Release of histamine from skin and muscle in the cat by opium alkaloids and other histamine liberators / by W. Feldberg and W.D.M. Paton.

[*Reprinted from the Journal of Physiology*,
1951, Vol. **114**, No. 4, p. 490.]
PRINTED IN GREAT BRITAIN

J. Physiol. (1951) 114, 490–509

RELEASE OF HISTAMINE FROM SKIN AND MUSCLE IN THE CAT BY OPIUM ALKALOIDS AND OTHER HISTAMINE LIBERATORS

By W. FELDBERG and W. D. M. PATON

From the National Institute for Medical Research, Mill Hill, London, N.W. 7

(*Received* 5 *February* 1951)

A number of widely different chemical compounds have the common property of releasing histamine from mammalian tissue without producing gross structural change (MacIntosh & Paton, 1949; Paton, 1951). These substances were called 'histamine liberators'. The present experiments are a continuation of their work and deal in the main with two new aspects of this subject. It has been shown that the opium alkaloids and the morphine derivative, apomorphine, have to be added to the class of histamine liberators. Furthermore, an identification of the tissues from which the histamine is released was begun. It was found in perfusion experiments that, in cats at least, histamine is released from muscles and skin by histamine liberators.

The possibility that morphine and other opium alkaloids release histamine was considered but rejected in 1917 by Sollman & Pilcher. Later however, Lewis (1927), found that strong concentrations of morphine pricked into the human skin elicited the triple response, and therefore concluded that morphine caused the release of histamine or H-substance in the human skin.

Evidence for the release of histamine by opium alkaloids was obtained independently at the same time by Nasmyth & Stewart, and their results as well as ours were shortly reported to the Physiological Society (Nasmyth & Stewart, 1949; Feldberg & Paton, 1949). A short account of the release of histamine from the perfused skin by various histamine liberators has also been communicated by us to the International Physiological Congress (Feldberg & Paton, 1950).

METHODS

All experiments were done on cats anaesthetized with chloralose. In those experiments in which the blood pressure was recorded, a mercury manometer was connected to a siliconed cannula tied into the carotid artery; clotting was prevented by the addition of heparin in the cannula. When samples of plasma were required, blood was withdrawn from the femoral artery into a heparinized syringe and at once centrifuged. The routine assays of histamine were done on the guinea-pig's ileum preparation suspended in a 15 ml. bath of Tyrode solution at 35° C. The values for histamine are expressed as base, but the doses of the liberators refer to the following salts: propamidine isethionate, D-tubocurarine chloride, morphine sulphate, apomorphine chloride, thebaine chloride, codiene sulphate, papaverine chloride, antrycide methylsulphate, compound 48/80 from

Burroughs Wellcome as chloride. The atropine was used as sulphate, acetylcholine as chloride, and mepyramine as maleate (Anthisan, May and Baker).

Perfusion of gastrocnemius muscle. The isolated muscle was perfused with Tyrode solution from a cannula tied into its artery, and the venous effluent collected from a venous cannula. The skin of the leg was cut from the popliteal region to the heel. The hamstring muscles were divided so that the knee joint could be hyperextended and the popliteal space made more accessible. All branches of the popliteal vessels not supplying the gastrocnemius muscle were then ligated and cut. The Achilles tendon was cut, the gastrocnemius separated from soleus and reflected upwards in order to ligate the vessels below the origin of the vessels to the gastrocnemius, after they have passed deep between the two heads of the muscle. The two heads were separated from the femoral condyles; the medial one was ligated, as near to its attachment as possible, and cut; the lateral one, having a tendinous attachment, could usually be detached without ligation by cutting through the cartilage. Care was taken to keep the muscle warm during the whole preparation and prevent it from drying by covering the muscle with cotton-wool soaked in warm paraffin. The arterial and venous cannulae were inserted only after the muscle had been detached; until this time its natural blood supply was maintained. The muscle was then transferred into a Petri dish filled with warm liquid paraffin, and the histamine liberators injected after the venous effluent had become free of blood and control samples had been collected. The perfusion pressure required for a flow of between 1 and 3 ml./min. was between 20 and 30 cm. saline solution. At the end of the perfusion, indian ink was injected through the artery to verify the extent of the perfusion. Apart from a small fascicle in the middle of the deep surface, the muscle was usually stained black throughout; occasionally the ink had penetrated only feebly to the distal parts of the muscle near the origin of the Achilles tendon. In a few experiments the non-perfused, or badly perfused, parts were dissected out and weighed; they never exceeded 15 % of the muscle weight. The volume of the muscle, in ml., was taken as equal to its weight in grams; this approximation overestimates the volume by about 7 %.

Skin perfusion. Large cats weighing between 3 and 4·5 kg. were used. An area of skin from the right hindleg, supplied by the saphenous artery was perfused with Locke solution. After shaving and marking out the area of skin to be isolated, the skin was divided with a thermocautery by two incisions right round the leg, one about 4–6 cm. above the knee joint, the other about 2 cm. above the ankle. The two incisions were joined by a further incision along the lateral aspect. At the border of the lower, circular incision, the saphenous vein was tied and cut. The skin was then dissected off from the underlying muscles and fascia, starting from the longitudinal incision and working from both sides to the centre of the piece of skin where the saphenous artery and vein enter and leave it. Anastomostic vessels from the popliteal space, the gluteal region, and the lateral aspect of the ankle were ligatured and cut. In addition, a few smaller vessels penetrating the muscles required ligature.

In order to prevent spasm of the saphenous artery, the preparation throughout the dissection was kept warm by radiant heat from a bowl fire, and drying of the inner surface of the dissected skin was prevented by repeated flooding with warmed liquid paraffin.

The saphenous artery and vein up to the femoral vessels were carefully isolated. Because of the length of this vascular trunk, handling the saphenous vessels was avoided to prevent spasm, and the saphenous nerve was left undissected with the vessels so that it gave additional support and protection. A length of the femoral artery and vein, 2 cm. above and 1 cm. below the junction with the saphenous vessels, was dissected free by ligating and cutting all side vessels. The dissected patch of skin with its vessels still attached to the animal was packed in warm saline swabs and kept warm by radiant heat for 30–60 min. to allow any arterial spasm to pass before perfusion was started.

For the perfusion the Locke solution passed from a reservoir whose heignt could be adjusted, through a hot water jacket near the injection cannula, to which it was connected by thick-walled rubber tubing through which the injections of the histamine liberators were made. A small, vertical side arm on the injection cannula served to trap any bubbles in the perfusion fluid. The

femoral artery below the origin of the saphenous was ligated and cut, the cannula was tied into the femoral artery above the saphenous, perfusion at once started, and the femoral artery divided above the cannula. Another cannula was tied into the femoral vein in the same way above the saphenous for the collection of the venous effluent. The entirely isolated patch of skin with its cannulated vessels was transferred to a paraffin bath warmed by a water jacket. The skin was spread out with its inner surface upwards on a perforated, immersed, celluloid platform, so that it was possible to observe the washing out of the blood from the perfused parts of the skin, as well as the formation of oedema if it should occur. The paraffin bath used was funnel-shaped, with an outlet at the bottom, so that any fluid escaping from the skin could be collected. It usually amounted to a few ml. only. Warm water was kept circulating through the water jacket near the injection cannula, and through that surrounding the paraffin bath by an 'air lift'. By this means the temperature of the fluid perfusing the skin and of the paraffin surrounding it was kept at about 37° C.

