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RELEASE OF 5-HYDROXYTRYPTAMINE AND HISTAMINE FROM TISSUES OF THE RAT

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5-Hydroxytryptamine (5-HT) occurs in many tissues of the rat, but its association with histamine in tissue mast cells remains in doubt. For example, 5-HT has been found to be concentrated in areas of the skin where histamine levels are low and mast cells are few (Parratt & West, 1957*a*). Experiments were therefore designed to study the changes in the 5-HT and histamine content of the tissues of the rat following treatment with drugs. The results reported in this paper show that the release of histamine and disruption of mast cells can occur without the simultaneous release of 5-HT, and vice versa, and it is most unlikely that the mast cells contain any considerable quantity of 5-HT.

METHODS

Groups of five or more male albino rats weighing 100–150 g were used. Preparation of the tissue extracts and assay methods for 5-HT and histamine have been described in detail (Parratt & West, 1957*a*). In general, the following tissues were extracted—ventral abdominal skin, ears, tongue, pads and dorsal skin of the feet, spleen, lung, liver and brain. The abdominal skin was usually separated into two parts—the inner scrapings, which contain most of the larger mast cells, and the residue or outer layers where mast cells are smaller and not so numerous.

The following drugs were used to produce depletion of the tissue amines—polymyxin B sulphate, compound 48/80, morphine hydrochloride and reserpine. Reserpine (100 mg) was dissolved in a few drops of glacial acetic acid, and then propylene glycol (2·5 ml.), ethanol (2·5 ml.) and water (15 ml.) were added. All the other drugs were dissolved in N-saline. 5-HT was used as the creatinine sulphate, histamine as the acid phosphate. With the exception of polymyxin B, all values in the text refer to the base.

RESULTS

Single injection of polymyxin B

Within a few minutes of a single intraperitoneal injection of polymyxin B (2·5 mg/kg), the rat is depressed, respiration is laboured, and some muscular weakness is seen. Within 30 min, the characteristic signs of histamine release are present (Bushby & Green, 1955). Scratching, erythema of the ears, and extensive vasodilatation with slight oedema of the muzzle and extremities

occurs in 90% of the animals, which by this time lie away from each other. There is an obvious lowering of body temperature during the period of acute shock but, if given abundant drinking water and kept in a warm atmosphere, most of them soon recover. Groups of rats were killed at given intervals of time after injection. Corresponding control groups of rats received N-saline solution. The 5-HT and histamine concentrations in the tissues were estimated and calculated as percentages of the control saline-injected values.

Mast cells. In as short a time as 30 min after the injection, a striking change occurs in the mast cells of the peritoneum (Parratt & West, 1957*b*). Whereas in the control animals, injected with saline, the mast cell picture is normal, the stained spread of mesentery from the test group shows that all the mast cells have swollen up and some have released most of their granules. The blood vessels in the mesenteric windows are dilated. Some hours later irregular blobs of metachromatically stainable material appear in the macrophages. As might be expected, disruption of the mast cells in regions remote from the injection site is less than in the mesenteric cavity, and is often patchy in distribution.

By day 2 (i.e. 24 hr after the injection) there are very few recognizable mast cells left in the mesentery. Such mast cells as remain possess a few small weakly staining granules situated at the periphery of greatly swollen cells (termed 'ghost cells'). Other cells such as the macrophages and fibroblasts exhibit irregular blobs of metachromatically stainable material which are very varied in size and shape. The spread of the subcutaneous connective tissue at this time shows some disruption of mast cells and scattering of the granules.

By day 9 further degranulation of the mast cells in the subcutis has taken place, particularly those nearer to blood vessels. Swollen 'ghost cells' are numerous. In the mesentery increased basophilia of the loose mesenchyme is prominent and a few new small mast cells have appeared, though they are intermingled with macrophages containing masses of material of varying sizes from the broken cells—material which by this time is stained much less densely by toluidine blue. Six days later (day 15) all the spreads contain many small new rounded mast cells with densely staining granules. These mast cells have originated from the adventitia of the blood vessels and now mix with the 'ghost cells' and macrophages containing debris from the original disruption of cells. By day 29, recovery in all areas is almost complete and the histological picture is normal.

