**The mechanism of histamine release / W.D.M. Paton.**

Reprinted from *Ciba Foundation Symposium on Histamine* 1956, pp. 59–73

# THE MECHANISM OF HISTAMINE RELEASE

W. D. M. PATON

*Department of Pharmacology, Royal College of Surgeons, London*

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Until recently the ways by which histamine could be mobilized in the body were relatively few. Sensitization, venoms, toxins and peptone accounted for the bulk of them (Feldberg, 1941). But now the field is enormously expanded. Not only this, but the release is achieved by a number of widely different techniques. Accordingly, the problems of anaphylaxis and allergy come to be interpreted in different and sometimes even in conflicting terms. If we wish, then, to enquire *how* histamine is released in allergic processes, we face, from the start, the possibility that there is in fact no single mechanism, even at the most fundamental level; and further that some of the methods of investigation used may bear only a coincidental relationship to anaphylaxis. Our first task is perhaps a Linnaean one, that of sorting out the various releasing processes, and grouping separately those which may involve different mechanisms.

**Table I**

Types of Histamine-releasing Agents

| | |
|---|---|
| 1. *Sensitizing Compounds* . . | Antigens. Reagents with proteins forming antigens. |
| 2. *Compounds Damaging Tissues* . | Venoms. Toxins. Traumatic agents. |
| 3. *Proteolytic Enzymes* . . | Trypsin. |
| 4. *Surface-active Agents* . . | Tween 20. Bile Salts. |
| 5. *Large Molecules* . . . . | Egg white. Dextran. P.V.P. Horse serum. "Anaphylatoxin". |
| 6. *Histamine Liberators* . . | Dibasic and polybasic compounds. Compound 48/80. |
| 7. *Monobasic Compounds* . . | Alkylamines. Antihistamines. Q.P.E. |

*First* we must mention those compounds causing sensitization, which typically need more than one application to an

animal to cause release, which produce specific antibodies, and which are usually of protein nature or are able to react with proteins in the body to form an effective antigen.

*Second* are those processes which produce frank damage to tissues, such as venoms, bacterial toxins, or direct trauma. One might, of course, argue that the mere release of the normal tissue constituent constitutes "damage". But we are concerned here with a more radical affair, such as the appearance of cellular débris in the effluent from a perfused organ; the pouring out of fluid from the trachea instead of a pulmonary vein; or the production of necrosis of a tissue.

*Third* are proteolytic enzymes. The idea that proteolysis plays a part in anaphylaxis is an old one, taken further when Rocha e Silva, Dragstedt and others found that trypsin, and other proteases, could mobilize histamine from the liver and produce other phenomena similar to anaphylactic shock; they went on to the idea, no longer fashionable but still interesting, that histamine is bound by a peptide bond. Linking up with this we have the complex story of fibrinolysin, which Dr. Ungar will be telling us about.

*Fourth* is a group of surface active materials. Typical members are the detergent Tween 20, which Krantz and his colleagues (1948) showed could cause generalized urticaria in the dog as well as other anaphylactoid signs; and bile salts which Schachter (1952) found to release histamine from cats' isolated perfused skin. An older representative of this group, of course, is lysolecithin; in their work on cobra venom, Feldberg and Kellaway (see Feldberg, 1941) raised the interesting possibility that a lytic substance of this kind was formed in the anaphylactic process, although attempts to demonstrate its presence were not successful. Whatever the physiological significance of such actions may be, there is no doubt that the interfacial activity of a compound when applied to tissues deserves attention.

*Fifth* is a curious and rather puzzling group of large molecular compounds. This includes dextran in the rat (Edlund *et al.*, 1952) (and very occasionally in man); polyvinyl-

pyrrolidone, in the dog (Halpern and Briot, 1953); horse serum, in the cat (Feldberg and Schachter, 1952); and perhaps the substance described long ago and prematurely christened "anaphylatoxin," which can be formed by incubating various sera with agents such as agar (Rocha e Silva, 1952). We have