The perfused skin weighed between 15 and 30 g. The perfusion pressure was adjusted so as to maintain a venous outflow of 2–4 ml./min. For this purpose the pressure required ranged between 60 and 200 cm. of water, usually about 100 cm.

The histamine content of samples of the skin from the perfused side and corresponding areas on the other limb was determined. The skin was freed from the underlying subcutaneous tissue and, after weighing, cut up in 2 ml. N-HCl/g. skin, and ground in a mortar with sand. This procedure does not fully macerate the tissue. This is achieved as follows: the partly macerated tissue is transferred into another mortar, again cut up and reground after the addition of distilled water (about 10 ml./g. tissue). The contents are returned to the original mortar, again ground and brought into a flask. The mortars are washed several times with saline solution and the washings added to the flask, which is boiled for a minute or two. The flasks are then stored in the cold until the assay is carried out (usually after 24 hr.). For this purpose they are centrifuged or filtered, the residue washed twice with saline solution, the supernatant or the filtrate respectively and the washings mixed, neutralized with N-NaOH, made up to a given, suitable volume and then assayed for histamine on the guinea-pig's ileum preparation.

RESULTS

The vaso-depressor action of morphine and codeine in the cat

Both morphine and codeine, when injected intravenously, cause a fall in arterial blood pressure. The effect does not start during the first few seconds after the injection, but only appears after a latency of 15–20 sec. which, as shown by MacIntosh & Paton (1949), is characteristic for histamine liberators. As seen from Fig. 1, the fall after a large dose of morphine (5 mg.) is steep, whereas after a smaller dose (1 mg.) it develops gradually. Once the blood pressure has fallen after morphine, recovery is either absent or much delayed, even with small doses of morphine. This prolonged action is unlike that of other histamine liberators. For example, after the injection of 0·5 mg. propamidine the immediate depressor action was greater than after 5 mg. of morphine, but the blood pressure was restored within 5 min. After the injection of 5 mg. of morphine, recovery had not set in 45 min. later, when the blood pressure was restored by the intravenous injection of dextran.

Large doses of morphine caused stoppage of respiration, so that artificial ventilation was required. After codeine (5 mg.) there were convulsive movements.

Plasma withdrawn during the first minutes after a large dose of morphine contained more histamine than normal plasma. The results of two experiments are given in Table 1. The histamine of the plasma was assayed on the cat's blood pressure and identified by the following tests:

(1) The depressor action was resistant to atropine, but abolished by mepyramine.

(2) The plasma caused a contraction of the guinea-pig's gut.

(3) The depressor and gut-contracting action of a given sample of plasma corresponded approximately to the same histamine equivalent.

The concentration of histamine in the plasma, even after large doses of morphine, was much smaller than that found after propamidine (MacIntosh & Paton, 1949). Release of histamine into the blood stream therefore can account only partly for the depressor action of morphine. This conclusion agrees with the observation that the intravenous injection of 1 mg./kg. of mepyramine did not produce even partial recovery of the persistently low blood pressure, whereas mepyramine has this action on the depressor effect of other histamine liberators (MacIntosh & Paton, 1949). Further, if large amounts of histamine had been liberated, we should also expect regular and extensive haemorrhages in the subendothelium of the heart and in the mucosa of the small intestine (Feldberg & Kellaway, 1937), as well as haemoconcentration (Dale & Laidlaw, 1918). Although such haemorrhages were, in fact, found in most experiments, they were never more than slight, and consisted of one or a few subendocardial haemorrhages and sometimes also a few haemorrhagic patches in the duodenal mucosa. Haemoconcentration did not occur.

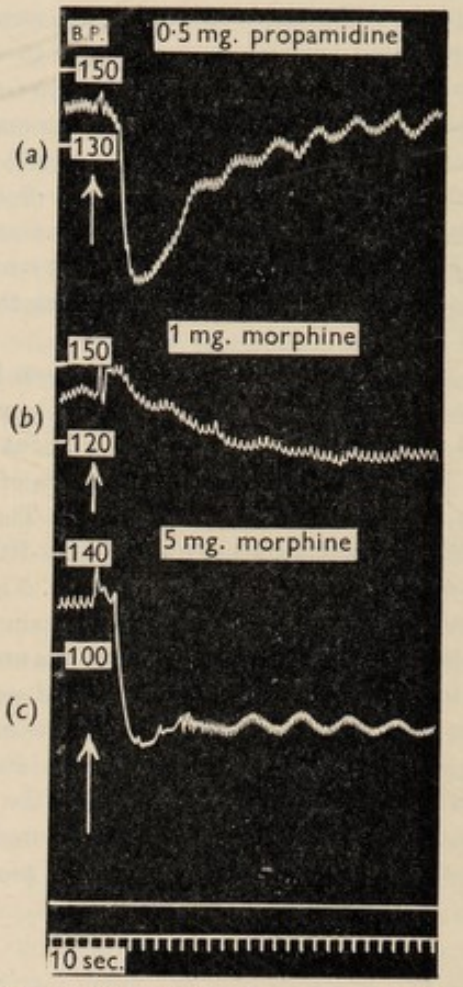

Fig. 1. Arterial blood pressure of 2·1 kg. cat in chloralose anaesthesia. Intravenous injections of 0·5 mg. propamidine (at *a*), of 1 and 5 mg. morphine (at *b* and *c*). Time in 10 sec.

TABLE 1. Histamine in cat's plasma (μg./ml.)

Exp. no.	Before morphine	Minutes after 20 mg./kg. morphine			
		2	4	7	10
1	0·02	0·13	0·08	—	0·08
2	0·025	0·115	—	0·005	—

Effects of histamine liberators on perfused cat's gastrocnemius muscle

The venous effluent collected for 5–10 min. before the injection of histamine liberators either had no effect when tested on the guinea-pig's ileum, or caused a contraction corresponding to a histamine content of less than 0·01 μg./ml. But, after the injection of histamine liberators, it caused a vigorous contraction of the intestine and had a histamine equivalent of 1 μg./ml. or higher.

The gut-contracting substance in the venous effluent was identified as histamine by the following tests. Its action was not abolished by atropine but was abolished by small doses of mepyramine. When the mepyramine was

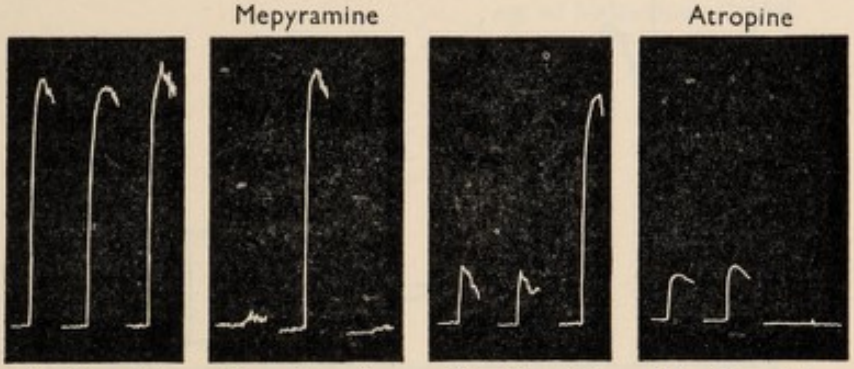

Fig. 2. Contractions of guinea-pig's ileum preparation in 15 ml. Tyrode solution. S., 0·045 ml. of effluent from cat's gastrocnemius after injection of 5 mg. thebaine (Exp. 10, Table 3); Hi., 0·04 μg. histamine; Ac., 0·03 μg. acetylcholine. At (*b*) in the presence of, at (*c*) during recovery from, 0·2 μg. mepyramine; at (*d*) in the presence of 0·4 μg. atropine.