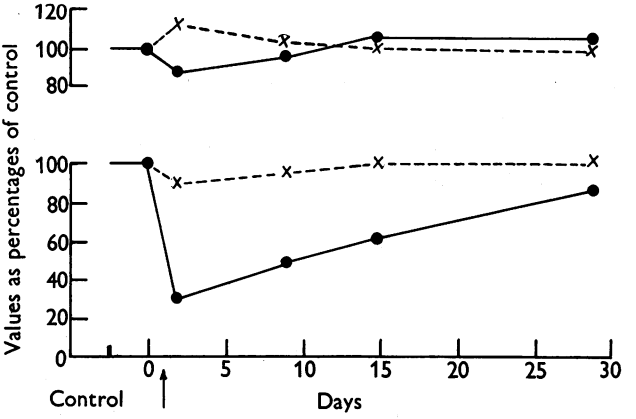
Histamine. The high histamine values for the ears, inner layers of abdominal skin, the pads and the dorsal skin of the feet fall in 24 hr to approximately 30% of control levels, whereas the loss of histamine is much less in the outer layers of the skin. These results are a reflexion of both the number and the reactivity of the mast cells in the tissues. Recovery is slow in all areas which by day 29 contain only about 80% of their initial histamine (Table 1 and Text-fig. 1).

The only other tissue studied which releases histamine under such treatment is the lung: by day 2 it has lost about 50% of its normal content and it requires about 13 days for full recovery.

5-Hydroxytryptamine. There is no release of 5-HT except for a small depletion of 30% in the pads of the feet and the inner layers of the skin (Table 1). Recovery of 5-HT to normal levels is a rapid process after polymyxin B and occupies only a few days.

TABLE 1. The effect of a single intraperitoneal injection of polymyxin B (2·5 mg/kg) on the 5-HT content and histamine content of the skin of the rat, together with the subsequent recovery of the amines. All values are expressed as percentages of the control levels

	Day 2		Day 9		Day 15	Day 29
Tissue	5-HT	Histamine	5-HT	Histamine	Histamine	Histamine
Skin, inner layers	70	22	90	53	54	85
Skin, outer layers	110	71	110	72	82	86
Feet, dorsal skin	115	41	100	50	52	72
Feet, pads	77	39	95	55	43	85
Ears	100	23	110	42	35	74



Text-fig. 1. The changes in the 5-HT (× - - - ×) and histamine (●—●) contents of the spleen (upper curves) and abdominal skin (lower curves) of the rat following a single intraperitoneal injection of polymyxin B (2·5 mg/kg, at the arrow). All values are expressed as percentages of the control levels.

Repeated injections of polymyxin B

In this group of experiments the rats received 5 doses of polymyxin B intraperitoneally. On the first day the dose was 2·5 mg/kg; on the second, two doses of 5 mg/kg were given; and on the third, the animals received two doses of 7·5 mg/kg. The rats of the first group were killed 24 hr after the last dose. After each dose, there is erythema and slight swelling of the extremities. Marked generalized vasodilatation, however, occurs even after the last dose of polymyxin when the skin histamine levels are maximally depleted.

Mast cells. Histological examination began on day 4, by which time the number of recognizable mast cells has been greatly decreased, even in regions remote from the injection site. Such cells as remain are 'ghost cells'. Many of the mast cells lying in the perineurium of the peripheral nerves however escape the action of polymyxin B. The numerous granular cells present in each spread are macrophages and fibroblasts containing metachromatically stainable debris.