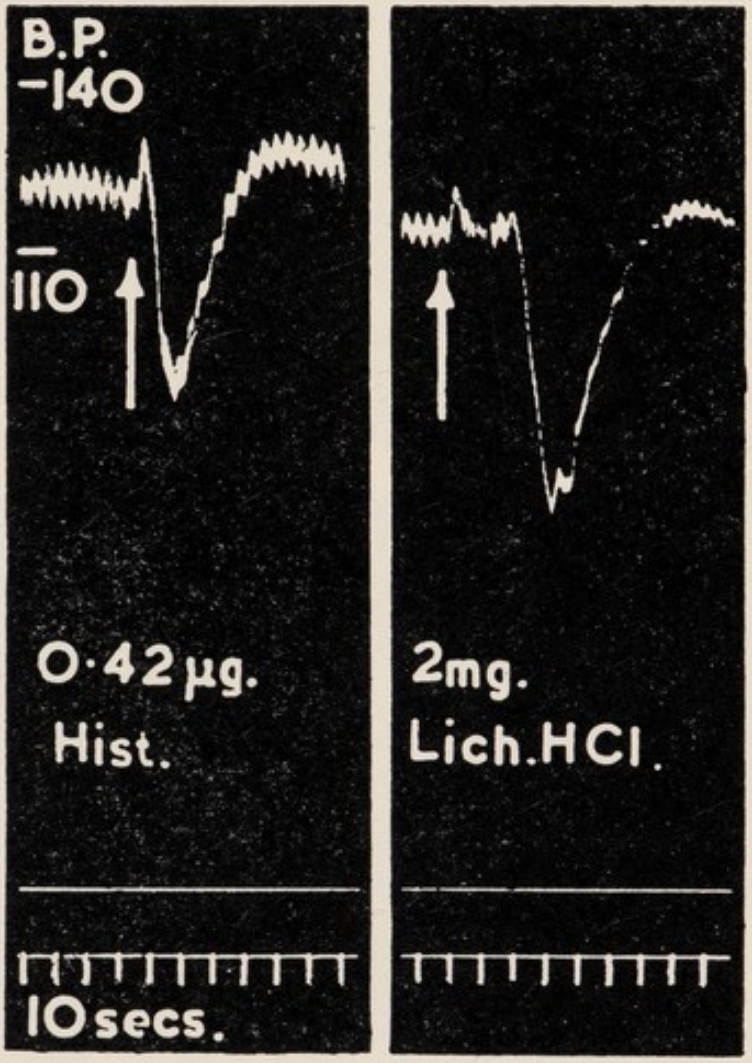



FIG. 1. Effect of histamine and of licheniformin on the blood pressure of the cat under chloralose, showing the delayed depressor response with licheniformin, a histamine liberator. (MacIntosh and Paton, 1949, by permission of the *Journal of Physiology*.)

no clear conception of how these substances can release histamine, not only in a whole animal, but also in an isolated perfused organ.

*Sixth* we have the group of bases known as histamine liberators, a name that serves usefully to distinguish them

within the general class of histamine releasers. Their pedigree starts with Anrep and his colleagues who showed in the course of studies on muscle that curarine was able to release histamine from it (Alam *et al.*, 1939). It was later found by MacIntosh and Paton (1949) that the ability to release histamine extended to a very wide range of organic bases. Indeed it seems that any compound possessing two or more basic groups carried on and separated by a sufficient aliphatic or aromatic scaffold is liable to have this property. The interest of these compounds however did not lie only in their number and their diversity of chemical structure, great as this was. They have a rather characteristic type of action exemplified in a simple way in the so-called "delayed depressor response". Injected into a cat under chloralose, histamine causes a fall of blood pressure in 5–8 seconds (Fig. 1). But during this time the histamine liberators showed no action at all; and it is not until 20 or 30 seconds have elapsed, a period which Gray and Paton (1949) found later to correspond closely to one circulation time, that a depressor response occurs. When it comes however it is often as rapid as with histamine alone. Fig. 2 shows the delayed depressor response with a number of other compounds. The absence of action during the initial latent period is a nice demonstration that the drug itself has no direct effect on the blood vessels, and the absence of such a direct action can be shown in other ways, on isolated tissues or in one's own skin. This can be shown too by the ineffectiveness of a liberator when all the mobilizable histamine has been released. The study of such depleted or refractory tissues is now almost a subject in itself (Feldberg and Talesnik, 1953).

The release process appears to take place in a sharp burst, which has been termed "explosive" and is rather rapid. This follows from a comparison of the interval from the injection to the delayed depressor response with the circulation time; the release process must take only a few seconds or even less. A similar conclusion was reached by Feldberg and Paton (1951) in studies on perfused muscle, where it was

found, for instance, that if a constant submaximal infusion of a histamine liberator was made, there was still obtained the

