictor and hypotensive actions of curarizing drugs. Anesthesiology **8:** 506–523, 1947.
122. Lecomte, J.: Libération de l'histamine endogène chez l'homme. Arch. int. Pharmacodyn. **101:** 375–376, 1955.
123. Lecomte, J.: Sur la pathogénie du choc nitritoïde bénin. Arch. int. Pharmacodyn. **92:** 241–251, 1952/53.
123a. Lecomte, J.: Propriétés antihistaminiques des dérivés décarboxylés de la cystéine (cystéamine et cystamine). Arch. int. Physiol. **63:** 291–304, 1955.
124. Lecomte, J. and Borensztajn, C.: Libérateurs d'histamine et intradermo-réaction tuberculinique chez l'homme. Acta allerg., Kbh. **6:** 89–95, 1953.
125. Léger, J. and Masson, G. M. C.: Studies on eggwhite sensitivity in the rat. Influence of the endocrine glands. Ann. Allergy **6:** 131–143, 1948.
126. Léger, J., Masson, G. and Prado, J. L.: Hypersensitivity to egg white in the rat. Proc. Soc. exp. Biol., N. Y. **64:** 366–370, 1947.
127. Leitch, J. L. and Haley, T. J.: Effect of 48/80 and X-irradiation on urinary histamine in the rat. Fed. Proc. **14:** 363, 1955.
128. Lewis, T.: The blood vessels of the human skin and their responses. Shaw and Son, London 1927.
129. Lindahl, K. M.: The binding of histamine to phosphatides. Acta physiol. scand. **35:** 146–152, 1955.
129a. Lindell, S. E. and Westling, H.: Potentiation of histamine effects by an antihistaminase. Acta physiol. scand. **32:** 230–237, 1954.
130. Loew, E. R. and Papacostas, C. A.: Comparative toxicity of histamine and a histamine liberator compound 48/80. Fed. Proc. **14:** 364, 1955.
131. Lourie, E. M.: Treatment of sleeping sickness in Sierra Leone. Ann. trop. Med. Parasit. **36:** 113–131, 1942.
132. Lundberg, A.: Adrenaline and transmission in the sympathetic ganglia of the cat. Acta physiol. scand. **26:** 252–263, 1952.
133. MacIntosh, F. C.: Histamine and intracellular particles. In: Ciba Foundation Symposium on Histamine, pp. 20–35. Churchill, London 1956.
134. MacIntosh, F. C. and Paton, W. D. M.: The release of histamine by amidines and other compounds. Abstr. Comm. XVII Internat. Physiol. Congress, Oxford 1947, pp. 240–241.
135. MacIntosh, F. C. and Paton, W. D. M.: The liberation of histamine by certain organic bases. J. Physiol. **109:** 190–219, 1949.

136. McCUBBIN, J. W. AND PAGE, I. H.: Nature of the hypotensive action of a thiophanium derivative (Ro 2-2222) in dogs. J. Pharmacol. **105:** 437–442, 1952.
137. McINTIRE, F. C.: The mechanism of histamine release. In: Ciba Foundation Symposium on Histamine, pp. 416–430. Churchill, London 1956.
138. McINTIRE, F. C., ROTH, L. W. AND RICHARDS, R. K.: The in vitro release of histamine from the blood cells of sensitized rabbits: Relationship to blood coagulation mechanisms. Amer. J. Physiol. **159:** 332–336, 1949.
139. McINTIRE, F. C., ROTH, L. W. AND SPROULL, M.: Histamine release in rabbit blood by simple molecules, inhibition and reaction rate studies. Amer. J. Physiol. **167:** 233–240, 1951.
140. MARSHALL, P. B.: The influence of adrenal cortical deficiency on the histamine content of rat tissues. J. Physiol. **102:** 180–190, 1943.
141. MARRAZZI, A. S.: Electrical studies on the pharmacology of autonomic synapses. II. The action of a sympathomimetic drug (epinephrine) on sympathetic ganglia. J. Pharmacol. **65:** 395–404, 1939.