washed out, the sensitivity of the preparation to histamine and to the substance in the perfusate returned together. This is illustrated in Fig. 2, which shows equal contractions produced by perfusate (0·045 ml.), by histamine (0·04 μg.) and by acetylcholine (0·03 μg.). After the addition to the bath of 0·2 μg. mepyramine, the contractions produced by histamine and perfusate were both practically abolished, whereas acetylcholine exerted its previous effect. The mepyramine was then washed out, and there was a partial and equal return of the effect of histamine and perfusate. These responses were not affected by a dose of atropine, which abolished the much stronger acetylcholine contraction. Similarly, when the intestinal preparation had been rendered relatively insensitive to histamine by previous exposure to a large dose of histamine, the activity both of histamine and the sample were equally reduced, and, as the gut recovered its sensitivity, the response to histamine and to the perfusate returned together (Barsoum & Gaddum, 1935). Further, the perfusate caused a fall of arterial blood pressure in the cat. When the histamine equivalent of a given sample was assayed on the cat, it corresponded to that obtained in the assay on the guinea-pig's ileum. The depressor effect of the sample resembled

histamine in its sensitivity to mepyramine and its resistance to atropine. Finally, during the assay on the cat's blood pressure, it was noted that the perfusate reproduced also the action of histamine on the suprarenal medulla, since the depressor effect was followed by a rise in blood pressure with cardio-acceleration. Since by all these tests the active substance in the perfusate was indistinguishable from histamine, it will be referred to as such.

Propamidine. In the first experiments, propamidine was used as a prototype of a strong histamine liberator. Its injection into the artery of the perfused muscle caused vasoconstriction and release of histamine. Vasoconstriction

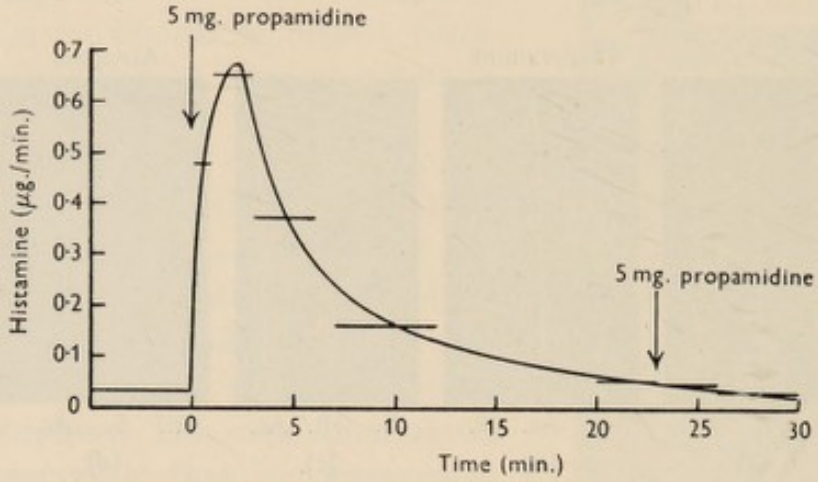

Fig. 3. Histamine release from perfused cat's gastrocnemius muscle after arterial injections of propamidine. Horizontal lines indicate mean values of histamine release in μg./min. during the periods shown by the lengths of these lines.

occurred within a few seconds after the injection and could cause cessation of outflow for several minutes, and in some experiments even for longer. It is probably an effect of the released histamine, since histamine was found to be vasoconstrictor in this preparation, and, in addition, there was a certain parallelism between vasoconstriction and the concentration of histamine found in the perfusate. For instance, when a second injection of propamidine failed to release histamine (see later), vasoconstriction was absent or trivial.

Fig. 3 shows the release of histamine by an injection of 5 mg. of propamidine. Of the total output of 3·9 μg. histamine, about 75 % was recovered in the effluent collected during the first 6 min. after the injection. In all experiments in which vasoconstriction was transient such a quick initial release was obtained.

The amounts of histamine released by 5 mg. propamidine in fourteen cats per gastrocnemius and per gram muscle, are given in Table 2. There are great differences in the amounts released by different muscles. Part of this variation is due to the different weights of the muscles, but even when the histamine is expressed per gram of muscle there is still a sevenfold variation in histamine output. This means it is not possible to make quantitative comparisons with

different doses of the same drug, or with different drugs, by using different cats; to make such a comparison possible the histamine outputs from the right and the left gastrocnemius muscles were compared with each other. Even then there is some variation, doubtless due to differences in the perfusion. For instance, in two experiments, the histamine outputs from the right and left gastrocnemius, after the injection of 5 mg. propamidine into each muscle, were compared. They amounted to 10·2 and 7·8 in the one, and to 18·5 and 12·2 μg. histamine in the other, the differences between the two sides being 23 and 34 % respectively.

TABLE 2. Release of histamine from gastrocnemius of the cat by 5 mg. propamidine

Weight of cat (kg.)	Weight of muscle (g.)	μg. of histamine released: Per gastrocnemius	μg. of histamine released: Per g. muscle
1·4	12	2·1	0·175
2·3	21	5·9	0·280
2·6	23	3·7	0·161
2·7	29	5·7	0·196
2·7	30	25·5	0·850
2·8	30	3·4	0·113
2·8	28	7·2	0·257
3·2	43	9·7	0·225
3·2	35	14·4	0·411
3·3	40	7·9	0·197
3·3	37	6·6	0·178
4·0	43	7·4	0·172
4·2	43	11·9	0·277
4·5	55	11·4	0·207
		Mean	0·264

TABLE 3. Release of histamine from perfused gastrocnemius of the cat

Substance	Exp. no.	Dose (mg.)	Weight of gastrocnemius (g.)	Histamine release: In μg.	Histamine release: As % of release by 5 mg. propamidine
Propamidine isethionate	1	10	52	12·2	107
	2	2·5	12	1·2	55
	3	1	21	3·0	51
	4	0·5	27	1·3	—
	5	0·25	29	2·6	46
Morphine sulphate	6	10	40	4·9	62
	7	5	29	3·7	52
	8	2	43	3·6	49
Codeine sulphate	9	5	43	6·2	52
Thebaine sulphate	10	5	37	7·8	118
Apomorphine hydrochloride	11	5	29	3·1	91
	12	1	33	2·8	11
Papaverine hydrochloride	13	5	24	3·2	85
	14	5	23	4·6	—
D-Tubocurarine chloride	15	5	48	9·2	70

In Table 3 the histamine released by various doses of propamidine is given in μg. per muscle and, in addition, expressed as percentage of the histamine

output obtained from the other gastrocnemius injected with 5 mg. of propamidine. Doses of over 5 mg. do not significantly increase the output, and in those experiments in which the effect of other histamine liberators was examined, it was therefore compared with that of 5 mg. propamidine on the other gastrocnemius.

When the histamine release has come to an end and a second dose of 5 mg. propamidine is injected, there is either no further release of histamine (see Fig. 3) or occasionally small amounts, less than 20 % of that released by the first injection. As will be seen later, however, the muscle still contains appreciable quantities of histamine.