Recovery of mast cells is remarkably slow. By day 11 (i.e. 8 days after the last injection), there are large 'ghost cells' in the mesenteric windows and subcutis, together with a few small new mast cells derived from the adventitia of blood vessels and septa of fat cells. The stainable debris in the cytoplasm of the macrophages and fibroblasts still predominates in each field (Pl. 1, fig. 2). Even by day 29 the picture is by no means normal. The mesentery and subcutis now contain normal mast cells (hypertrophied small cells), 'ghost cells' refilling with granules, and clumps of stainable material in the macrophages. The subcutaneous connective tissue contains, in addition, many new elongated mast cells which lie with their long axes parallel to the vessel walls. By day 56 the histological picture is almost back to normal; in the mesentery a few of the many mast cells have not completely refilled with granules, and some clumps of metachromatically stainable material still remain, whereas the subcutaneous connective tissue shows complete recovery.

Histamine. At the end of the injection period less than 6% of the initial histamine concentration remains in those areas of the skin which are normally rich in mast cells. On the other hand, the depletion is between 30 and 50% in the spleen, lung and liver; in the stomach 75% remains after this treatment. This latter finding is of interest, since much of the histamine in the stomach is not contained in mast cells. The experiment is noteworthy for the very slow rate of recovery of histamine in the skin areas (Table 2 and Text-fig. 2). For example, the histamine levels at day 29 are only 30–50% of the control levels, and even by day 57 those in the feet and ears are only 60% of the control levels.

5-*Hydroxytryptamine.* As in the acute experiment, the inner layers of the skin and the pads of the feet are the only areas studied where appreciable losses of 5-HT occur. Recovery of these losses occupies about 25 days (Table 2).

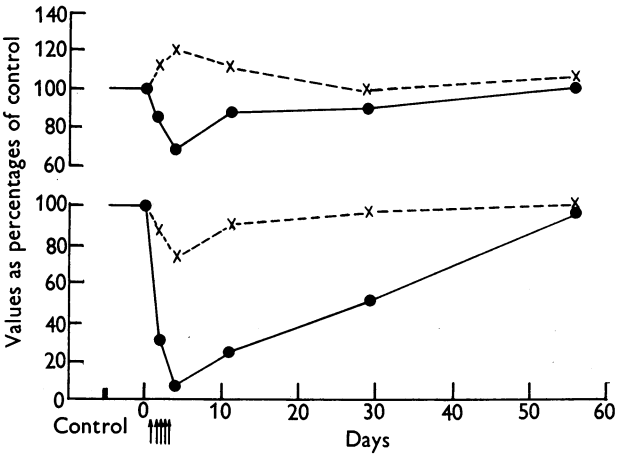
Injections of compound 48/80

In the first set of experiments with this drug two doses of compound 48/80 were injected intraperitoneally, one of 1 mg/kg in the morning and one of 2 mg/kg in the afternoon. One group of rats was killed 24 hr later, the remainder being killed after further intervals of time. Depletion of both amines to about 40% of the control values occurs in the abdominal skin, the ears and the dorsal skin of the foot, accompanied by widespread degranulation

and disruption of the many mast cells in these areas. On the other hand, no release of either amine occurs from the spleen, lung or liver—areas where mast cells are scanty in distribution. The 5-HT content (0·42μg/g) and histamine content (0·41μg/g) of the brain are also unchanged by this treatment with compound 48/80. The results for abdominal skin are shown in Text-fig. 3 and contrast sharply with those for spleen. The return of both amines to most areas

TABLE 2. The effect of five intraperitoneal injections of polymyxin B (over 3 days) on the 5-HT content and histamine content of the skin of the rat, together with the subsequent recovery of the amines. All values are expressed in percentages of the control levels

	Day 4		Day 11		Day 29		Day 57
Tissue	5-HT	Histamine	5-HT	Histamine	5-HT	Histamine	Histamine
Skin, inner layers	55	3	58	22	110	47	100
Skin, outer layers	90	14	100	44	91	68	96
Feet, dorsal skin	83	6	83	22	100	52	63
Feet, pads	39	11	46	15	78	27	48
Ears	102	4	100	18	100	38	60