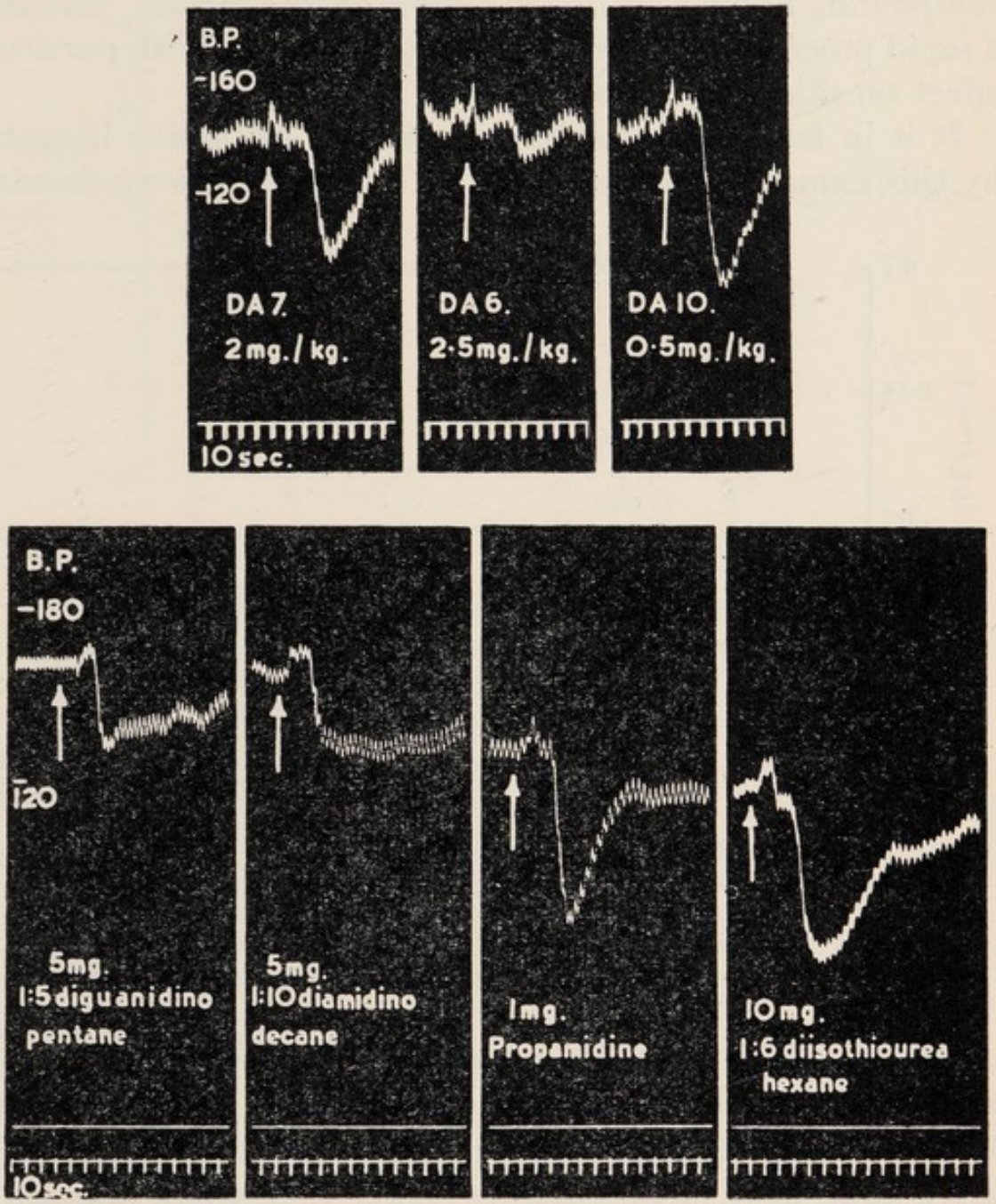



FIG. 2. Delayed depressor responses in the cat under chloralose, produced by a number of dibasic compounds. (MacIntosh and Paton, 1949, by permission of the *Journal of Physiology*.)

same abrupt rise and then fall of histamine in the effluent that is seen with a single sharp dose. Further the whole time course of the release corresponded well to what would be

observed if it had taken place rapidly and the histamine was then, exponentially, rinsed out (Fig. 3).

One can characterize the histamine liberators, therefore, as compounds having no direct action on the tissues, initiating a rapid process of histamine release, and acting with particular effect on skin and muscle.

It is in fact a slight misnomer to call histamine liberators by this name since it does not quite describe the whole of the