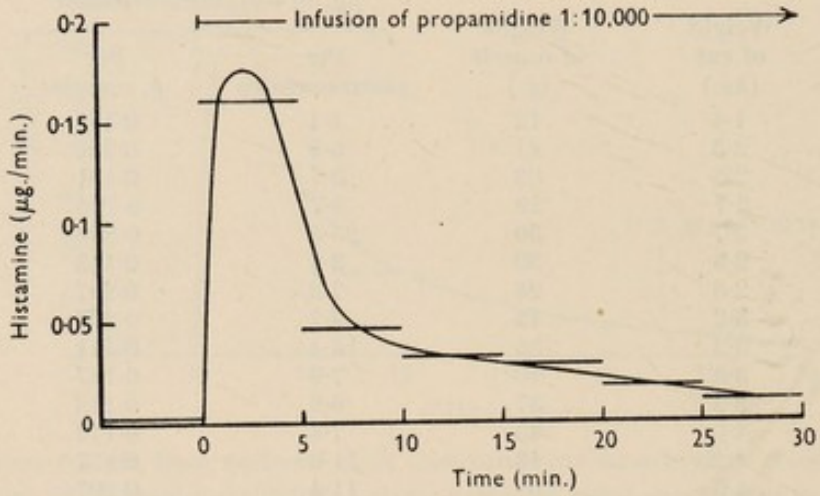

Fig. 4. Histamine release from perfused cat's gastrocnemius during infusion of propamidine 1×10^{-4}. Horizontal lines indicate mean values of histamine release in μg./min. during each 5 min. period.

When the propamidine is administered, not by a single injection but by slow infusion, the course of histamine release does not differ and most of the histamine released is collected in the first few ml. of effluent. This is shown in the experiment of Fig. 4, in which the perfusion fluid contained propamidine 1 in 10,000.

D-*Tubocurarine.* Of the known histamine liberators D-tubocurarine chloride was examined once on the perfused gastrocnemius muscle. Five mg. caused the release of 9·2 μg. of histamine as compared with 13·1 μg. after 5 mg. propamidine from the gastrocnemius of the other leg. Although this difference is within the normal range of variation between the two gastrocnemii, it may be due to the fact, found by MacIntosh & Paton (1949), that, weight for weight, D-tubocurarine chloride is a weaker histamine liberator than propamidine.

Opium alkaloids and apomorphine. Table 3 includes a new group of histamine liberators: the opium alkaloids, morphine, codeine, thebaine and papaverine, as well as the morphine derivative apomorphine.

All the opium alkaloids as well as apomorphine caused vasoconstriction and an output of histamine, when injected in doses from 1 to 10 mg. From the few

results obtained it appears that morphine, codeine and apomorphine are weaker histamine liberators than propamidine.

These substances, when dissolved in saline solution, gave acid reactions, and papaverine in particular would not dissolve in a concentration of 1 % except when the saline was acidified to a pH of about 4·3. Such an acid reaction, however, did not cause release of histamine or the release of minute amounts only. For instance, an injection of 0·5 ml. N/3 acid sodium phosphate (pH 4·37) did not cause any release of histamine, or traces only (0·07 μg.). Even the injection of 0·5 ml. of N/30-HCl (pH 1·56) released not more than 0·3 μg. of histamine. In addition, most of the other opium alkaloids and apomorphine could be injected in neutral or approximately neutral injection by neutralizing the solution with NaOH shortly before the injection.

TABLE 4. Diminution of histamine in muscle after propamidine

Histamine content of muscle (μg./g.)		Histamine collected in effluent (μg./g. muscle)	
Control muscle	Perfused muscle	After 1st injection	After 2nd injection
1·8	1·6	0·2	0·03
0·8	0·3*	0·4*	0·06*

*Values corrected for oedema.

Histamine content of muscle. From Table 2 it will be seen that large doses of propamidine release on the average 0·264 μg. histamine per gram muscle. This is less than the histamine known to be present in cat's muscle (Feldberg & Schilf, 1930). In two experiments, the results of which are given in Table 4, the histamine in the non-perfused and perfused muscle was determined. It was shown that the muscle histamine could not be exhausted even after two injections of 5 mg. propamidine. The second injection was found to release only a fraction of the histamine still present in the muscle. It therefore appears that some of the histamine in muscle is inaccessible to the action of histamine liberators, at least when they are injected arterially into the perfusion fluid.

The course of release of histamine in muscle. In different experiments the time taken for all the histamine released after a given dose of liberator to appear in the effluent varied considerably. In Fig. 5 *a*, the histamine output, expressed as a percentage of the total released, is plotted against the time of perfusion for nine typical experiments. It is seen that two-thirds of the total histamine output was collected in times ranging from 5 to 20 min. These variations are accounted for by the varying degrees of vasoconstriction in the different experiments, and, when the histamine output is related not to the time of perfusion but to the volume of perfusate, the curves of all the experiments become practically indistinguishable. This has been done in Fig. 5 *b*, in which the abscissae give the volume of perfusate, expressed as percentage of the volume of the muscle to allow for the varying size of the different muscles.

From the curve it is possible to say that, after the injection of a histamine liberator, two-thirds of the total output of histamine will be obtained when a volume of perfusate equal to 15–25 % of the muscle volume has been collected. This is independent of the extent of vasoconstriction, the size of the muscle,

