REVIEW ARTICLE

ON SOME PHYSIOLOGICAL ASPECTS OF HISTAMINE*

By W. Feldberg, M.D., F.R.S.

National Institute for Medical Research, Mill Hill, London.

If I were to ask a student in an examination about histamine, I would expect him to know that it is the amine of histidine, that it is a naturally occurring constituent of the body, present in many tissues, and one of the most active substances known. I would also expect him to be acquainted with its main effects: that it causes contraction of many smooth muscles, secretion of many gland cells, increased permeability of the capillary wall with fluid exudation, independent of whether there is simultaneous vasodilation or not; further, that it has a peculiar action on sensory nerve endings which, in the human skin, is responsible for the red flare of the triple response and the associated itching. But if I were to probe deeper and ask him what is the physiological function of histamine, what does it do in the skin, in skeletal muscles, in nerve fibres, in the wall of the digestive tract, in liver and lung, I should not be surprised if, after a few sentences he started to stammer, or, if he were one of those with a lively imagination, he told me some most interesting functions for which there is unfortunately no experimental evidence. This naturally would not mean that he was not right.

It is not possible to give a definite and satisfactory answer to the question: what is the physiological function of histamine? It may rightly be asked why, then, do I choose to write about it? I felt that even if we do not known the answer, we can at least make an attempt to discuss the ideas which are at the back of our minds when setting up experiments. You all know Chinese puzzles; you fiddle around, you know there is a solution; you think you have got it and then again it does not work. Then you try again and again, but without success. That about describes the situation in which I am continuously finding myself when trying to find experimental evidence for the function of histamine in this or that tissue.

The Histamine Liberators

We possess a new tool for studying the histamine problem. Work during the last few years has provided us with numerous substances which liberate histamine from the tissues: the so-called histamine liberators or histamine releasers. They are chemical compounds capable of releasing histamine without producing visible damage of the tissues. In this respect they differ from snake venom, bee venom and trypsin, which have long been known to release histamine. But these agents destroy or digest the tissues and the histamine release is associated with, in fact caused by, this gross cell damage.

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The histamine in the tissues is somehow linked to the proteins and lipins. All attempts to get a clear understanding of this linkage by which the histamine is kept in the tissues in an inactive form have so far failed. But by destroying either the proteins or the lipins the histamine is freed. Trypsin and some snake venoms which are proteolytic enzymes digest the proteins and in this way release the histamine[1,2]. Other snake venoms, as well as bee venom, release the histamine by the following mechanism: they are lecithinases, and by splitting off oleic acid from lecithin (see Fig. 1), they produce a powerful lytic substance, lysolecithin, which dissolves the tissue lipins and in doing so sets free the histamine[3,4,5].

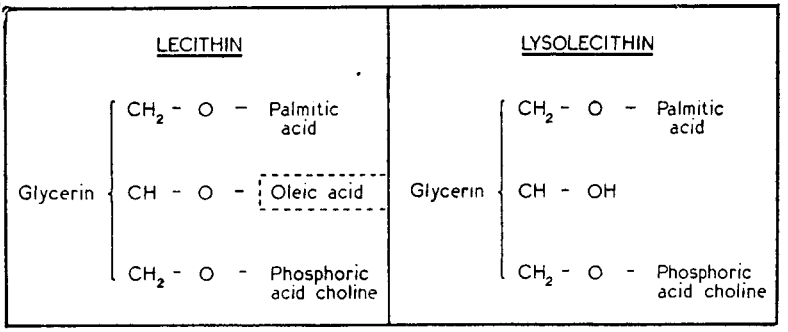

FIG. 1. Conversion of lecithin into lysolecithin by cobra or bee venom.

The histamine liberators, on the other hand, manage to release histamine without causing these deep structural changes in the tissues. The first substance found to have an effect of this kind was *d*-tubocurarine the observation being made by Alam, Anrep, Barsoum, Talaat and Weininger[6] as far back as 1939. Ten years later, MacIntosh and Paton[7] published their extensive study on histamine liberators. By showing that this property is shared by a great variety of organic bases, they started a new line of research on histamine.

Many of the organic bases which they found active were diamines or mono- and diamidines. Since histamine itself may be regarded as having a diamine or amidine structure, they suggested that it is because of this structural similarity with histamine that these basic substances are able to displace histamine from its attachment to an acidic residue in the tissues by some kind of cationic exchange. This displacement hypothesis has the advantage of explaining why no cell damage accompanies the histamine release. It does not easily explain, however, why histamine liberators also release heparin, at least from the dog's liver; but on the assumption that heparin and histamine form a tissue complex, the displacement of one component, histamine, could be assumed to mobilise the other component, heparin, as well.

In the last few years, the number of known histamine-releasing compounds has greatly increased and now includes morphine and other opium alkaloids, straight chained mono-alkylamines, tryptamine, 5-hydroxytryptamine, and a substance called compound 48/80 which is a

condensation product of *p*-methoxyphenethylmethylamine with formaldehyde. This compound is, at the present time, the most potent histamine liberator known, being active in fractions of a μg.[8,9,10,11,12,13].

It was the correct analysis of a very small difference between the depressor effect of histamine and that of a substance under examination which led to the discovery of this new class of substances, the histamine liberators.

MacIntosh and Paton studied the effect of licheniformin, an antibiotic base extracted from *Bacillus licheniformis*, on the cat's arterial blood pressure. When injected intravenously it produced, like histamine, a fall in arterial blood pressure. But there was this difference. With histamine the fall started within a few seconds, with licheniformin there was a delay of 20 to 25 seconds between injection and the depressor effect (Fig. 2), and this characteristic delay occurred always, whether small or large doses were injected. The delay pointed to an indirect mechanism of action, and when they removed the blood of a cat which had been given a large dose of licheniformin and tested the plasma of this blood pharmacologically for histamine, it was found to contain nearly 0·1 μg. of histamine per ml.

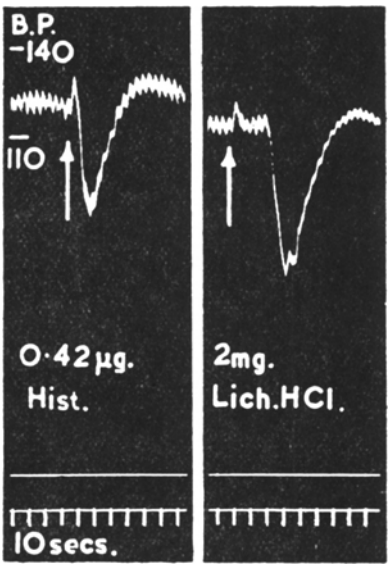

FIG. 2. Arterial blood pressure of cat in choralose anæsthesia. Illustration of the delayed depressor effect of a histamine liberator, licheniformin, in comparison to the depressor action of histamine. (After MacIntosh and Paton[7].)