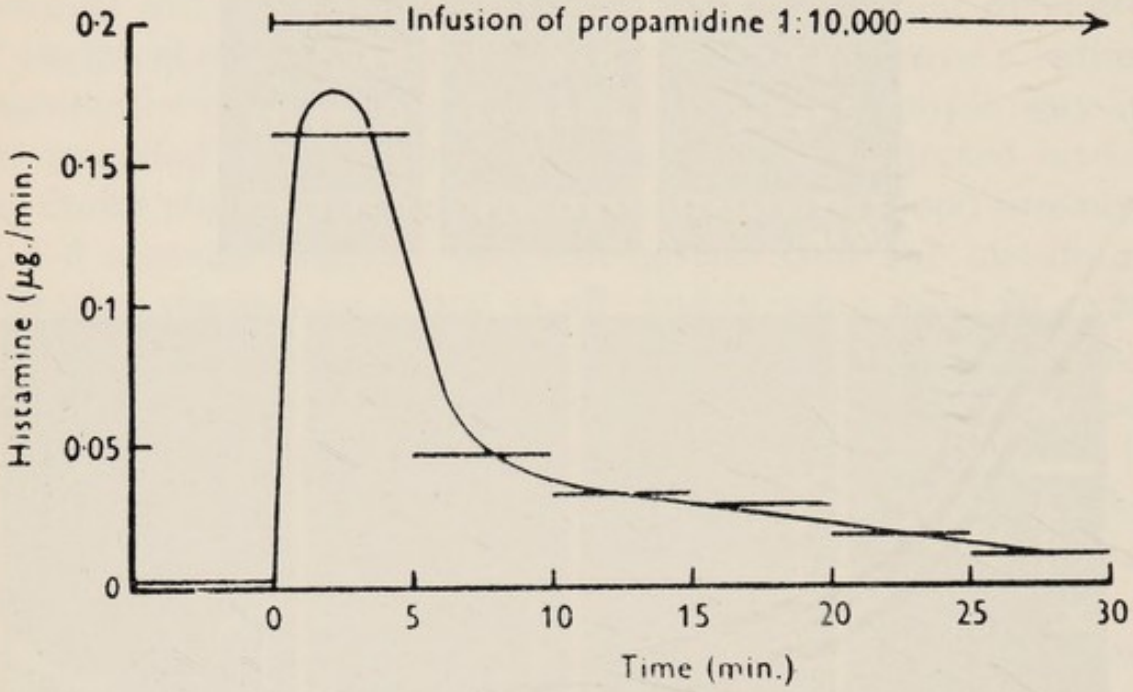



FIG. 3. Histamine release from perfused cat's gastrocnemius during infusion of propamidine $1 \times 10^{-4}$. Horizontal lines indicate mean values of histamine release in μg./min. during each 5 min. period. (Feldberg and Paton, 1951, by permission of the *Journal of Physiology*.)

events to which they give rise. At the same time as they release histamine in the body, then at least in the dog they release heparin (Fig. 4); and in the cat and the dog, if a fairly large dose of liberator is given, then there appears in the plasma what one refers to as a "slow reacting substance," resistant to antihistamines and corresponding to a similar substance appearing in anaphylactic shock, described by Beraldo (1950). Despite these qualifications, the liberators are quite a specific group of drugs; this is in itself important, not only as a fact about liberators, but as a fact about

histamine, since it implies that histamine exists in such a form that it can be selectively chiselled away from its normal attachment.

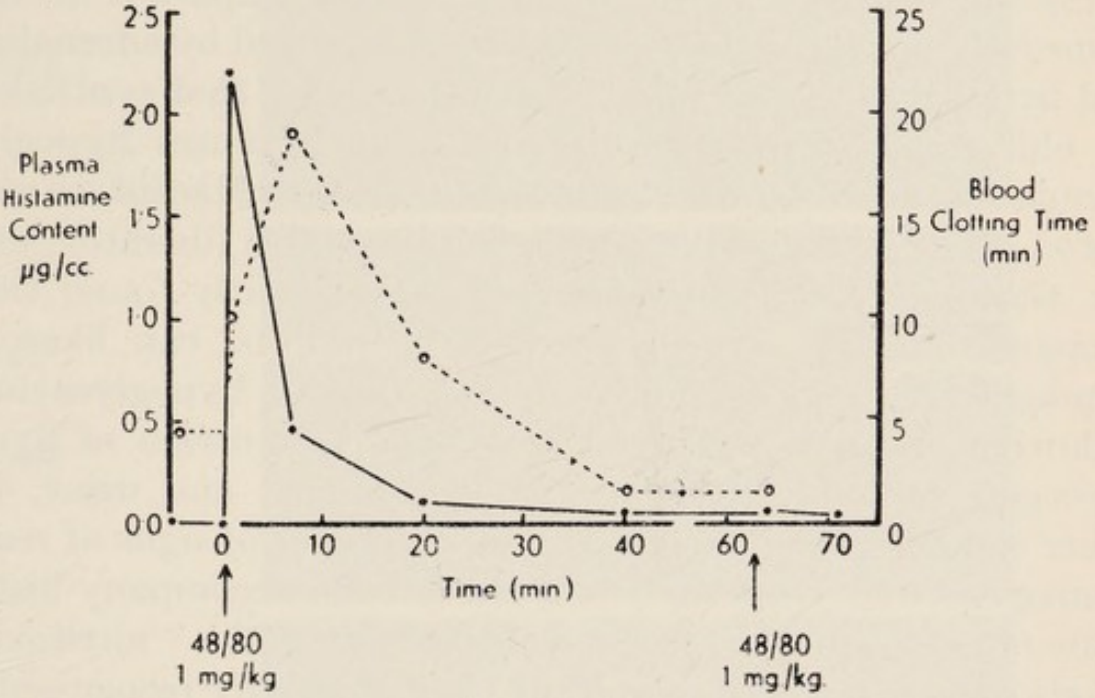



FIG. 4. Histamine concentration (continuous line) and blood clotting time (dotted line) after injection of 1·0 mg./kg. 48/80 intravenously at arrows, in dog under chloralose. (Paton, 1951, by permission of the *British Journal of Pharmacology*.)