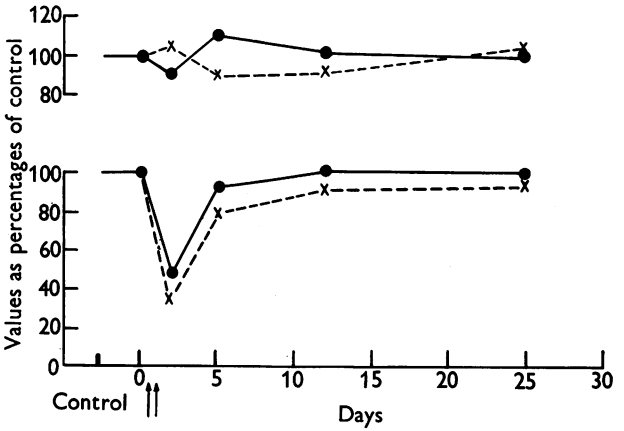
Text-fig. 2. The changes in the 5-HT (×- - -×) and histamine (●—●) contents of the spleen (upper curves) and abdominal skin (lower curves) of the rat following five doses of polymyxin B (at the arrows). All values are expressed as percentages of the control levels. The time scale differs from that used in the other figures.

of the skin is almost complete by day 5 (4 days after the last injection) by which time the degranulated cells have refilled with metachromatically stainable granules. In contrast, the return of both amines to the dorsal skin of the foot is slower, over 12 days being required for complete recovery. This slow recovery in the feet has been noted previously for histamine (Feldberg & Talesnik, 1953; Riley & West, 1955), and it is important to note that the return of 5-HT in this tissue follows very closely that of histamine.

In the second set of experiments with compound 48/80, seven doses were injected intraperitoneally, as follows:

	Dose of 48/80 (mg/kg)			
Day...	1	2	3	4
Morning	1	2	4	5
Afternoon	2	3	5	

Oedema of the extremities was seen in all animals after about the third injection, but this decreased as the dose was increased until few symptoms were noted after the last dose. The rats of the first group were then killed 8 hr later (i.e. on day 4). By this time mast cells in the skin are no longer intact and histamine depletion is maximal. Depletion of 5-HT, however, is no more

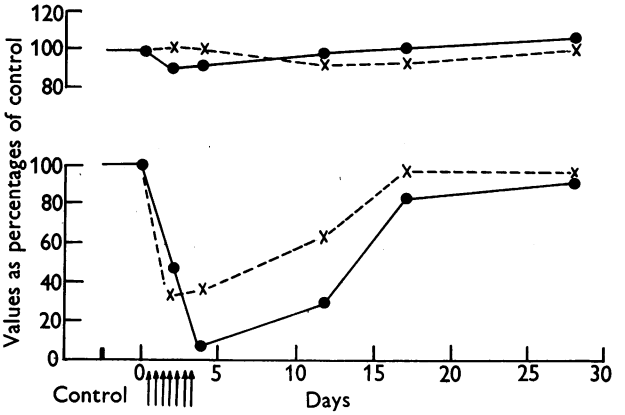


Text-fig. 3. The changes in the 5-HT (×- - - ×) and histamine (●—●) contents of the spleen (upper curves) and abdominal skin (lower curves) of the rat following two intraperitoneal injections of compound 48/80 (at the arrows). All values are expressed as percentages of the control levels.

than that found following only two doses of compound 48/80 (compare Text-figs. 3 and 4). Again no release of either amine occurs from the spleen, liver or brain. When the skin is divided into specialized areas, histamine depletion is found to be general, whereas the loss of 5-HT is relatively small in certain areas (Table 3). In the outer layers of the skin, for example, compound 48/80 has disrupted mast cells, releasing histamine (values down to 7% of control), but 60% of the 5-HT remains (see also Text-fig. 7). This remaining 5-HT does not therefore appear to be of mast cell origin. The return of the amines to these tissues takes about 28 days (Table 3), both amines returning at about the same rate and closely following the production of new mast cells.