Licheniformin concentrates give a positive test (Sakaguchii) for guanidine derivatives. Therefore, MacIntosh and Paton examined various bases containing the guanidine group and later diamines and so on, in order to see whether they produced the characteristic delayed depressor response. It is interesting to note here that 6 years before MacIntosh and Paton described their results, the depressor effects of 2 of the most active diamidines, propamidine and stilbamidine, were published with tracings showing the characteristic latent period[14]. However, its importance was not recognised; in fact, no reference was made to it.

We realise to-day that whenever we encounter a delayed depressor effect on injection of a new substance, it may be the first item of evidence that this substance is a histamine liberator. But in order to study the histamine-releasing properties of such compounds, we employ now more direct methods.

One method consists of perfusing an isolated organ through its artery

with physiological salt solution, injecting the histamine liberator arterially and assaying the histamine in the venous effluent. The perfused isolated gastrocnemius muscle and isolated skin flaps from the hind legs perfused from the saphenous artery have been widely used for this purpose. Figure 3 illustrates the result of such a perfusion experiment on a skin flap from the cat's hind leg. In addition, we can measure the histamine content at the end of the perfusion and show the reduction in histamine content of the tissue. In fact, it has been possible to deplete perfused skin regions of their histamine by this means.

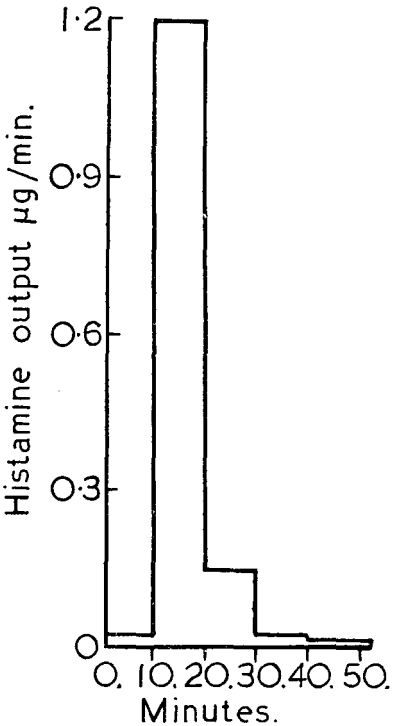

FIG. 3. Output of histamine from perfused cat's skin preparation by 2 µg. of compound 48/80 injected 10 minutes after beginning of perfusion.

The output of histamine from a perfused tissue on a single injection of a histamine liberator is maximal during the first minutes but continues in decreasing amounts for long periods. That does not mean that the release itself is prolonged. On the contrary, it looks rather as if the release occurs explosively into the tissue spaces from which it then diffuses out gradually into the capillaries and is washed away with the perfusion fluid. This view is based on the finding that the time course of the histamine output is the same whether we give a single injection or infuse the liberator for long periods. In other words, a given concentration of a histamine liberator has the same effect whether it is in contact with the tissue only momentarily or for minutes. What does that mean? My view is as follows. The histamine in a given tissue shows different degrees of resistance against histamine liberators. The most susceptible histamine is released by very low concentrations, the most resistant by high concentrations of liberator. We do not know if this difference resides in the same cell or is due to the fact that the histamine in different cells of the same tissue has a different resistance, but the release is always explosive. Therefore, single injections and prolonged infusions lead to the same result.

Another problem which also concerns the resistance to histamine liberators is as follows: if we compare different organs, for instance, lung, liver, digestive tract, skin and skeletal muscle, does the histamine in these behave in the same way when subjected to known histamine liberators? The answer is definitely no. The histamine in skin and skeletal muscle

is more sensitive to liberators than that of any other organ so far examined, but the relative sensitivity varies for different liberators. For instance, when comparing the histamine-releasing potency of the histamine liberator known as 48/80 and octylamine on perfused skin and lung preparations, we find that on the perfused skin 48/80 is several hundred times as active as octylamine but on the perfused lung it is only a few times as active. We therefore reach different conclusions about the relative potencies of two histamine liberators according to the tissue used for studying the release. From results obtained by McIntire, Roth and Sproull[11], it also appears that the ability of a compound to release histamine from rabbit's blood does not run parallel to that of releasing it from other animal tissues. There is a further complication. Mongar and Schild[12] determined the histamine release from incubated minced tissue particles and found that with this method octylamine was even more effective than 48/80, because the histamine in the tissue particles of the skin and skeletal muscle is no longer highly sensitive to 48/80 (Feldberg and Mongar, 1954). Why this is so we do not know, but it illustrates the difficulty in comparing results obtained with different methods.

Depletion and Restoration of Tissue Histamine

We can deplete a perfused skin flap of its histamine content when we inject a histamine liberator. Can we obtain the same result when we inject the histamine liberator into a living animal? In that case we would be able to study a problem about which we know very little, namely how long it takes to restore the histamine in a histamine depleted tissue, and, if the restoration were a slow process, it would give us, in addition, an opportunity to study reactions which are attributed to release of histamine in conditions where the tissue contains only little histamine or none.

Talesnik and I[15] succeeded in greatly reducing and practically depleting the histamine in the skin of rats by intraperitoneal injections of 48/80 in increasing dosage. We found, further, that it takes a long time before the skin histamine is restored. This is shown in Figure 4, and similar results were obtained for the histamine in skeletal muscle, but there was no definite reduction in the histamine content of the viscera. This difference was particularly striking for the wall of the stomach and intestine, because these tissues have a high histamine content. Again we see that skin and skeletal muscle histamine are more susceptible to release by histamine liberators than the histamine of the viscera.

Similar results were obtained by Smith[16] in cats, but he was unsuccessful in guinea-pigs. One factor, but probably not the only one, responsible for his failure was the strong gastric secretion after 48/80, which cannot be abolished by antihistamine drugs and which leads to perforating stomach ulcers. We were anxious to obtain a histamine-depleted skin in the guinea-pig because this species is particularly suitable for studying allergic and related skin phenomena in histamine-depleted skin. At present such a study has to be confined to the rat.