These compounds have some interest apart from the questions of allergy and anaphylaxis since a large number of

**Table II**

HISTAMINE LIBERATORS IN HUMAN USE

| | | | |
|---|---|---|---|
| *d*-Tubocurarine<br>Laudexium<br>Mytolon | Quaternary salts | Propamidine<br>Pentamidine<br>Phenamidine<br>Stilbamidine<br>Antrycide | Chemotherapeutic agents |
| Morphine<br>Codeine<br>Papaverine<br>Thebaine<br>Pethidine<br>Atropine<br>Strychnine | Centrally active compounds | Arfonad<br>Apresoline<br>Priscol | Depressor drugs |
| | | Amphetamine<br>Tyramine<br>Phenylethylamine | Sympathomimetic agents |

Lobster, mussel and crayfish extracts
Protein hydrolysates and basic amino acids

them occur in human use. Here they give rise to the inappropriately named "nitritoid" reactions and provide useful "experiments" in man confirmatory of animal experience. These are usually regarded as unimportant, apart from the immediate urticarial reaction which is controlled by adrenaline and by antihistamines. But it is worth noting that synthalin, an old proposed insulin substitute, which causes hypoglycaemia in rabbits by damaging the liver, is (as MacIntosh and Paton, 1949, found) a perfectly good histamine liberator; and Dr. Gaitonde and I (unpublished) have recently found that propamidine too, given repeatedly to rabbits, can likewise damage the liver, as shown by a profound hypoglycaemia following on an initial hyperglycaemia, exhaustion of liver-glycogen, amino-acid accumulation in blood and urine, by other signs of liver damage, and by a number of signs of renal damage as well. If in fact such phenomena accompany histamine release on any frequent scale, then even the " nitritoid " reaction may be more important than is usually recognised.

*Seventh*, are the group of monoamines which Mongar and Schild (1953) and McIntire, Roth and Sproull (1951) have recently shown to possess histamine-releasing power. These will be discussed in more detail by Dr. Mongar. But I must allude here briefly to the question of whether they should be distinguished for the time being from histamine liberators. There are in fact a number of differences :

(1) If one compares them on the cat's blood pressure, then octylamine, a typical member of the group, either has no depressor action, or else an early and transient one which passes over into the pressor effect at a time when with a histamine liberator the delayed depressor effect would just be starting. They seem to lack the specificity attainable in some histamine liberators (Fig. 5).

(2) The monoamines seem to initiate a slower and more prolonged releasing process, seen in experiments on perfused skin, and rather clearly shown in Wilson's experiments on urinary histamine excretion in rats (1954). He found that 48/80 produces a short sharp increase in urinary histamine