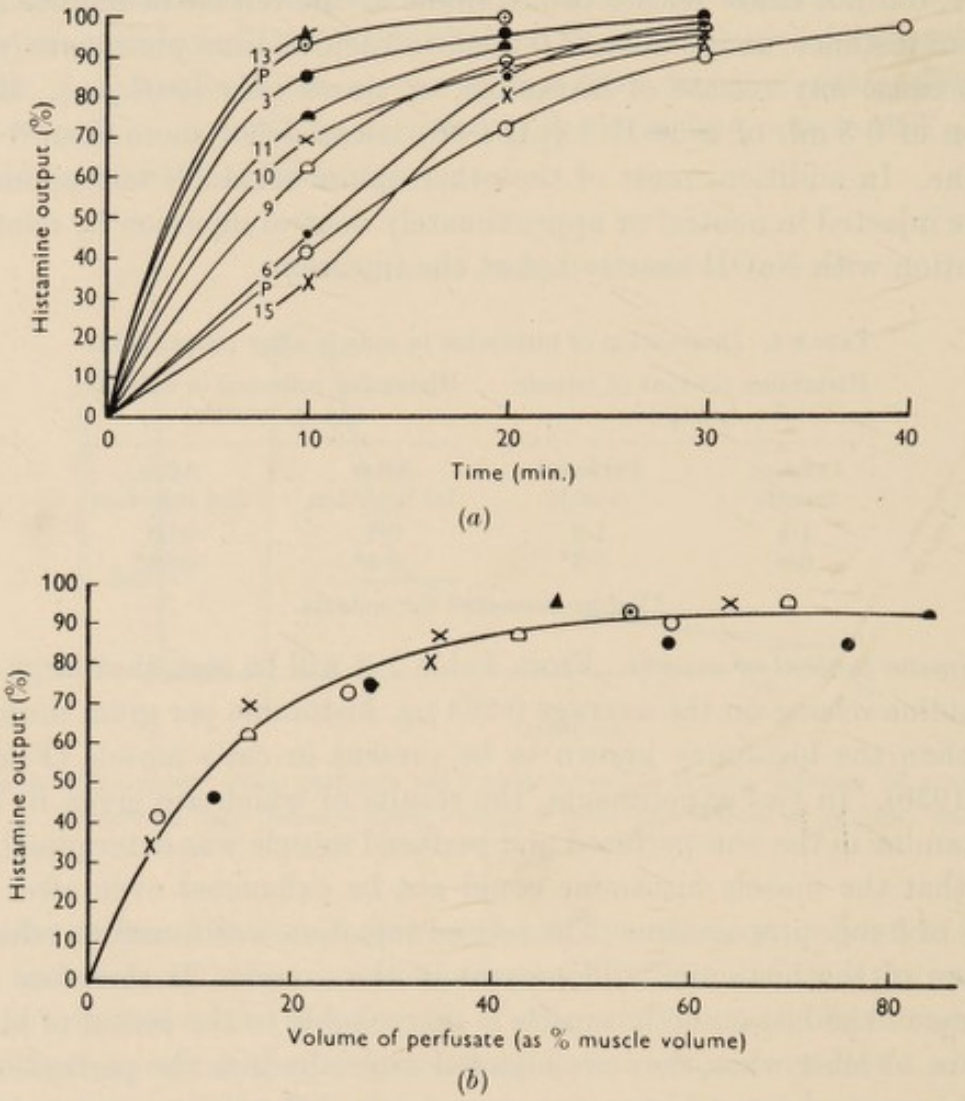

Fig. 5. Histamine output from perfused cat's gastrocnemius muscles after the injection of histamine liberators. The numbers refer to the experiments of Table 3, and *P* refers to experiments with 5 mg. propamidine. The ordinates give the histamine output as percentage of total histamine released. In (*a*) the abscissae give time in minutes, in (*b*) volume of perfusate collected as percentage of muscle volume. Histamine liberators injected at 0.

the dose of liberator, the nature of the liberator, or the amount of histamine released. Further, the course of release is a regular one of approximately exponential type such that equal fractions of the histamine remaining in the muscle are released into equal, successive volumes of perfusate. Fig. 6 shows the output of three representative experiments with different histamine liberators plotted in this way.

Effects of histamine liberators on perfused cat's skin

The arterial injection of histamine liberators—propamidine, D-tubocurarine, morphine, codeine, antrycide and compound 48/80—caused intense vasoconstriction, development of oedema, and the appearance of large amounts of histamine in the venous effluent, with a corresponding loss of histamine from the perfused skin.

Vasoconstriction. The vasoconstriction after a large dose of histamine liberator, for instance 5 mg. of propamidine, occurs after a latency of about half a minute after the injection, and may be so intense as to cause cessation of outflow for periods varying from a few minutes up to an hour in one experiment. During

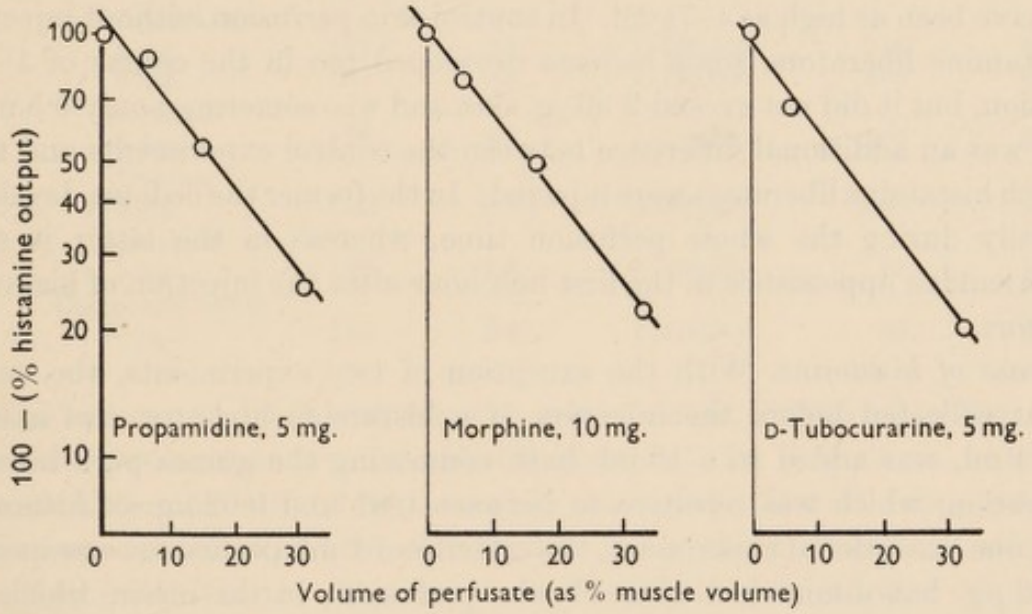

Fig. 6. Histamine output from perfused cat's gastrocnemius muscle after propamidine, morphine and D-tubocurarine. The two latter experiments are the same as nos. 7 and 15 in Table 3. Ordinates: the total histamine output is taken as 100, and the figures refer to the percentage of this histamine still remaining in the muscle. Logarithmic scale. Abscissae: volume of perfusate as percentage of muscle volume.

this period of intense vasoconstriction the outflow can be accelerated to some extent, or if it has stopped, started by increasing the perfusion pressure or by repeated compressions of the rubber tubing from the reservoir, but this procedure never restores the original rate of outflow. This often happens rather abruptly during the spontaneous, gradual passing off of the vasoconstriction, and the original flow can then be maintained for hours.

When the same dose of histamine liberator is injected a second time after the effect of the first injection has worn off, the vasoconstriction is only slight. As will be seen later, the release of histamine by a second injection is also very much reduced. Therefore, the vasoconstriction is either wholly, or at least to a great extent, accounted for by the action of the released histamine. This

conclusion is supported by the observation that an injection of 100 μg. of histamine, an amount comparable to that released by a large dose of a histamine liberator, produced the same intense and long-lasting vasoconstriction.

Oedema. In all experiments in which histamine liberators were injected intense oedema developed. At the end of the perfusion the subcutaneous tissue in the central region had swollen greatly and hung placenta-like as a gelatinous mass, on the inner surface of the skin. The dermis itself was also definitely thicker than normal. The subcutaneous, oedematous tissue could be dissected off, and the fluid in the skin proper pressed out between filter-papers. The weight of the oedema fluid was usually three to five times the weight of the skin. Since the oedema developed only over about two-thirds of the skin, that part which had been best perfused, the oedema fluid per gram of perfused skin may have been as high as 4–7½ ml. In control skin perfusion without injections of histamine liberators, some oedema developed too in the course of 1–3 hr. perfusion, but it did not exceed 2 ml./g. skin and was sometimes only 0·5 ml./g. There was an additional difference between the control experiments and those in which histamine liberators were injected. In the former the oedema developed gradually during the whole perfusion time, whereas in the latter it made a more sudden appearance in the first half hour after the injection of histamine liberators.

Release of histamine. With the exception of two experiments, the venous effluent collected before the injection of a histamine liberator was inactive when 1 ml. was added to a 15 ml. bath containing the guinea-pig's intestine preparation, which was sensitive to between 0·01 and 0·02 μg. of histamine. In the one exceptional experiment, the activity of 1 ml. perfusate corresponded to 0·02 μg. but diminished after 10 min. perfusion; in the other, which was from a pregnant cat, the activity of 1 ml. perfusate was as high as 0·11 μg. histamine. But even this activity was low compared with the activity of the samples collected after the injection of the histamine liberators. These samples caused such strong contractions of the intestine that they often had to be diluted ten to twenty times before testing. The activity was shown to be due to histamine by the same tests as were applied to the effluent from the gastrocnemius muscle. We shall therefore refer to the active substance in the venous effluent as histamine.