142. MAYEDA H.: Release of histamine by sinomenine. Jap. J. Med. Sci., IV. Pharmacol. **3:** 62–72, 1953.
143. MAYEDA, H.: The site of histamine release by sinomenine. Jap. J. med. Sci., IV. Pharmacol. **3:** 73–81, 1953.
144. MILES, A. A. AND MILES, E.: Vascular reactions to histamine, histamine-liberator and leukotaxine in the skin of guinea-pigs. J. Physiol. **118:** 228–257, 1952.
145. MITCHELL, R. G., BUTT, H. R. AND CODE, C. F.: Histamine metabolism in diseases of the liver. J. clin. Invest. **33:** 1199–1203, 1954.
146. MITCHELL, R., NEWMAN, P. J., MacGILLIVRAY, D. AND CLARK, B. B.: Evaluation of histamine liberator activity illustrated by a thiophanium compound, Ro 2-2222. Fed. Proc. **10:** 325–326, 1951.
147. MONGAR, J. L. AND SCHILD, H. O.: A comparison of the effects of anaphylactic shock and of chemical histamine releasers. J. Physiol. **118:** 461–478, 1952.
148. MONGAR, J. L. AND SCHILD, H. O.: Quantitative measurement of the histamine-releasing activity of a series of mono-alkylamines using minced guinea-pig lung. Brit. J. Pharmacol. **8:** 103–109, 1953.
149. MONGAR, J. L. AND SCHILD, H. O.: Mode of action of a series of diamines as histamine releasers and potentiators. Abstr. Comm. XIX Internat. Physiol. Congress, Montreal 1953, p. 67.
150. MONGAR, J. L. AND SCHILD, H. O.: The effect of histamine releasers and anaphylaxis on intracellular particles of guinea-pig lung. J. Physiol. **126:** 44P–45P, 1954.
151. MONGAR, J. L. AND SCHILD, H. O.: Inhibition of histamine release in anaphylaxis. Nature, Lond. **176:** 163, 1955.
152. MONGAR, J. L. AND SCHILD, H. O.: Effect of antigen and organic bases on intracellular histamine in guinea-pig lung. J. Physiol. **131:** 207–219, 1956.
153. MONGAR, J. L. AND WHELAN, R. F.: Histamine release by adrenaline and d-tubocurarine in the human subject. J. Physiol. **120:** 146–154, 1953.
154. MORRISON, J. L., BLOOM, W. L. AND RICHARDSON, A. P.: Effect of dextran on the rat. J. Pharmacol. **101:** 27–28, 1951.
155. MORRISON, J. L., RICHARDSON, A. P. AND BLOOM, W. L.: The effects of antihistaminic agents on the reaction of the rat to dextran. Arch. int. Pharmacodyn. **88:** 98–105, 1951.
156. MOTA, I., BERALDO, W. T. AND JUNQUEIRA, L. C. U.: Protamine-like property of compounds 48/80 and stilbamidine and their action on mast cells. Proc. Soc. exp. Biol., N. Y. **83:** 455–457, 1953.
157. MOTA, I., BERALDO, W. T., FERRI, A. G. AND JUNQUEIRA, L. C. U.: Intracellular distribution of histamine. Nature, Lond. **174:** 698, 1954.
158. MOTA, I., BERALDO, W. T., FERRI, A. G. AND JUNQUEIRA, L. C. U.: Action of 48/80 on the mast cell population and histamine content of the wall of the gastro-intestinal

tract of the rat. In: Ciba Foundation Symposium on Histamine, pp. 47–50. Churchill, London 1956.
159. MOTA, I. AND VUGMAN, I.: Effects of anaphylactic shock and compound 48/80 on the mast cells of the guinea-pig lung. Nature, Lond. **177**: 427–429, 1956.
160. NORTON, S.: Quantitative determination of mast cell fragmentation by compound 48/80. Brit. J. Pharmacol. **9**: 494–497, 1954.
161. NORTON, S. AND DE BEER, E. J.: Effect of some antibiotics on rat mast cells *in vitro*. Arch. int. Pharmacodyn. **102**: 352–358, 1955.
162. ORMEROD, W. E.: A study of basophilic inclusion bodies produced by chemotherapeutic agents in trypanosomes. Brit. J. Pharmacol. **6**: 334–341, 1951.