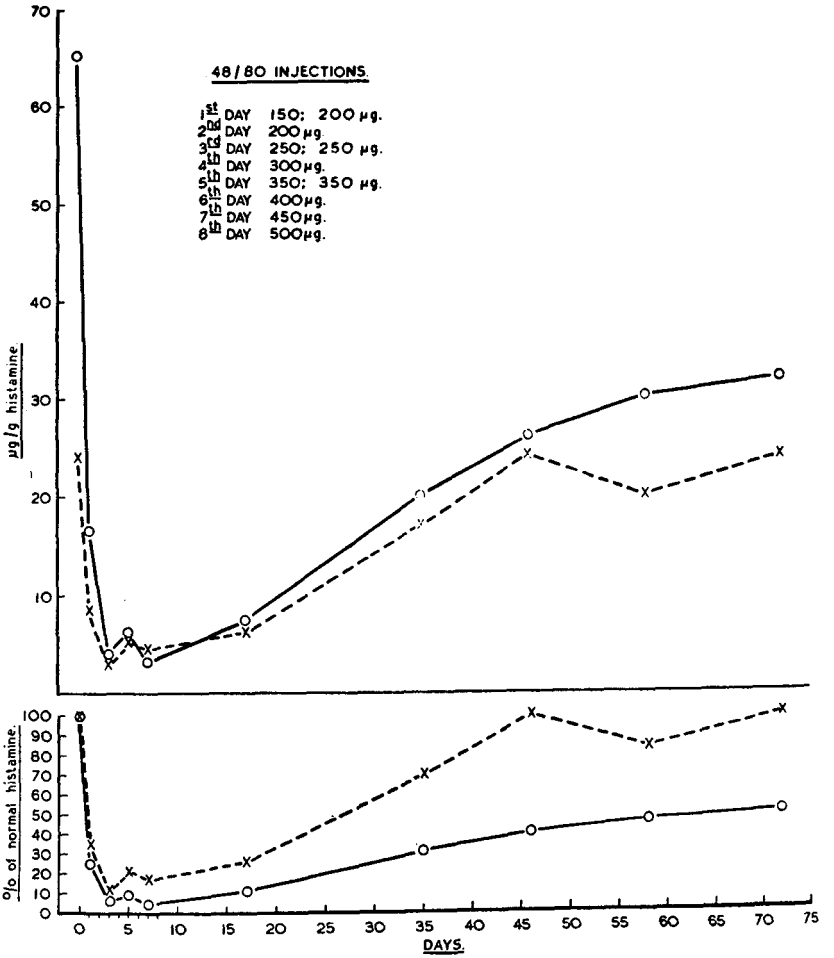

FIG. 4. Effect of intraperitoneal injections of 48/80 on histamine content of rat's skin from abdomen (x – – – x) and feet (o———o). Histamine content expressed in µg./g. skin in the upper tracings and in percentage of normal values in the lower tracings. 8 days' treatment with increasing doses of 48/80 as indicated. (From Feldberg and Talesnik[15].)

HISTAMINE CONTENT AND MAST CELL POPULATION OF TISSUES

So far we have spoken of the histamine content in different tissues, but perhaps we shall have to change our approach in the future and shall have to give closer consideration to the cellular elements when discussing the histamine content of a tissue.

Riley and West[17,18] have shown that there is a striking positive correlation between the histamine content and the mast cell population of many normal tissues. They found that tissues with a high population of mast cells have a high histamine content. These results have been confirmed by Graham, Lowry, Wahl and Priebat[19]. I shall cite 4 of the striking

instances given by Riley and West: (1) The ox pleura is packed with an enormous number of mast cells and contains between 200 and 300 μg./g. of histamine. (2) The mast cell population of many tissues is small in very young mammals but increases in the adult animal, and so does the histamine content of the tissues. (3) By painting the skin of the adult mouse with a carcinogenic hydrocarbon, the number of mast cells in the skin increases and so does its histamine content. (4) Mast cell tumours yield extremely high histamine values on extraction. One human tumour of this kind contained nearly 1 mg. of histamine per g. of tumour; this is certainly the highest histamine value found in a tissue.

Unfortunately we know very little about the mast cells which were discovered by Ehrlich. The textbooks of histology tell us that they are present, often in groups or clumps, in the connective tissue around the small blood vessels, that they contain granules which stain with basic aniline dyes like the granules of the basophil leucocytes, but that the two types of cells are independent of each other. The function of these histiogenous or tissue mast cells is unknown. According to Jorpes[20], the granules consist of heparin. There is a parallelism between extractable heparin and mast cell content of certain tissues. Heparin is not only an anticoagulant but has also the ability to cause rapid clearing of the visible fat from the blood of lipæmic animals, as was first shown by Hahn[21]. Heparin is assumed to produce this effect by releasing a "clearing factor"[22,23], and recent experiments of Levy and Swank[24] show that heparin increases the esterase activity in blood and suggest that the "clearing factor" and the "esterase activator" are either one and the same or are closely interdependent. There is the possibility that the tissue mast cells normally secrete small amounts of heparin into the blood to maintain its fluidity and enhance fat metabolism.

According to Riley and West, as well as Graham, the tissue mast cells contain not only heparin but histamine as well. They point out that there is a close connection between the release of histamine and heparin, for instance in peptone and anaphylactic shock. In dogs histamine liberators also release heparin; but the problem is more complicated, because this does not occur in cats. Riley[25] injected a fluorescent liberator, stilbamidine, into rats and observed that some of it was temporarily trapped in the mast cells which then underwent disruption.

The histologists tell us that the mast cells are found particularly round the blood vessels; therefore, if the histamine in the tissues resides in the mast cells, it should be concentrated around the vessels. It is interesting that Miles and Miles[26] concluded from entirely different experiments a similar location for the histamine in the guinea-pig's skin.

They worked on capillary permeability and used the following method. A colloidal dye, pontamine sky blue, was injected intravenously; drugs were then injected intradermally into the animals with the circulating dye; if the drugs caused increased permeability, there was local exudation of dye shown by local blueing. With both histamine and 48/80 local blueing occurred, but there was this difference: with intradermal histamine local blueing occurred throughout the whole thickness of the skin; with

48/80 the blueing accumulated at two levels which corresponded to the two regions in the skin where the two plexuses of the vessels were situated. There were other observations which also suggested that the histamine was released from the regions of the small arteries and veins. So there appears to be a good correlation between tissue mast cells and histamine.

But even if the mast cell population reflects the histamine content of many tissues, not all tissue histamine originates in these cells and this was never claimed by Riley and West. Therefore we may still speak of the histamine of a given tissue when discussing the physiological function.

Histamine in Skin

The release of H-substance or histamine is the first reaction or "defence mechanism" of the human skin to any kind of injury or irritation and leads to the triple response. Whenever we encounter an uncomplicated triple response, the local redness, the wheal and the surrounding flare, and have not administered histamine itself, it is due to its release from the tissue cells. This was one of the fundamental conclusions which the late Sir Thomas Lewis drew from his classical experiments. He spoke of H-substance, probably histamine itself; I think, as far as the uncomplicated triple response is concerned, we can drop the term H-substance and can call it histamine. The triple response is a local reaction to locally released histamine in the skin; to-day we also have information about the reactions when the release in the skin is more general.

Fig. 5. Blueing of the skin after intravenous injections of 48/80 into guinea-pigs with circulating pontamine sky blue. The animals were killed after full blueing had developed, and depilated. (From Feldberg and Miles[27].)