The total histamine in the venous effluent collected after the injection of a sufficiently large dose of liberator varied between 100 and 350 μg. as seen from the figures of Table 5. The highest concentration of histamine in the effluent of the different experiments varied between 6 and 8 μg./ml. These concentrations were obtained in samples collected during 10–15 min., so that even higher concentrations would have been obtained with shorter sampling periods.

The rate of histamine output per minute varied in different experiments,

independently of the total histamine released. It was to a great extent dependent on the initial vasoconstriction after the injection of the histamine liberator.

TABLE 5

Exp. no.	Histamine liberator	Histamine liberated (μg.)	Highest histamine concentration obtained		Time of collection (min.)	Volume collected (ml.)
			μg./ml.			
	Propamidine					
1	0·1 mg.	42	2·4	1:420,000	30	60
2	0·1 mg.	59	1·2	1:830,000	75	175
3	5 mg.	107	2·0	1:500,000	117	213
4	5 mg.	140	1·9	1:530,000	153	176
5	5 mg.	246	6·7	1:150,000	120	185
6	5 mg.	347	3·8	1:260,000	215	207
	D-Tubocurarine					
7	5 mg.	157	2·1	1:480,000	115	300
8	5 mg.	160	0·8	1:125,000	300	462
	Morphine					
9	5 mg.	113	2·2	1:450,000	107	232
	Codeine					
10	5 mg.	112	1·8	1:560,000	85	216
	Antrycide					
11	5 mg.	156	2·5	1:400,000	94	190
	Compound 48/80					
12	10 μg.	159	2·3	1:430,000	90	202

In the two experiments with 5 mg. D-tubocurarine, illustrated in Fig. 7, the total histamine output was practically identical. In Exp. 7, in which the maximal histamine output was reached during the first 20 min., vasoconstriction was moderate and the average flow during this period of collection was about 2 ml./min. On the other hand, in Exp. 8 the flow stopped completely for 30 min. and the average flow during the following 15 min. was less than 0·3 ml./min. The intense vasoconstriction also delayed the histamine output during the later stages of perfusion, when the outflow had again reached normal values of about 2 ml./min.

The experiments with propamidine are illustrated in Fig. 8. The initial delay in all three experiments with 5 mg. propamidine was accounted for by a greatly reduced outflow during the first 20 min. after the injection. The dependence of the initial and later rate of histamine output on the vasoconstriction after the injection is also evident in the two experiments with 0·1 mg. propamidine. In Exp. 1, vasoconstriction was over in 4 min., whereas in Exp. 2 it took about 20 min. before the outflow had reached its normal value of about 2 ml./min.

Fig. 9 illustrates the histamine output after codeine, morphine, and compound 48/80. Again, vasoconstriction was smallest in Exp. 12; during the first 10 min. after the injection 14·3 ml. perfusate was collected, whereas in Exps. 10 and 9 the outflow during the first 10 min. amounted to 8·5 and 5·7 ml. respectively.

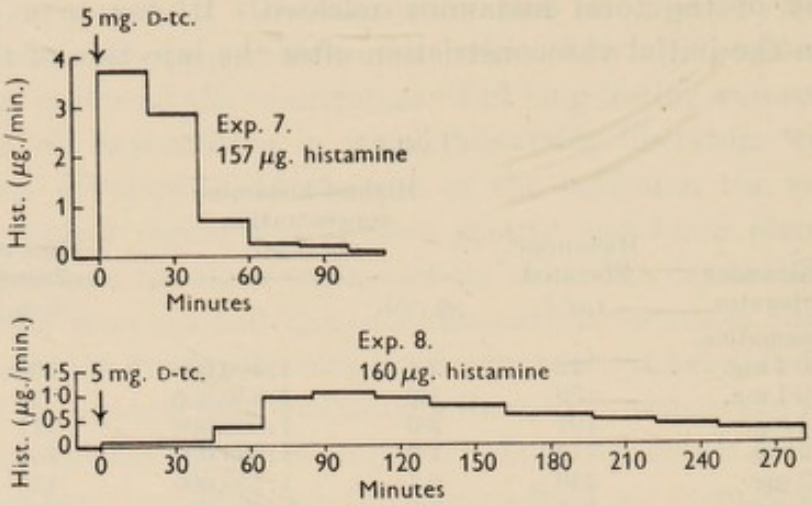

Fig. 7. Histamine output from perfused cat's skin by 5 mg. D-tubocurarine. The numbers of the experiments refer to those of Table 5.

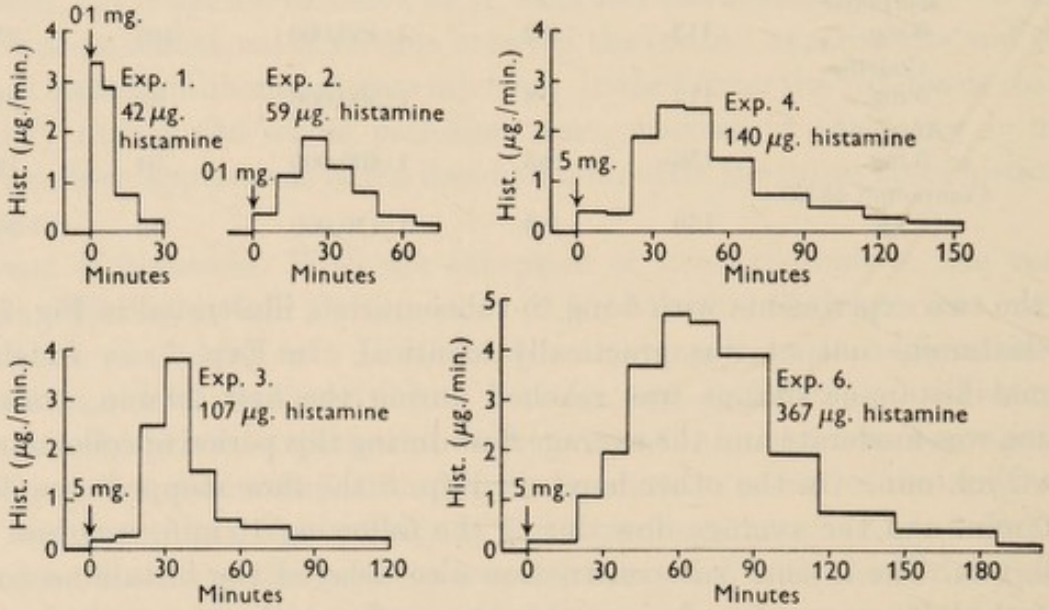

Fig. 8. Histamine output from perfused cat's skin by 0·1 mg. propamidine (Exps. 1 and 2) and by 5 mg. propamidine (Exps. 3, 4 and 6). The numbers of the experiments refer to those of Table 5.

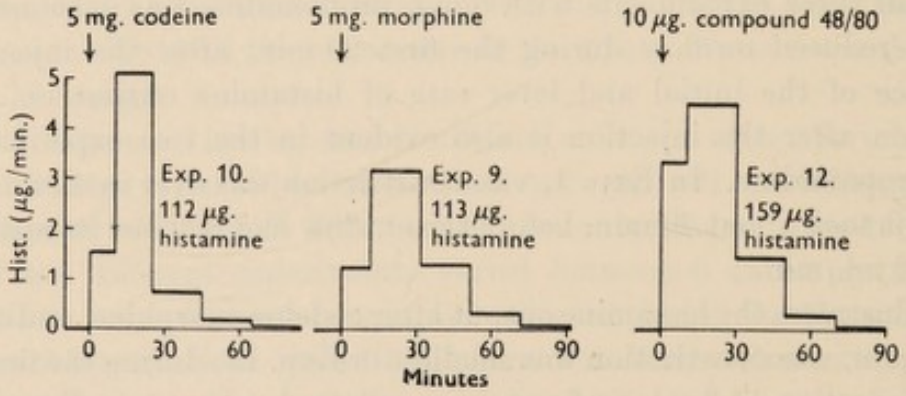

Fig. 9. Histamine output from perfused cat's skin by 5 mg. codeine, 5 mg. morphine and 10 μg. compound 48/80. The numbers of the experiments refer to those of Table 5.