TABLE 3. The effect of seven intraperitoneal injections of compound 48/80 (over 4 days) on the 5-HT content and histamine content of the skin of the rat, and the subsequent recovery of the amines. All values are expressed as percentages of the control levels

	Day 4		Day 17		Day 28	
Tissue	5-HT	Histamine	5-HT	Histamine	5-HT	Histamine
Skin, inner layers	15	13	83	60	100	89
Skin, outer layers	60	7	65	75	100	95
Feet, dorsal skin	36	4	73	40	110	100
Feet, pads	21	4	37	25	82	90
Ears	26	6	66	43	100	91
Tongue	13	7	80	50	85	110



Text-fig. 4. The changes in the 5-HT (×- - -×) and histamine (●—●) contents of the spleen (upper curves) and abdominal skin (lower curves) of the rat following seven doses of compound 48/80 (at the arrows). All values are expressed as percentages of the control levels.

Injections of morphine

Morphine is known to release histamine in mammals (Feldberg & Paton, 1951; Evans, Nasmyth & Stewart, 1952), and recently it has been shown to release 5-HT from perfused tissues of the rat (Bhattacharya & Lewis, 1956*a*). We have therefore given groups of rats repeated injections of morphine (each of 20 mg/kg) and then examined the tissues for amine levels. As in the experiment with compound 48/80, seven doses have been given over 4 days and the rats of the first group have been killed 8 hr after the last injection. Reduction of histamine to 60–80 % of the control values occurs in most tissues (Table 4) and this is accompanied by patchy mast-cell degranulation in the skin areas. Yet the only major change in the 5-HT levels is in the outer layers of the skin and the pads of the feet. In these two areas, where mast cells are scarce, morphine releases about 50 % of the 5-HT present, and it appears probable that most of this released 5-HT is derived from locations other than mast cells (see also Text-fig. 7). The return of both amines to all depleted areas is complete in 5 days, the time expected from degranulated but not disrupted mast cells

(West, 1956). Thus morphine differs from polymyxin B and compound 48/80 in the following ways: (1) it has little damaging effect on tissue mast cells and therefore releases less histamine; (2) it releases 5-HT from areas of the skin where mast cells are scarce; and (3) it does not produce visible tissue oedema.

TABLE 4. The effect of seven intraperitoneal injections of morphine on the 5-HT content and histamine content of tissues of the rat. All values are expressed as percentages of the control levels

Tissue	5-HT	Histamine
Skin, inner layers	100	87
Skin, outer layers	50	100
Feet, dorsal skin	100	61
Feet, pads	43	60
Ears	100	63
Tongue	100	80

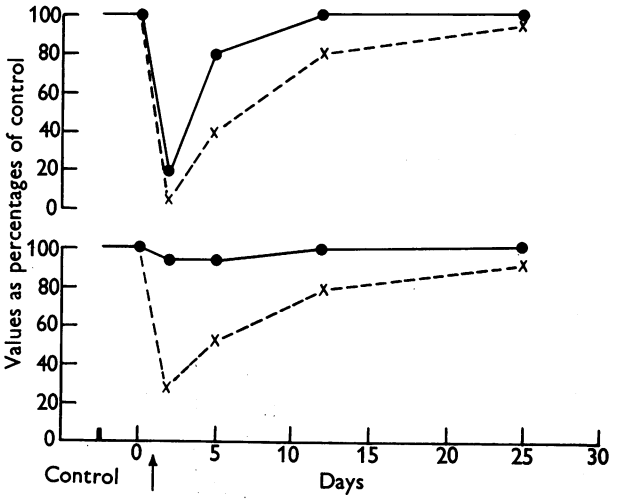
Injections of reserpine

Reserpine causes a conspicuous decrease in the 5-HT content of blood platelets, spleen and intestinal tract of rats (Erspamer, 1956). More than half of the total 5-HT of the rat, however, resides in the skin. Groups of rats therefore have received single or multiple doses of reserpine to study the release of 5-HT in this tissue. Other groups similarly treated have been left for given times after the injections so that the return of the tissue amines may be studied. Control groups of rats received only the reserpine solvent.