When 48/80 is injected into an animal, the histamine released in the skin causes increased permeability of the skin capillaries at the site of its liberation; and if the consequent fluid exudation is sufficient, œdema results. If not, the increased capillary permeability can still be detected by exudation of a colloidal dye, for instance, pontamine sky blue. Skin areas in which the permeability of the capillaries is increased become quickly blue. The experiments with intravenous or parenteral injections of histamine liberators have brought out two new facts. (1) The increased permeability of the skin vessels, whether associated with œdema or not, does not occur evenly over the whole surface of the body, but shows

certain regions of predilection, and (2) these skin areas are characterised by a high histamine content.

I shall start with experiments on guinea-pigs in which the increased permeability of the capillaries does not cause visible œdema. Miles and I[27] injected 48/80 intravenously into guinea-pigs with circulating pontamine blue. The skin did not blue at once all over the body. Blueing started, and became intense, around the mouth, at the base of the ear, in the submental region; the blueing spread all over the head and neck region and was intense around the areolar area of the nipples; there was also some deep blueing in the perineum; but the trunk and hind legs were only faintly stained and the staining was often patchy. This is shown in Figure 5. In addition to these signs of increased permeability, 48/80 produced another effect on the skin, signs of severe itching.

If the degree of blueing is, as we assume, dependent on the increase in permeability of the capillaries, then these findings would represent a regional pattern of increased permeability of the skin capillaries after 48/80; and the question was, what is the cause? Miles and I determined the skin histamine in these regions and found that the regional pattern of blueing was, with one exception (the distal parts of the legs), directly related to the histamine content of the skin. The regional variations of the histamine in the guinea-pig's skin are given diagrammatically in Figure 6. Miles has shown that, in guinea-pigs sensitised with various antigens, the simultaneous injection of antigen and pontamine sky blue produces blueing which extends to the feet as well, so that in this condition the agreement is even better than after 48/80.

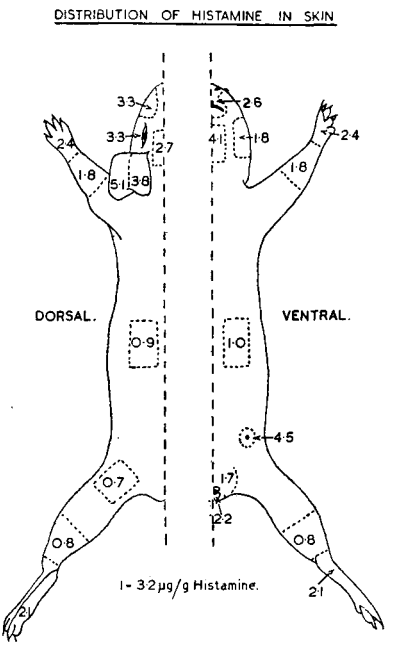

FIG. 6. Diagram of guinea-pig's body surface to illustrate distribution of histamine in various regions of the skin. The figures represent mean values for each region, the abdominal skin being taken as unity. (From Feldberg and Miles[27].)

Similar regional patterns concerning histamine content and proneness to increased permeability of the skin capillaries exist in those species in which 48/80 leads to skin œdema. For instance, Paton and Schachter[28] describe the œdema which occurs in dogs after subcutaneous injections of large doses of 48/80. There was facial sweling "particularly marked in the bristle area near the mouth, the eyelids, and the pinna of the ears. These areas also showed considerable erythema. Œdema and erythema of other regions were not observed except for the nipple area in one

animal". Miles and I found that the 5 skin regions with the highest histamine content in dogs were again the bristle-bearing area, the eyelids, the ears, the lips, and the areola of nipples; i.e., those regions which became œdematous in Paton and Schachter's dogs. Again the injections of 48/80 produced signs of severe itching.

One more species: the rat. Talesnik and I found that an intraperitoneal injection of 48/80 leads to œdema of a characteristic distribution, namely in the face, the ear, the back of the head, the perineal region and the paws, sometimes spreading over the whole leg, and again to signs of itching. Figure 7 illustrates the œdema of the mouth and paws in a rat after 48/80. Again, the skin regions which become œdematous after 48/80 are those which, in normal control animals, yield higher histamine values on extraction than the abdominal skin. However, although there is a direct relation in several species between skin histamine and proneness to increased capillary permeability, it has to be emphasised that other factors must not be neglected. For instance, the mechanical factor, the looseness of a tissue will determine the extent of œdema, provided increased permeability occurs.

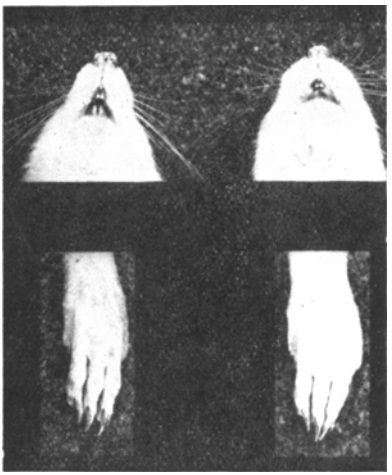

FIG. 7. Development of characteristic œdema in face and paws of a rat after intraperitoneal injection of 48/80; on the left, rat before, on the right, after 48/80.

Apart from regional differences in skin histamine, there are great species differences in the histamine content of the skin. The meaning of these differences is not clear, but as far as the evidence is available, the regional differences seem to occur, at least to a certain extent, whether the general level of skin histamine of a species is high or low. The question arises whether similar regional differences in skin histamine exist in the human and, if so, whether they can be related to regional differences of allergic and other dermatological manifestations.

To return to the development of the characteristic distribution of œdema which occurs in dogs, cats, and rats after the injection of histamine liberators. Are we really justified in attributing this œdema to histamine release, because we cannot imitate the effect by injections of histamine itself? I think we are: these effects are effects of histamine acting locally at the site of release. The evidence for this view is twofold. The effects are abolished by antihistamine drugs, and the effects no longer occur when the skin is depleted of its histamine. In rats, a dose of 48/80 which on first injection causes severe œdema of the characteristic distribution and itching becomes ineffective after several injections; but at this stage

the rats respond again to larger doses of 48/80 until these also become ineffective on repeated administration. When the rats are killed in this condition, the skin histamine is found to be low, as illustrated in Figure 4.

A rat with depleted skin histamine is not only insensitive to 48/80 but also to other agents which cause the characteristic distribution of œdema with itching.

For instance, Selye[29] had shown that rats are hypersensitive to egg white which, on its first intraperitoneal injection, that is in the non-sensitised animal, leads to itching and œdema in the face and paws. The rat, when treated with egg white, is practically indistinguishable from that treated with 48/80.

Schachter and Talesnik[30] showed that egg white releases histamine in rats, and when Talesnik and I[15] tested the effect of egg white on rats depleted of their skin histamine, the œdema no longer occurred. The rats had become resistant to egg white, and we concluded there was no longer sufficient histamine available in the skin to be released by the egg white and to cause an increased capillary permeability at the site of release.