Compound 48/80, which acted in amounts of a few micrograms, is so far the most active histamine liberator known (Paton, 1951).

Depletion of skin histamine. The histamine appearing in the perfusate is derived from the preformed histamine in the skin. Table 6 gives a balance sheet comparing the histamine found in perfused skin and in the perfusate after injection of propamidine with the total histamine available in a similar area of normal skin from the other leg. In the two experiments, nos. 3 and 4, with 5 mg. propamidine, the histamine content of the perfused skin is greatly reduced and the loss corresponds to the amounts found in the perfusate. In all experiments in which a balance sheet was made, the histamine released could be fully accounted for by the histamine loss. It follows, therefore, that no new histamine is produced by, or during, the process of histamine liberation, and that none is destroyed, unless we were to assume that, independent of the amount released, production and destruction exactly balanced each other.

TABLE 6

Exp. no.	Control skin (histamine in μg.)	Perfused skin (histamine in μg.)		
		In skin	In perfusate	In skin + perfusate
2	332	320	59	379
3	307	200	107	307
4	210	112	140	252

In Exp. 2, with a small dose of propamidine (0·1 mg.), less than 20 % of the available histamine was released. It is not possible to determine with certainty such a small loss of skin histamine if the histamine content of the whole perfused skin is determined *in toto*; but a loss is demonstrable when the best perfused area of the skin is extracted separately.

The perfused piece of skin was never equally well perfused in all parts, but those regions near the entrance of the saphenous artery were best perfused, as judged by the rapidity with which the blood was washed out from these regions by the perfusion fluid. The central parts were also those which suffered the greatest loss of histamine, and where, therefore, a loss was easily detectable even after a small dose of histamine liberator. In Exp. 2 of Table 6, the skin, after the perfusion, was divided into several portions and the histamine content of each part determined separately, together with the histamine content of corresponding areas of skin from the opposite leg. On the control side, the histamine content of skin from different areas did not vary significantly and was about 25 μg./g., and the same content was shown by two portions of skin from regions which were scarcely perfused at all. But a small area of skin from the perfused side round the point of entry of the artery contained only 8·4 μg./g. and an adjacent area 10 μg./g.; the remaining perfused skin contained 15 μg./g. These 15 μg./g. are not entirely a sign of histamine loss, because there was some oedema and the perfused skin weighed about 25 % more than the same area of the control side.

Even after the injection of a large dose of histamine liberator, the loss of histamine from the skin was not uniform but was greatest in the central regions which often became practically histamine free. In the experiment of Fig. 10 the control skin from the other leg contained 15 μg./g. histamine. On the perfused side three areas of skin—central, intermediate and peripheral—yielded 0·3, 1·5 and 10 μg./g. respectively. Results of the same kind were obtained with

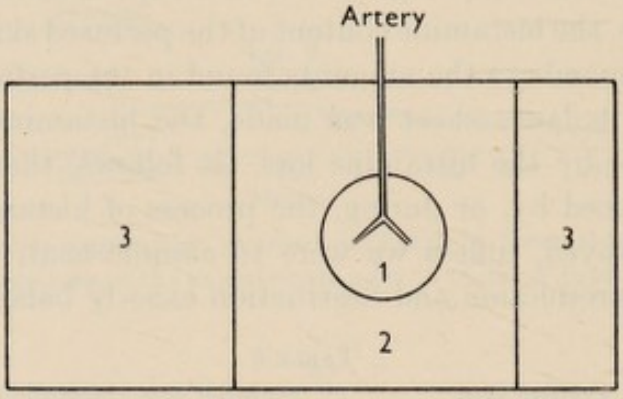

Fig. 10. Diagram of a perfused piece of skin with saphenous artery, showing the areas from which the histamine was extracted after injection of 5 mg. of propamidine (Exp. 4 of Table 5).

all histamine liberators tested, as seen from the results of Table 7. In this table the histamine content of normal skin has been compared with that of the central parts of the perfused skin in which the greatest histamine loss was found after the injection of the histamine liberator.

TABLE 7. Histamine content of skin in μg./g.

Exp. no.	Control skin	Central parts of perfused skin	Percentage depletion
1	23	<1·0	>96
2	25	8·4	66
3	21	4·8	77
4	25	0·3	99
7	21	2·0	90
8	13·5	1·0	93
9	20	1·0	95
10	24	2·0	92
11	23	0·4	98
12	21	5·9	72

DISCUSSION

It had previously been shown that a variety of compounds have the property in common of releasing histamine from mammalian tissue. Some of these compounds, such as D-tubocurarine, the trypanocidal diamidines and antrycide, are of clinical or veterinary importance. A new group of substances, widely used therapeutically, has to be added to the histamine liberators: the opium alkaloids and apomorphine.

Whereas, with certain histamine liberators such as propamidine or compound

48/80, the effects of the released histamine account for most of their pharmacological actions, this is certainly not true for the opium alkaloids and apomorphine, in which the property of releasing histamine is a side action independent of the main central and peripheral effects which these substances exert in the animal body. We have seen that even the long-lasting fall of arterial blood pressure in cats after large doses of morphine, which superficially resembles a histamine shock, is only partly accounted for by release of histamine. Nevertheless, this property of the opium alkaloids may have practical importance. The well-known 'idiosyncrasy' to morphine and codeine, particularly when applied by the intravenous route, the hypersensitivity of skin in certain patients to opium preparations, the pruritus and sneezing of the opium addict, may all be attributable to this property of releasing histamine. There is also the possibility that the hypersensitivity of the patient with bronchial asthma to morphine is due, or partly due, to the release of histamine, to which these patients are particularly sensitive (Herxheimer, 1950). It may become necessary to re-examine some of the effects of the opium alkaloids to find out if, or how far, release of histamine accounts for them. Such an examination may also give us information about what, in fact, are the effects of histamine liberators in general when given to man.

So far, little is known about the tissues mainly responsible for the release of histamine in different species. It is not even certain whether the 'shock organs' of anaphylaxis are the same as those on which the histamine liberators exert their main action. In cats anaphylaxis is difficult to produce; nevertheless, they respond well to the histamine liberators. MacIntosh & Paton concluded from indirect evidence that, in this species, the histamine must be derived, or derived mainly, from the skeletal muscle and skin. Our perfusion experiments fully support this conclusion and show further that of the two tissues the skin is by far the greater source of released histamine, and can therefore be regarded as the 'shock organ' of cats. It was found that the histamine liberators liberate per gram skin up to between 15 and 30 μg. of histamine, but per gram muscle only between 0·1 and 0·3 μg. This difference is due partly to the higher content of histamine in skin than in muscle, but also to the fact that most of the muscle histamine, unlike that of the skin, is resistant to the action of the histamine liberators. Even if we take into account the fact that skeletal muscle makes up about 50 % of the body weight, and skin only about 15 %, muscle would only be able to contribute per kg. body weight less than 0·2 mg. histamine, whereas skin might contribute 3 mg. or more, provided that the histamine liberator was evenly distributed by the bloodstream in these tissues.