The results shown in Table 5 indicate that a single dose of 0·1 mg/kg produces little histamine release but a reduction of about 50% in tissue 5-HT, from the same two tissues which responded to morphine treatment—outer layers of the skin and pads of the feet. No visible mast-cell damage or degranulation occurs. Splenic 5-HT levels are much depleted, only 16% of the control value remaining. When the dose of reserpine is increased one hundred times, the rats tend to isolate themselves and sleep much of the day. Further losses of 5-HT from most skin areas result but mast-cell damage and histamine loss are minimal. Traces of spilling of the granules may be occasionally seen but no oedema. The return of the 5-HT to normal levels takes place in 4 days following the small dose of reserpine, and in about 12 days following the larger dose (see Text-fig. 5). The spleen is one of the last tissues to regain its original level of 5-HT. Repeated injections of the high dose (10 mg/kg) gives maximal depletion of the 5-HT, values for skin and spleen then being under 10% of normal (Text-figs. 6, 7). Even lung, liver and brain show marked depletion of 5-HT following this dosage of reserpine. Thus reserpine is a more powerful depleter of tissue 5-HT than compound 48/80. On the other hand, the maximum release of histamine by reserpine is no more than 34% in any tissue except the spleen. In the skin some mast-cell degranulation can be seen following this dosage of reserpine and also spilling of the granules into the subcutaneous area (Pl. 1, fig. 3), but it is never extensive.

TABLE 5. The effect of intraperitoneal injections of reserpine on the 5-HT content and histamine content of tissues of the rat. All values are expressed as percentages of the values obtained from solvent-treated control rats. Animals killed 24 hr after the last injection

	Dose of reserpine							
	0·1 mg/kg				10 mg/kg			
	1 dose		4 doses		1 dose		4 doses	
Tissue	5-HT	Histamine	5-HT	Histamine	5-HT	Histamine	5-HT	Histamine
Abdominal skin, inner layers	100	100	110	100	35	92	6	66
Abdominal skin, outer layers	48	100	80	83	20	86	3	72
Feet, dorsal skin	91	100	100	91	89	90	18	92
Feet, pads	48	100	75	100	56	95	11	85
Ears	61	67	60	67	56	80	8	81
Tongue	60	90	90	90	50	100	12	100
Spleen	16	62	37	62	5	17	2	40
Lung	60	100	60	100	60	90	8	91
Liver	86	100	90	100	73	100	27	100

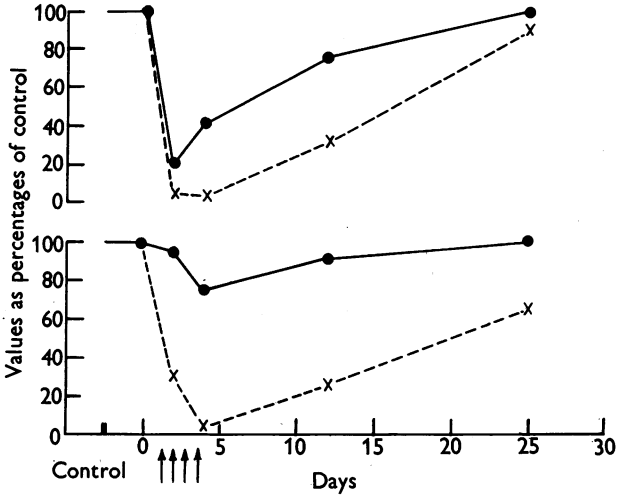


Text-fig. 5. The changes in the 5-HT (×- - -×) and histamine (●—●) contents of the spleen (upper curves) and abdominal skin (lower curves) of the rat following a single intraperitoneal injection of reserpine (10 mg/kg, at the arrow). All values are expressed as percentages of the values obtained for solvent-treated control rats.