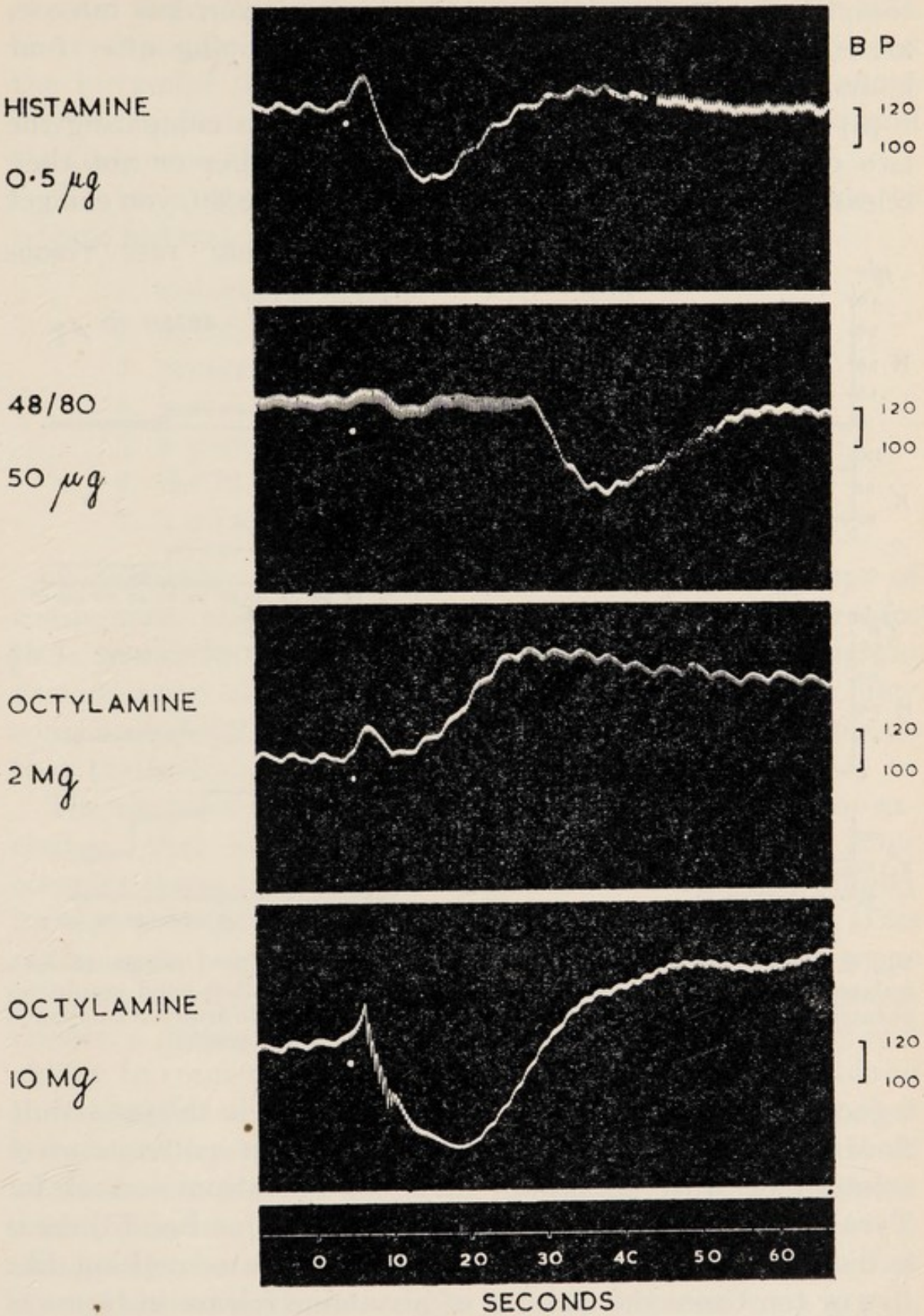



FIG. 5. Blood pressure recording from cat under chloralose. Effect of histamine, compound 48/80, and octylamine.

complete in one hour, but octylamine a slower, less intense, more prolonged one, sometimes still developing after four hours.

(3) I have recently done some experiments comparing the two on perfused skin with respect to whether or not they release potassium (Fig. 6). With compound 48/80, you can get

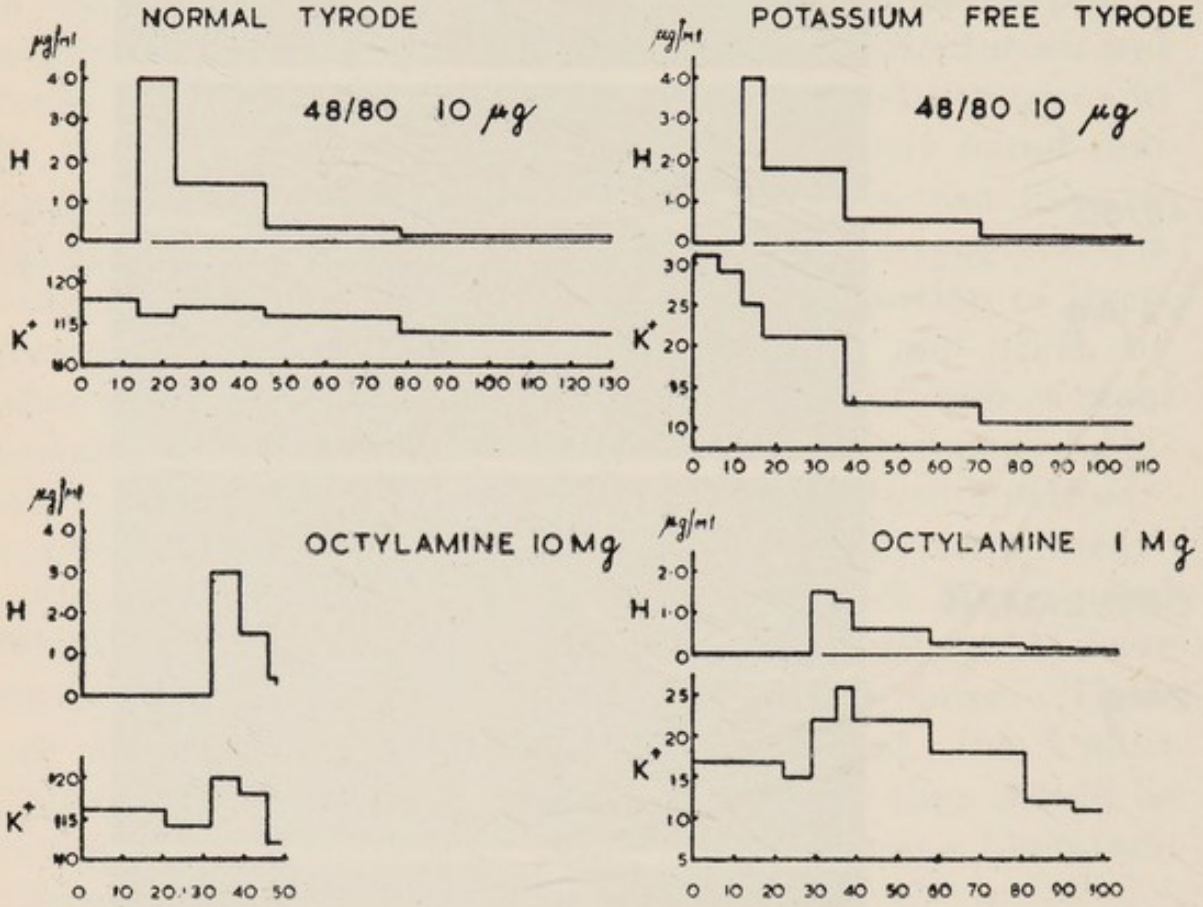



FIG. 6. Experiments on histamine (H) and potassium ($K^+$) release in cat's isolated skin preparation perfused with normal Tyrode (left-hand graphs) or potassium-free Tyrode (right-hand graphs). Injections into artery of compound 48/80 (upper graphs) or octylamine (lower graphs).