When these results were communicated to the Physiological Society, Sir Henry Dale suggested that we also test the effect of light in photo-sensitised rats after treatment with 48/80. When normal rats were exposed to the light from a carbon lamp 20 hours after an intraperitoneal injection of hæmatoporphyrin, they became restless and started to scratch violently. One group of rats then became listless and weak and was found to sit with ruffled fur and intense cyanosis in their cages; some of these rats died. The other group of rats developed œdema in the face, head, ears, and in the paws, as after 48/80 or egg white.

On the other hand, rats after prior treatment with 48/80 and depletion of their skin histamine, when injected with hæmatoporphyrin were found to show a definite resistance to light. Apart from some scratching during the exposure to light, some of the rats showed no further signs; other rats became slightly swollen in the ears, but all other effects seen in the control rats were absent, and none died. So at least two kinds of skin phenomena closely related to allergic reactions can be more or less prevented in rats in which the skin histamine has been greatly reduced.

In the œdema produced in rats by 48/80, egg white or light, we encounter a phenomenon which is due to histamine but which cannot be reproduced by intravenous histamine injections, because they do not imitate the local effect which histamine exerts at the site of its release. I want to go even further. The characteristic distribution of œdema associated with severe itching which is seen in rats after 48/80 and egg white, although not reproducible by injected histamine, is probably as characteristic for histamine release in the rat's skin as is the triple response in the human skin. Whenever we encounter this characteristic œdema distribution with itching, we may assume that the agent which produced it acted by release of histamine. In each species so far examined, cats, dogs, and mice, there is a pattern of skin reactions, not quite the same as in rats but very similar, which is also apparently typical of histamine release in the skin and associated with the regional variations of skin histamine.

Histamine and Itching

In all the animals so far examined, the histamine released in the skin causes severe itchiness and there is this problem: is release of histamine the main physiological stimulus for itching? If we ask this question, we naturally mean it to apply only to itching originating in the skin, because there is this question: is there itching of central origin; have we a central representation for itching? Sherwood and I[31] have recently studied, in the unanæsthetized cat, the effects of anticholinesterases injected into the lateral brain ventricle through a permanently implanted cannula and found that eserine, as well as di*iso*propyl phosphorofluoridate, produced intense scratching of head and face, wiping of the front legs over the face, and licking of the front legs. The cats gave every appearance of tremendous itching, particularly in the face, head, and forelegs. These effects were certainly central effects due to an action of the anticholinesterases on the nerve cells situated on the floor of the fourth ventricle and probably also of the third.

When we consider the problem of whether histamine is the main physiological stimulus for itching, we naturally mean only itching originatin the skin. Everyone who is himself allergic and has once undergone treatment with desensitising pollen extracts and has by chance been given too high a dose, or has seen patients in whom this has happened, will agree that the itching brought about by such an injection, which leads to release of skin histamine, is one of the most effective means of producing a nearly unbearable condition of itchiness over the whole body surface.

The possibility that histamine may be a general physiological stimulus for itching has been envisaged by Schachter[32] and by Broadbent[33]. Schachter tested whether bile salts release histamine, because of the well-known phenomenon of pruritus in jaundice, and he found that bile salts do liberate histamine.

Histamine injected intradermally causes itching, but at the same time a flare and a wheal. Itching, however, can occur unassociated with these skin reactions. Broadbent, who worked in Bain's laboratory in Leeds, has tried to overcome this difficulty. He examined the mechanism of itching powder which consists of the hairs of a plant, cowhage, containing very fine spikes which make minute mechanical wounds. His results suggest that these spikes contain a histamine liberator which can be extracted from the mechanical minute needle-like instruments. The powder no longer produces itching once it is deprived of its histamine liberator. The inactive powder can, however, be reactivated by bringing it into a concentrated solution of a histamine liberator, for instance, morphine. Glass wool can also be converted into an effective itching powder when first treated with a histamine liberator. Itching so produced is not always associated with the full development of the triple response. It may thus be that histamine released in minute concentrations near the specific sensory nerve endings, i.e., in the superficial layers of the epidermis, can produce itching without bringing about the triple response. The

epidermis contains, in fact, a 6 times higher concentration of histamine than the dermis[34].

As Sherwood and I[31] pointed out, it will not always be easy to assess whether the itching produced on systemic administration of a drug is a central or a peripheral effect, for a drug may produce itching when applied to the ventricular system and be a peripheral histamine liberator as well. For instance, morphine is a known histamine liberator and by this action produces itching originating in the skin, but it also causes itching on cisternal injection in doses which would not allow sufficient amounts to reach the general circulation for producing the effect in the skin[35,36,37]. Therefore, the well-known pruritus in man after morphine administration may, in some cases, be of peripheral origin, in others of central, and again in others be a combination of both mechanisms. Similar considerations apply to the pruritus seen in obstructive and hepatic jaundice.

Histamine in Skeletal Muscle, Liver, and Lung

The histamine in skeletal muscle is as easily released by histamine liberators, and under the same conditions, as the skin histamine, but we do not know whether it serves the same "defence mechanism" in this tissue and whether its release is the first reaction to any kind of injury. The finding by Anrep and Barsoum[38] that it is released from the contracting muscle has not found general acceptance. Histamine would not only act directly on the muscle vessels but, as in the skin, it seems able to produce dilatation of the muscle arteries by an axon reflex. In fact, Fleisch[39] showed that it is a very active agent in producing such an effect, or as he called it, such a nutritive reflex.

I have little to say about the histamine in liver and lung. In dogs the liver is the main "shock organ" for the release of histamine in anaphylactic and peptone shock. About the mechanism of this release we have very little definite knowledge. We know from the work of Rocha e Silva and his co-workers[40,41,42] that the antigen-antibody reaction, as well as peptone, produces a strong release of histamine from the dog's liver only in the presence of fresh blood to which no heparin has been added but which has not clotted. There is this problem: does the antigen-antibody reaction cause the release of histamine by the formation of an intermediary histamine liberator? The same question applies to the release of histamine by peptone. No convincing evidence has yet been brought forward for this attractive suggestion. In fact, it is not even certain, as Paton and I pointed out[9], whether the "shock organs" of anaphylaxis are the same as those on which the known histamine liberators exert their main action. This problem has recently been taken up by Schachter[43] in the rabbit, and he found a striking difference. Whereas antigen caused the release of histamine from the perfused liver, 48/80 failed to do so.

It is astonishing how little we really know for certain about the function of histamine in liver and lung. If we assume that the release of large amounts of histamine and of heparin during anaphylaxis from liver and lung is nothing but an exaggeration of a normal process, we should have

to assume that there is not only a constant release of heparin but also of histamine. This comes, perhaps, from the tissue mast cells and may not only maintain the fluidity of the blood and enhance fat metabolism, but may also help vascularisation and other local regulatory mechanisms.