The different histamine liberators had to be injected in different amounts in order to release approximately the same amounts of histamine. The opium alkaloids had to be injected in amounts of a few mg. in order to release from the skin amounts of about 100 μg. of histamine or more. A tenth of a mg. of

propamidine released about 50 μg. of histamine, but 10 μg. of compound 48/80 were sufficient to release over 150 μg. of histamine. Compound 48/80 consists of several, probably three, phenylethylamine nuclei with a molecular weight of about 180 each. Each molecule of compound 48/80, therefore, released approximately 70–90 molecules of histamine from the skin, or, if calculated for each phenylethylamine nucleus of the molecule, 20–30 histamine molecules. The statement of Rocha e Silva & Schild (1949) with regard to D-tubocurarine, that several molecules of a histamine liberator are required for the release of one molecule of histamine, certainly does not apply to compound 48/80.

It is interesting to compare quantitatively the degree of depletion of histamine in different tissues by histamine liberators. Snake and bee venoms release histamine by enzymic destruction of the tissue and produce complete depletion. In contrast to these enzymes, the histamine liberators we examined do not produce visible structural damage. Nevertheless, the cat's skin could be fully depleted by them. On the other hand, the cat's gastrocnemius lost only a part of its histamine. According to Rocha e Silva & Schild, the muscle of the rat's diaphragm loses at most about 70 % of its histamine when exposed to D-tubocurarine. It appears that the histamine of different muscles, or of muscles of different species, is to a varying degree susceptible to the action of histamine liberators. This problem of the susceptibility of the tissue histamine in different organs, and in different species, and possibly also to different histamine liberators, is one which can fruitfully only be discussed when more data are available, but the ease with which the whole skin histamine can be released by histamine liberators without visible structural damage may be related to the fact that release of histamine in the skin is a normal response to a wide variety of stimuli and is continuously called into play under physiological conditions.

The time course of histamine output from skin and from muscle differs. In perfused skin all the histamine is accessible to the action of histamine liberators, but the output is more prolonged than in perfused muscle and extremely variable. Nevertheless, the actual release from the skin may have occurred as quickly as in muscle. In the latter, the release regularly followed a typical exponential course, which suggests that the whole histamine release took place in a very short time into some space within the muscle, from which it was afterwards slowly rinsed out by the perfusing fluid.* If the process of release

* Suppose that the histamine released diffuses rapidly throughout a space (V) which is then slowly rinsed out by perfusion, and that the histamine concentration in the space is h (initially h_0) when the volume of perfusate which has passed after the moment of release is r ml. Then

$$h = h_0 \exp(-r/V).$$

Therefore total output from the muscle in a volume of perfusate $R = Vh_0(1 - \exp(-R/V))$.

Therefore fraction of the total output which is still within the muscle after a volume of perfusate R has perfused $= \exp(-R/V)$, and V = that volume of perfusate which contains the first $1 - 1/e = 63\,\%$ of the total output.

were not abrupt, but were to proceed gradually during the perfusion, a smaller proportion of the histamine would be obtained in earlier stages of the perfusion than was actually found. It appears that the release itself is, therefore, of an explosive type. This would explain why the histamine release followed the same exponential course when the liberator, instead of being given by single injection, was slowly infused with the perfusing fluid.

It is possible to calculate the hypothetical space into which the histamine is released in the muscle from the exponential constant of the release.* It was found to be on the average 21% of the muscle volume. It cannot be argued that this means that only 21% of the muscle was actually perfused, because arterial injections of indian ink at the end of each experiment showed that the whole substance of the muscle was well perfused. Therefore either the histamine liberator or the histamine itself is freely diffusible only in 21% of the volume of the cat's muscle. This volume can hardly be other than the extra-cellular space, and it is into this volume that the explosive release of histamine appears to take place according to our view. If we assume that it is the histamine liberators which are freely diffusible only in 21% of the muscle volume, most of the muscle histamine would be inaccessible to them; this could explain why so much of the histamine content of muscle is 'resistant' to the histamine liberators.

SUMMARY

1. The depressor action of morphine on the cat's blood pressure is associated with the appearance of small amounts of histamine in the plasma, but these amounts are insufficient to account fully for the depressor action of morphine.

2. A number of histamine liberators were examined on the isolated, perfused gastrocnemius muscle and on an isolated, perfused piece of skin from the medial aspect of the thigh in cats. The preparations were perfused from the artery and the venous effluent collected and assayed for histamine. When no histamine liberators were given, the effluent contained no detectable histamine, or only traces. After the injection of histamine liberators, histamine appeared in the effluent; in the effluent from the skin its concentration could be higher than 1 in 200,000. In addition, histamine liberators produced vasoconstriction and oedema, effects which may both be due to the released histamine.

3. Propamidine, D-tubocurarine, morphine, codeine, papaverine, thebaine and apomorphine, when injected into the artery of the perfused gastrocnemius muscle in amounts of a few mg., released several micrograms of histamine. The amounts that could be released, even with repeated injections, represented only a fraction of the total histamine content of the muscle.

4. Propamidine, D-tubocurarine, morphine, codeine and antrycide, when injected into the saphenous artery of the perfused skin in amounts of a few mg.,

* See footnote on previous page.

released between 100 and over 300 μg. of histamine. The much stronger histamine liberator, compound 48/80, released over 150 μg. when injected in an amount of 10 μg. only.

5. With compound 48/80 about 70–90 molecules of histamine were released by a single molecule of the histamine liberator from the perfused skin.

6. The perfused skin, unlike the perfused gastrocnemius, could be almost completely freed of its histamine by the arterial injection of any of the histamine liberators tested.

7. It is suggested that the release of histamine from the perfused tissues by the histamine liberators occurs explosively, and that the released histamine is then washed out gradually by the perfusing fluid.

8. It is suggested that the property of the opium alkaloids and apomorphine of releasing histamine may account for some of the 'allergic' and histamine-like side actions of these compounds in man.

REFERENCES

Barsoum, G. S. & Gaddum, J. H. (1935). *J. Physiol.* **85**, 1.
Dale, H. H. & Laidlaw, P. P. (1918). *J. Physiol.* **52**, 355.
Feldberg, W. & Kellaway, C. H. (1937). *Aust. J. exp. Biol. med. Sci.* **15**, 441, 461.
Feldberg, W. & Paton, W. D. M. (1949). *J. Physiol.* **111**, 19*P*.
Feldberg, W. & Paton, W. D. M. (1950). *Abstr. XVIII int. physiol. Congr.* p. 198.
Feldberg, W. & Schilf, E. (1930). *Histamin.* Berlin: Springer.
Herxheimer, H. (1950). *Brit. med. J.* ii, 167.
Lewis, T. (1927). *The Blood Vessels of the Human Skin and their Responses.* London: Shaw and Sons.
MacIntosh, F. C. & Paton, W. D. M. (1949). *J. Physiol.* **109**, 190.
Nasmyth, P. A. & Stewart, H. C. (1949). *J. Physiol.* **111**, 19*P*.
Paton, W. D. M. (1951). *Brit. J. Pharmacol.* (in the Press).
Rocha e Silva, M. & Schild, H. O. (1949). *J. Physiol.* **109**, 448.
Sollman, T. & Pilcher, J. D. (1917). *J. Pharmacol.* **9**, 309.