163. PAPACOSTAS, C. A., MOZDEN, P. AND LOEW, E. R.: Influence of histamine and histamine liberator, compound 48/80, on blood glucose of unanesthetized animals. Fed. Proc. **15**: 466, 1956.
164. PARKER, J. T. AND PARKER, F., JR.: Anaphylaxis in the white rat. J. med. Res. **44**: 263–287, 1924.
165. PARRATT, J. R. AND WEST, G. B.: Tissue histamine and 5-hydroxytryptamine. J. Physiol. **132**: 40P–41P, 1956.
166. PARRATT, J. R. AND WEST, G. B.: The location and possible function of tissue 5-hydroxytryptamine in the rat. J. Physiol. **134**: 11P–12P, 1956.
167. PARRATT, J. R. AND WEST, G. B.: Oedema-producing substances in the rat. J. Physiol. **135**: 10P–11P, 1957.
168. PARROT, J. L. AND BURSTEIN, M.: Atténuation du choc peptonique chez le chien par l'association de l'atropine et d'un antagoniste de l'histamine. C.R. Soc. Biol., Paris **144**: 395–398, 1950.
169. PARROT, J. L. AND REUSE, J.: Rôle physiologique et physiopathologique de l'histamine. J. Physiol., Paris **46**: 99–221, 1954.
170. PATON, W. D. M.: Compound 48/80: a potent histamine liberator. Brit. J. Pharmacol. **6**: 499–508, 1951.
171. PATON, W. D. M.: Histamine liberation and lymphagogue action. J. Physiol. **123**: 58P–59P, 1954.
172. PATON, W. D. M.: Histamine metabolism. Int. Arch. Allergy, N. Y. **6**: 203–229, 1955.
173. PATON, W. D. M.: The mechanism of histamine release. In: Ciba Foundation Symposium on Histamine, pp. 59–73. Churchill, London 1956.
174. PATON, W. D. M.: Unpublished.
175. PATON, W. D. M. AND SCHACHTER, M.: The influence of an antihistamine drug on the release of histamine in the unanaesthetized dog. Brit. J. Pharmacol. **6**: 509–513, 1951.
176. PATON, W. D. M. AND THOMPSON, J. W.: The mechanism of action of adrenaline on the superior cervical ganglion of the cat. Abstr. Comm. XIX Internat. Physiol. Congress, Montreal 1953, p. 664.
177. PAYNE, J. P.: Histamine release during controlled hypotension with Arfonad. Proc. World Congress Anaesthesiol. 1955, pp. 180–181.
178. QUIVY, D.: La coagulabilité sanguine chez le chien après injection d'un libérateur d'histamine (composé 48/80). J. Physiol., Paris **47**: 267–269, 1955.
179. RANDALL, L. O., PETERSON, W. G. AND LEHMANN, G.: The ganglionic blocking action of thiophanium derivatives. J. Pharmacol. **97**: 48–57, 1949.
180. REID, G.: The liberation of histamine and heparin by tubocurarine and its circulatory effects in the dog. Aust. J. exp. Biol. med. Sci. **28**: 465–476, 1950.
181. REID, G.: Pharmacology of tryptamine. Aust. J. exp. Biol. med. Sci. **29**: 101–116, 1951.
182. REUSE, J. J.: Modifications circulatoires au cours du choc anaphylactique et après administration d'un libérateur d'histamine (composé 48/80). Arch. int. Pharmacodyn. **93**: 122–125, 1950.
183. RILEY, J. F.: The relationship of the tissue mast cells to the blood vessels in the rat. J. Path. Bact. **65**: 461–469, 1953.

184. RILEY, J. F.: The effects of histamine-liberators on the mast cells of the rat. J. Path. Bact. **65:** 471–479, 1953.
185. RILEY, J. F.: Pharmacology and functions of the mast cells. Pharmacol. Rev. **7:** 267–277, 1955.
186. RILEY, J. F. AND WEST, G. B.: The presence of histamine in tissue mast cells. J. Physiol. **120:** 528–537, 1953.
187. RILEY, J. F. AND WEST, G. B.: Histamine liberation in the rat and mouse. Arch. int. Pharmacodyn. **102:** 304–313, 1955.
188. RILEY, J. F. AND WEST, B. G.: Tissue mast cells studies with a histamine-liberator of low toxicity (compound 48/80). J. Path. Bact. **69:** 269–282, 1955.