One cannot help feeling that in the past we have concentrated so much on obtaining evidence for the release of histamine in the antigen-antibody reaction of anaphylaxis and on assessing the role of histamine in the symptomatology of anaphylactic shock, that in doing so we have forgotten to think what role, if any, the release of histamine may have in these organs under physiological conditions.

Histamine in the Wall of the Digestive Tract

The wall of the stomach and intestine has a high content of histamine which is apparently very resistant to the known histamine liberators, because it has not been possible, with these compounds, to release more than a fraction of the total histamine. In those species, like dog and cat, in which it is easy to separate the various layers of the wall, histamine is found in all of them but the greater part resides in the mucosa, whereas in the muscularis externa the histamine is present in relatively low concentrations. What then is the function of histamine in these different layers?

Muscularis Externa. Does the presence of histamine in this layer signify a motor function of histamine in the wall of the gastro-intestinal tract, at least in those species in which the muscles of the wall are sensitive to histamine? If so, one might expect this action to be accentuated by the administration of histamine liberators. So again these compounds provide a useful tool with which to re-examine the problem of the role of histamine in motor activity of the gastro-intestinal tract. Smith and I[44] have carried out experiments from this point of view on the guinea-pig's ileum preparation, using mainly compound 48/80 as histamine liberator.

The guinea-pig's ileum is very sensitive to histamine, but rather large doses of 48/80 are required to produce motor effects. This might seem strange because 48/80 is such a potent liberator; we must, however, remember the great resistance of the histamine in the wall of the digestive tract to histamine liberators.

With large doses of 48/80, two motor effects were regularly obtained: an immediate contraction when the 48/80 was added to the bath, which passed off as soon as the 48/80 was washed out again, and secondly, an after-effect; the previously quiescent gut began to exhibit motor activity which increased after each administration of 48/80; in addition, the tone of the longitudinal muscle gradually increased and the intestine shortened. These two effects are illustrated in Figures 8 and 9. In Figure 8 the immediate contractions are seen; the 48/80 is given every 30 minutes and washed out after 1½ minutes. The after-effects are omitted. These are shown in Figure 9, which, on the other hand, omits the immediate, direct contractions. How far can all these motor effects be accounted for by release of histamine?

Each time the bath fluid is replaced the muscle relaxes but the motor

activity only diminishes, as seen in the experiment of Figure 9 at (w). The relaxation of tone must be explained by the accumulation of a muscle-contracting substance in the bath fluid. We know that choline and acetylcholine continuously diffuse from the wall of the intestine into the bath fluid, but the substance released after 48/80 cannot be choline or acetylcholine, because the effect also occurs on the atropinised preparation. In fact, the experiment of Figure 9 was on an atropinised preparation.

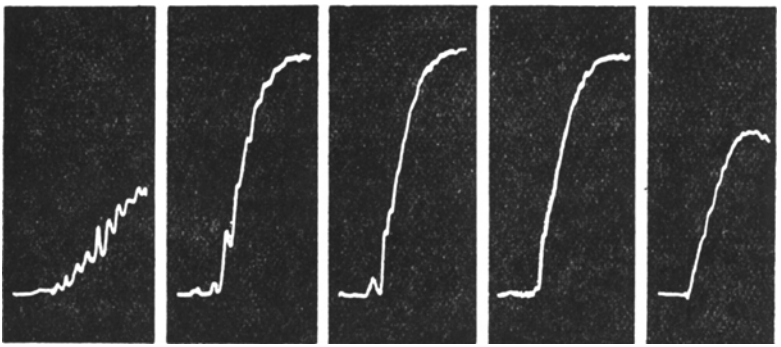

FIG. 8. Guinea-pig's ileum preparation suspended in 15 ml. of magnesium-free Tyrode solution containing 0·04 μg. of atropine sulphate. Contractions to 5 successive applications, at 30 minute intervals, of 2 mg. of 48/80 kept in the bath for 90 seconds. (From Feldberg and Smith[44].)

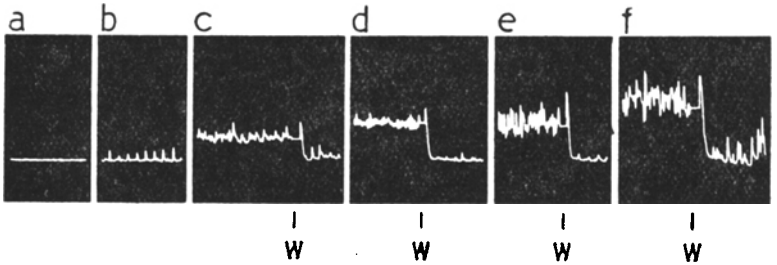

FIG. 9. Guinea-pig's ileum preparation suspended in 15 ml. of magnesium-free Tyrode solution containing 0·04 μg. of atropine sulphate. Development of motor activity and tone after successive doses of 2 mg. of 48/80, the direct contractile effects of which were omitted from the tracing, (a) before, (b to f) after the successive applications of 48/80. At (w) the bath fluid was siphoned off whilst the lever was arrested. (From Feldberg and Smith[44].)

The substance diffusing into the bath fluid after 48/80 and causing the development of tone behaves like histamine and is probably histamine itself. It is therefore not surprising that small doses of mepyramine produce the same relaxation of the muscle and reduction of its spontaneous activity as replacement of the bath fluid. Figure 10 shows the output of histamine per minute from an intestinal preparation after two administrations of 48/80 during the development of activity and tone. The figure was obtained by syphoning off the bath fluid, after the 48/80 had been washed out, every 30 minutes, measuring its volume and assaying it against histamine on another atropinised guinea-pig's ileum preparation.

The histamine which diffuses into the bath fluid certainly fully accounts for the development of tone, but what about the motor activity which is such a characteristic after-effect not only of 48/80 but of other histamine liberators as well, and which is not abolished by replacement of the bath fluid or by mepyramine which relaxes the tone. Smith and I suggested that the increased activity is also an effect of histamine but not of the histamine after it has diffused into the bath fluid, but when it acts at the site of its release within the muscle coat. Replacement of the bath fluid would not remove this histamine, and mepyramine added to the bath might be unable to reach this "intrinsic" histamine acting within the muscle coat at the site of release. By making this distinction between the action of "intrinsic" and "extrinsic" histamine, all motor effects of 48/80 on the guinea-pig intestine could be accounted for by release of histamine.