a good histamine release without any change in the potassium flowing from skin, whether perfused with potassium-free solution or with potassium in a concentration normal for Tyrode solution. With octylamine on the other hand there is a distinct potassium release, in magnitude something like five or ten times the amount of histamine release in terms of potassium ion and histamine base. The interpretation of this is not clear but it both supports the general view that there

is some important difference between these monoamines with their depressor action and potassium-releasing ability and the histamine liberators, and makes one reluctant to adopt any view of mechanism for liberators involving cell lysis and the histamine liberators.

We have therefore seven classes of substances able to release histamine:

1. sensitizing agents;
2. agents damaging the tissues;
3. proteases;
4. surface active substances;
5. a group of large molecular weight substances;
6. the histamine liberators; and
7. a group of simple monobasic substances.

A particular interest focuses on the last two groups of compounds, and particularly on the histamine liberators, for their specificity and activity, since such compounds might provide a sort of pharmacological final common path for more complicated modes of release. But if this is the case, how do they themselves act?

The simplest theory is that based on the idea of ion exchange: that histamine as a base is associated with some complex tissue acid, and that any base with a greater affinity for that acid will free the histamine from its attachment. This notion rested initially on two facts; first that so wide a range of bases existed which possessed the property for histamine release, a curious observation unless it was their basicity and ability to react with acids, as such, which conferred much of the histamine-releasing power upon them; and secondly that heparin, at least in the dog, is also released, presenting itself as an obvious possible candidate for the rôle of complex tissue acid. This ion exchange theory has gained support, in a small way, from Miss Eldridge and my finding (1954) that among amino acids, otherwise a fairly homogeneous group, only the basic ones were active. But it was strengthened in a more important way by the demonstrations by Riley and

West (1953, 1954), and later by Graham and co-workers (1953) that heparin and histamine are regularly and quantitatively associated in many tissues. Prof. MacIntosh has marshalled some of the arguments in favour of such a hypothesis in one of its forms.

But there are one or two difficulties. One is that compound 48/80 is more active than it ought to be, since it releases much more than its own molecular weight of histamine, usually something like 10 molecules of histamine to each basic nucleus of compound 48/80. This, however, is an objection that need not be taken too seriously, since one can find elsewhere examples where one drug can compete with ten times or more than ten times its molecular weight of another drug; it only presents a difficulty if a completely static idea of the reaction is adopted.

A second difficulty, perhaps, arises if one believes that some of the histamine of the body is not contained in mast cells. The evidence seems quite clear now that some of the histamine is contained there, but it also seems likely that some of it is in other sites. For instance, Harris (1927) was able to show by scalding human skin, so obtaining epidermis split off from the dermis at the *stratum germinativum*, that histamine was still in the epidermis, where there are no mast cells. Such an experiment cannot be applied to cat skin in general, because the hair follicles hold the epidermis in place. But I have been able to confirm it for the skin of the pad and the skin of the ear, which can be treated in this way. Further, on the dorsal skin of a cat, one can show that a thin layer removed with a MacIndoe dermatome, which just cuts the tops of the papillae, has a higher histamine content (30 μg./g.) than a slightly thicker layer which cuts deeper into the corium (20 μg./g.). This of course is the reverse of what you would expect if it was the corium which contained the mast cells (and thence histamine), while the epidermis was histamine-free. To make these experiments decisive of course, one needs a histological control that mast cells are in fact absent from the scalded epidermis, and that the distribution of mast cells is as expected in the corium.

Suppose, then, that there is histamine in the epidermis (or in some other area free of mast cells). We know that virtually all the histamine can be removed from the skin by a liberator, yet so far as we know heparin is not present in the epidermis. The question arises as to what tissue acid we must now think of. Now Leach, Peters and Rossiter (1943), during the war, showed in their studies on burning that, with a gentle burn, the epidermis very rapidly (within a minute) begins to lose its basophil properties and the nuclei begin to degenerate. This prompted the notion that nucleic acids might be involved, and RNA (the nucleic acid in cytoplasm) is an obvious candidate. If this were the case, the histamine liberators should form complexes with RNA just as they do with heparin (as MacIntosh showed us yesterday). In fact they do this; and furthermore their readiness to do so runs parallel with their activity in releasing histamine. In Table III I have given an estimate of the concentration of liberators producing a distinct visible precipitate with RNA against the dose which is