189. RILEY, J. F. AND WEST, G. B.: A binding site for histamine in hog pyloric mucosa. Experientia **12:** 153–154, 1956.
190. RILEY, J. F. AND WEST, G. B.: Mast cells and histamine in hog stomach. J. Physiol. **130:** 3P, 1955.
191. RILEY, J. F. AND WEST, G. B.: Mast cell and histamine profiles in the skin of various species. J. Physiol. **130:** 28P, 1955.
192. ROCHA E SILVA, M.: Concerning the mechanism of anaphylaxis and allergy. Brit. Med. J. *1:* 779–784, 1952.
193. ROCHA E SILVA, M.: Histamine: its role in anaphylaxis and allergy. C. C. Thomas, Springfield, Ill. 1955.
194. ROCHA E SILVA M. AND ARONSON, M.: Histamine release from the perfused lung of the guinea pig by serotoxin (anaphylatoxin). Brit. J. exp. Path. **33:** 577–586, 1952.
194a. ROCHA E SILVA, M. AND DRAGSTEDT, C. A.: Observations on the trypan blue capillary permeability test in rabbits. J. Pharmacol. **73:** 405–411, 1941.
195. ROCHA E SILVA, M. AND ROTHSCHILD, A. M.: Anaphylatoxin, histamine depletion and skin reactions in the rat. Nature, Lond. **175:** 987–988, 1955.
196. ROCHA E SILVA, M. AND SCHILD, H. O.: The release of histamine by d-tubocurarine from the isolated diaphragm of the rat. J. Physiol. **109:** 448–458, 1949.
197. ROSE, B. AND BROWNE, J. S. L.: The distribution and rate of disappearance of intravenously injected histamine in the rat. Amer. J. Physiol. **124:** 412–420, 1938.
198. ROWLEY, D. A. AND BENDITT, E. P.: 5-Hydroxytryptamine and histamine as mediator of the vascular injury produced by agents which damage mast cells in rats. J. exp. Med. **103:** 399–412, 1956.
199. SALTER, W. T. AND WHITE, M. L.: Morphine "sensitivity". Anesthesiology **10:** 553–561, 1949.
200. SCHACHTER, M.: The release of histamine by pethidine, atropine, quinine, and other drugs. Brit. J. Pharmacol. **7:** 646–654, 1952.
201. SCHACHTER, M.: Anaphylaxis and histamine release in the rabbit. Brit. J. Pharmacol. **8:** 412–419, 1953.
202. SCHACHTER, M. AND TALESNIK, J.: The release of histamine by egg white in non-sensitized animals. J. Physiol. **118:** 258–263, 1952.
202a. SCHAYER, R. W.: Studies on histamine-metabolizing enzymes in intact animals. J. biol. Chem. **203:** 787–793, 1953.
203. SCHAYER, R. W.: Binding and release of histamine. Fed. Proc. **14:** 275–276, 1955.
203a. SCHAYER, R. W.: Formation and binding of histamine in vitro. Fed. Proc. **15:** 347–348, 1956.
204. SCHAYER, R. W., KENNEDY, J. AND SMILEY, R. L.: Studies on histamine-metabolizing enzymes in intact animals II. J. biol. Chem. **205:** 739–748, 1953.
205. SCHAYER, R. W. AND SMILEY, R. L.: Binding and release of radioactive histamine in intact rats. Amer. J. Physiol. **177:** 401–404, 1954.
206. SCHILD, H. O.: Release of histamine by ammonia. Nature, Lond. **164:** 24–25, 1949.
207. SCHILD, H. O. AND GREGORY, R. A.: Liberation of histamine from striated muscle by curarine, strychnine and related substances. Abstr. Comm. XVII Internat. Physiol. Congress, Oxford 1947, p. 208.

208. SCHOENBACH, E. B. AND GREENSPON, E. M.: The pharmacology, mode of action and therapeutic potentialities of stilbamidine, pentamidine, propamidine and other aromatic diamidines. Medicine **27:** 327–377, 1948.
209. SEAGER, L. D. AND CASTELNUOVO, G.: Toxicity of stilbamidine. A study of the effects of chronic intoxication. Arch. Path. (Lab. Med.) **44:** 287–296, 1947.