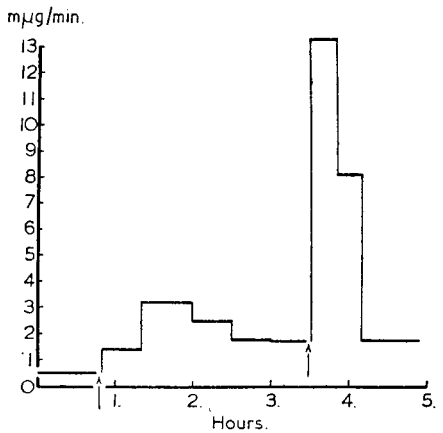

FIG. 10. Histamine output in mμg./minute from guinea-pig's ileum preparation after two consecutive administrations of 2 mg. of 48/80 at the arrows. (From Feldberg and Smith[44].)

When we discuss the possible role of histamine in motor intestinal activity, we must not forget that atropine-resistant motor activity occurs also in intestinal preparations which are insensitive to histamine, for instance, the rabbit or rat intestine, and it is interesting to note that the intestinal preparations from these species are also insensitive to 48/80. Histamine is not the only smooth muscle-stimulating substance which is found in the wall of the digestive tract and which, when released, could account for atropine resistant motor activity. von Euler[45], for instance, suggested the release of substance P, a polypeptide, as the cause of the spontaneous movements of the intestine. Since the smooth muscle stimulating effects of substance P are resistant to both atropine and mepyramine, the fact that the spontaneous movements of the intestine are also resistant to these drugs would be satisfactorily explained by this theory. We are at present unable to say under which special conditions the various pharmacologically active substances present in the intestinal wall are released and what function they serve. We cannot even say if the release of histamine by 48/80 is an accentuation of a physiological phenomenon or if we imitate with this method pathological changes, for instance, allergic reactions and related phenomena. It is feasible to assume that the histamine in the muscular wall is released in allergic conditions, as it is in the skin; there it produces a triple response, here increased motor activity.

Mucosa. Recently Harris and I[46] have tried to localise the mucosal histamine of the dog's gastro-intestinal tract more exactly in terms of

histological structure. For this purpose we flattened pieces of mucosa on the freezing microtome and cut the frozen pieces in the horizontal plane in serial sections. The frozen sections were either weighed, extracted, and assayed for histamine, or stained and examined histologically. From the results "histamine profiles" were constructed in which the ordinates represented histamine content of the sections and the abscissæ position and thickness of the sections.

Figure 11 shows histamine profiles of mucosa plus submucosa of the body and the pyloric region of the dog's stomach, and, placed below, microphotographs of transverse sections through these regions.

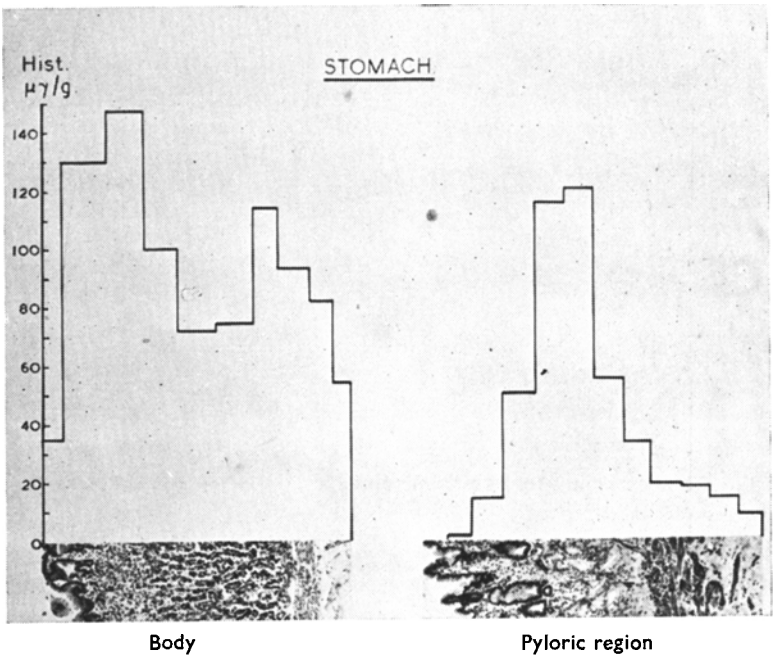

FIG. 11. Histamine profiles and microphotographs of mucosa and submucosa from the body and the pyloric region of the stomach of the same dog. (From Feldberg and Harris[46].)

The main structures of the mucosa of the body in passing from the lumen towards the submucosa are: surface epithelium—gastric pits—glands (towards the neck of the glands parietal cells mainly; deeper parts consisting mainly of zymogenic cells)—smooth muscles (muscularis mucosæ)—connective tissue (submucosæ). The histamine profile shows two peaks: one is located in the region of the parietal cells, the other in the region of the muscularis mucosæ. There is also some histamine in the connective tissue of the submucosa. It may reside here in the mast cells. In the pyloric region of the stomach, the mucosa has only one peak located in the region of the pyloric glands. On the other hand, the histamine profile of the duodenum shows again two peaks: one near the lumen in the region of the villi and one near the submucosa in the region

of the muscularis mucosæ (Fig. 12). The overall histamine content of mucosa was found to decrease in passing from stomach to colon, but the mucosal glands retained a relatively high histamine content throughout.

What do these facts mean when considering the physiological or pathological role of the mucosal histamine in the wall of the digestive tract? I can envisage four possible ways in which to account for the presence of histamine in the mucosa of the digestive tract.

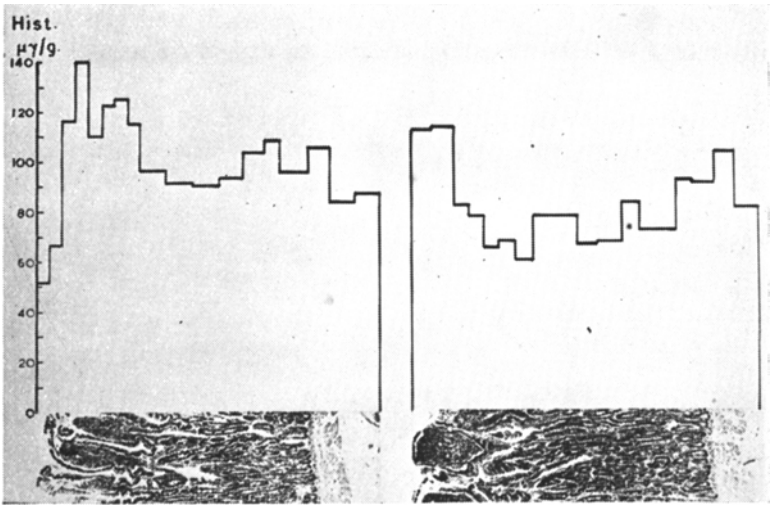

FIG. 12. Histamine profiles and microphotographs of mucosa and submucosa from the duodenum of two dogs. (From Feldberg and Harris[48].)