**Table III**

COMPARISON OF HISTAMINE-RELEASING ACTIVITY WITH ABILITY TO REACT WITH RIBOSE-NUCLEIC ACID

| *Compound* | *Concentration of base forming visible precipitate with RNA* | *Dose causing delayed depressor response in cat* | 10 × *Dose active on cat's isolated perfused skin* |
|---|---|---|---|
| | mg./cc. | mg./kg. | mg. |
| 48/80 | 0·03 | 0·01 | 0·01 |
| Stilbamidine | 0·3 | 0·3 | |
| Propamidine | 0·3 | 0·3 | |
| *d*-Tubocurarine | 0·4 | 0·6 | |
| Arfonad | 2 | 1 | |
| Octylamine | 3 | — | 2 |
| Morphine | 15 | 3 | |
| Amphetamine | 30 | — | c. 50 |
| Arginine | 50 | — | c.100 |
| Hexamethonium | 100 | 70 | |

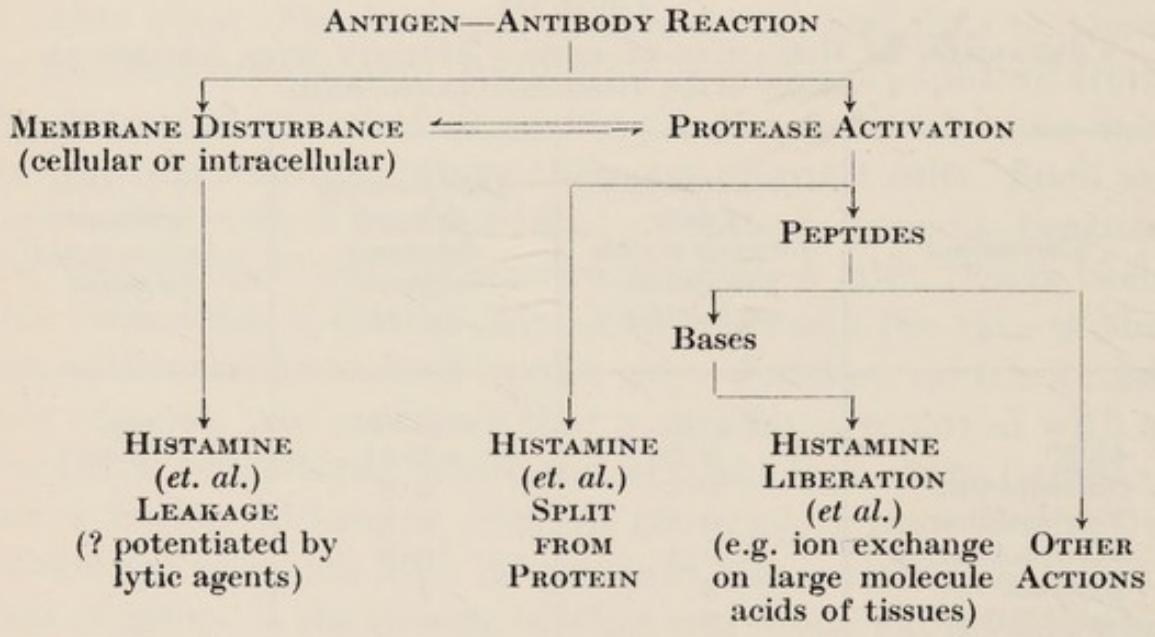
(Data on release from MacIntosh and Paton (1949), Feldberg and Mongar (1954), and unpublished).

active on the cat's blood pressure, or when this figure is not available, on the cat's skin (scaled up on the basis of the 48/80 figures to bring the results numerically into the same range).

Such *in vitro* work is a long way from experiments directly on histamine release in a living tissue. But the fact that a widely distributed substance like RNA can form complexes with liberators, in proportion to their histamine-releasing activity, seems important, still more so since a riboside of a histamine metabolite has now been isolated by Dr. Tabor and Dr. Schayer; and it suggests that in considering the ion exchange theory, or indeed the binding of any base in the tissues, substances like RNA may play an important part.

Finally I would like to return to the more general interaction of the different methods of histamine release. How are they connected? It is useful to have some provisional scheme of these connections, simply to keep in mind the various and conflicting aspects of the subject. The scheme shown (Table IV)

**Table IV**




assembles some of the better known concepts: firstly, it brings out some of the possible interactions of the different histamine releasing processes; secondly it shows that there are at least three basic ideas about how histamine is released (by leakage

through a damaged membrane, by splitting off from a protein, and by release from some retaining acid within a cell); and thirdly it indicates one way at least how the properties of histamine liberators, which for such simple substances provide such a striking analogy to anaphylaxis, can be fitted into the whole antigen-antibody story. But a critical scrutiny reminds one that a diagram of this sort indicates no more than the main cards dealt us in the histamine pack, a large pack which we all shuffle in different ways. I must admit to a suspicion that there are too many jokers and that the ace of trumps is still missing.

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