210. SELYE, H.: Studies on adaptation. Endocrinology **21:** 169–188, 1937.
211. SELYE, H.: The prevention of adrenaline lung edema by the alarm reaction. Amer. J. Physiol. **122:** 347–351, 1939.
212. SMITH, A. N.: The effect of compound 48/80 on acid gastric secretion in the cat. J. Physiol. **119:** 233–243, 1953.
213. SMITH, A. N.: Release of histamine by the histamine liberator compound 48/80 in cats. J. Physiol. **121:** 517–538, 1953.
214. SMITH, A. N.: Gastrin and histamine release. J. Physiol. **123:** 71P–72P, 1954.
215. SMITH, J. A., LIND, A. H. AND RYZOFF, R. I.: Production of bronchoconstriction by thiamine in guinea pigs. Fed. Proc. **14:** 141, 1955.
216. SNAPPER, I., MIRSKY, A. E., RIS, H., SCHNEID, B. AND ROSENTHAL, M.: Development of inclusion bodies containing ribose nucleic acid in myeloma cells after injections of stilbamidine. Determination of stilbamidine in myeloma tissue. Blood **2:** 311–322, 1947.
217. SNIPER, W.: The estimation and comparison of histamine release by muscle relaxants in man. Brit. J. Anaesth. **24:** 232–237, 1952.
218. SQUIRE, J. R., BULL, J. P., MAYCOCK, W. D'A. AND RICKETTS, C. R.: Dextran. Blackwell Scientific Publications, 1955.
219. STAUB, H.: Die Adrenalin-Histamin-Regulation, gleichzeitig Beitrag zum Antistinmechanismus. Helv. physiol. acta **4:** 539–550, 1946.
220. STONE, J. L., MERRILL, H. M. AND MENEELY, G. R.: Distribution of histamine in human tissues. Fed. Proc. **14:** 147–148, 1955.
221. TRETHEWIE, E. R.: "Free" and "bound" histamine and acetylcholine. J. Physiol. **94:** 11P–13P, 1938.
222. UNGAR, G. AND DAMGAARD, E.: Tissue reactions to anaphylactic and anaphylactoid stimuli; proteolysis and release of histamine and heparin. J. exp. Med. **101:** 1–15, 1955.
223. UVNÄS, B.: Personal communication.
224. VAUGHAN, V. C., VAUGHAN, V. C., JR. AND VAUGHAN, J. W.: Protein split products in relation to immunity and disease. Lea & Febiger, Philadelphia and New York 1913.
225. VOORHEES, A. B., BAKER, H. J. AND PULASKI, E. J.: Reactions of albino rats to injections of dextran. Proc. Soc. exp. Biol., N. Y. **76:** 254–256, 1951.
226. WEBB, R. L.: Peritoneal reactions in the white rat with special reference to the mast cells. Amer. J. Anat. **49:** 283–334, 1931.
227. WEISS, S., ROBB, G. P. AND ELLIS, L. B.: The systemic effects of histamine in man, with special reference to the responses of the cardiovascular system. Arch. int. Med. **49:** 360–396, 1932.
228. WEST, G. B.: Histamine in mast cell granules. J. Pharm., Lond. **7:** 80, 1955.
229. WIEN, R.: The pharmacological actions of certain aromatic diamidines possessing trypanocidal activity. Ann. trop. Med. Parasit. **37:** 1–18, 1943.
230. WIEN, R., FREEMAN, W. AND SCOTCHER, N. M.: The metabolic effects produced by certain aromatic diamidines. Ann. trop. Med. Parasit. **37:** 19–33, 1943.
231. WILSON, C. W. M.: The metabolism of histamine as reflected by changes in its urinary excretion in the rat. J. Physiol. **125:** 534–545, 1954.
232. WILSON, C. W. M.: Factors influencing the urinary excretion of histamine in the rat. J. Physiol. **126:** 141–154, 1954.
233. ZELLER, E. A.: Zur Kenntnis der Diamin-oxydase. 3. Mitteilung über den enzymatischen Abbau von Poly-aminen. Helv. chim. acta **21:** 1645–1665, 1938.