(1) *Absorption of histamine from the lumen of the digestive tract.* Intestinal bacteria form histamine from histidine, and many food substances contain histamine. Thus the histamine in the mucosa could be the result of absorption from the contents. Probably part, but only part, of the mucosal histamine is accounted for in this way. In fact, Wilson[47] has obtained some evidence in rats that the histamine in the mucosa of the small intestine is in part due to absorption from bacterial histamine in the intestine. But this mechanism would scarcely explain the histamine of the stomach mucosa, because there are few and probably no histamine decarboxylating bacteria in the stomach. Furthermore, the stomach is not an absorbing surface; and lastly, it is difficult to visualise that the histamine which is absorbed passes through the whole thickness of the mucosa. The absorbed histamine might well be found in the surface epithelium of the villi and its presence in this region of the duodenum, for instance, could be accounted for in this way; but how could it reach the deeper layers? Once absorbed it would be transported away into the blood or lymph stream. But even if one were to assume that the absorbed histamine could diffuse freely into the deeper layers of the mucosa, it is difficult to see how it could escape destruction by the histaminase present

in such high concentrations in the intestinal mucosa. Therefore, in my opinion, absorption can only be a minor contribution to the source of the intestinal mucosal histamine of the digestive tract.

(2) *Release of histamine: the first reaction to injury.* Does the histamine in the mucosa serve the same function as it does in the skin, and does

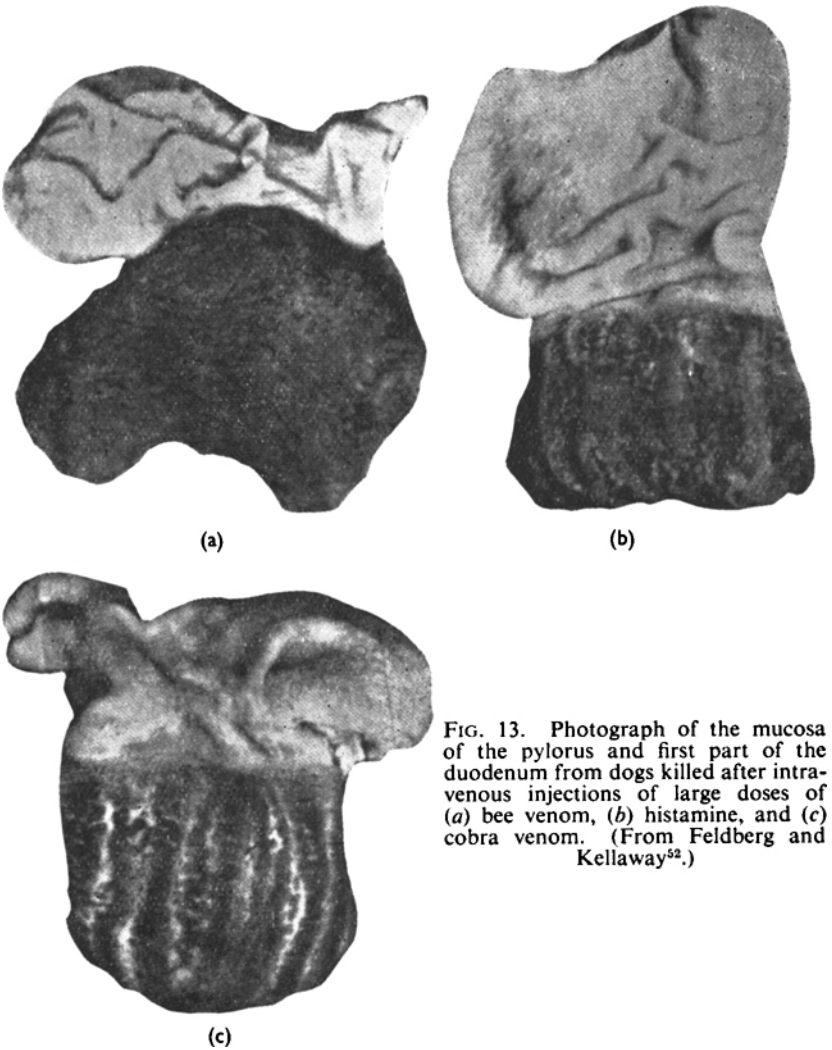

FIG. 13. Photograph of the mucosa of the pylorus and first part of the duodenum from dogs killed after intravenous injections of large doses of (*a*) bee venom, (*b*) histamine, and (*c*) cobra venom. (From Feldberg and Kellaway[52].)

its release represent the first reaction of the mucosa to any form of injury or irritation? Unfortunately we lack experiments on the mucosa similar to those carried out by the late Sir Thomas Lewis on the human skin. I think Figure 13, however, has a bearing on the problem. It shows the appearance of intense hæmorrhagic congestion in the mucosa of dogs after intravenous injection of bee venom (*a*), cobra venom (*c*), and large

doses of histamine (*b*), and raises the question of whether this reaction of the mucosal vessels to histamine represents something akin to the triple response of the human skin. On microscopic examination, the capillaries in the villi are found to be distended with blood. These changes do not occur in the stomach mucosa and are most intense in the duodenum; they diminish in intensity as we proceed distal to the ileum and colon.

(3) *A local physiological secretogogue.* Histamine is one of the strongest secretory stimuli for acid secretion of gastric juice; it also causes secretion of mucus in the stomach and, which is often forgotten, it is a powerful stimulus for secretion of succus entericus. It could therefore well play a physiological role in the secretion of these juices. The possibility that it is released as an intermediary substance in the nervous and hormonal secretion has been envisaged by various authors, at least for the secretion of gastric juice. MacIntosh[48] long ago tried, however without success, to obtain evidence that vagus stimulation causes gastric secretion *via* the release of histamine, and Emmelin and Kahlson[49] and Kahlson[50] suggested that the hormone gastrin acts *via* the release of histamine. This problem has recently been taken up by Smith[16], who could show that gastrin is a weak general histamine releaser and exerts this property also on the gastric mucosa. Whether release of histamine is the full explanation of the secretory effect of gastrin, however, is not yet certain.

(4) *Histamine excretion from the mucosa.* If we assume that small amounts of histamine are continuously released from various parts of the body and enter the circulation, the question arises as to how it is inactivated so as not to accumulate and to produce its general toxic effects. The main mechanism is probably enzymic destruction and excretion in the urine as conjugated histamine. But, in addition, part of the histamine might also be excreted with the gastro-intestinal juice, then acetylated in the intestinal lumen and thus inactivated. According to this view, which was envisaged by Smith[51], the histamine in the mucosa, or at least in the region of the gland cells, would represent a pre-excretory stage and the strong secretory action of histamine would serve the purpose of getting rid of surplus histamine entering the circulation. There is certainly one finding which would be accounted for on these lines. Gastric juice, whatever the stimulus for its secretion, always contains histamine (Emmelin and Kahlson[49]).

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