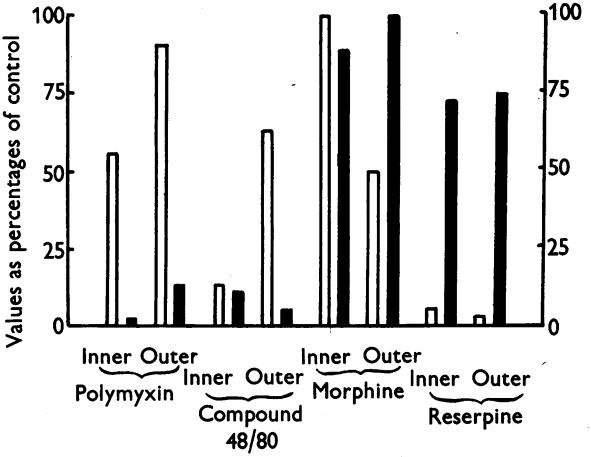
Recovery of 5-HT after repeated doses of reserpine (10 mg/kg) is slow, particularly in the skin (Text-fig. 6) and pads of the feet. Whereas histamine levels return to normal in under 12 days, the 5-HT level in the skin at day 25 is still only 62% of the normal level.

To indicate that 5-HT is not necessary for the release of histamine by compound 48/80, we have given rats two doses of 10 mg/kg reserpine and then

subjected them to two doses of the histamine releaser (1 and 2 mg/kg). They were killed 24 hr later and their tissues assayed. The results shown in Table 6 illustrate that this reserpine treatment produced 83% depletion of skin 5-HT and 17% depletion of skin histamine. Subsequent treatment with compound 48/80 released only histamine (depletion now 76%).



Text-fig. 6. The changes in the 5-HT (×- - -×) and histamine (●—●) contents of the spleen (upper curves) and abdominal skin (lower curves) of the rat following four doses of reserpine (at the arrows). All values are expressed as percentages of the values obtained for solvent-treated control rats.



Text-fig. 7. The effects of intraperitoneal injections of different substances on the 5-HT (□) and histamine (■) contents of parts of the abdominal skin of the rat. All values are expressed as percentages of the control levels.

TABLE 6. The effect of two injections of reserpine, followed by two injections of compound 48/80, on the 5-HT content and histamine content (μg/g) of the abdominal skin of the rat (for doses, see text)

Amine	Controls	After reserpine	Percentage depletion	After 48/80	Percentage depletion
5-HT	1·68	0·29	83	0·22	87
Histamine	23	19	17	5·5	76

DISCUSSION

The hypothesis that 5-HT is a natural constituent of the mast cell of the rat (Benditt, Wong, Arase & Roeper, 1955) has received indirect support from the findings of Bhattacharya & Lewis (1956*a*) on perfused tissues and from our own preliminary experiments using compound 48/80 (Parratt & West, 1956). The results in the present paper show, however, that only a small part, if any, of the 5-HT content of the skin can be associated with tissue mast cells and therefore with histamine. For example, polymyxin B releases a maximal amount of histamine from most areas of the rat with complete disruption of tissue mast cells yet has little or no effect on the tissue 5-HT from the same areas. Conversely, reserpine releases a maximal amount of 5-HT from most areas of the rat (including the lung, liver and brain) yet has little or no effect on tissue mast cells or on tissue histamine in general. Of nine tissues tested, only in the spleen did reserpine lower the histamine content; since it did this in a manner apparently not related to the dose, the significance of this anomaly is obscure. When both 5-HT and histamine are released, as for example following administration of compound 48/80, we conclude that they do not originate from the same cell, and further that the release of one amine is not dependent upon the release of the other.

A histamine-releaser like polymyxin B releases maximal amounts of histamine in the rat with only a small release of 5-HT. Compound 48/80 likewise releases maximal amounts of histamine in the rat but it is a much more active releaser of 5-HT. A further difference between the effects of polymyxin B and compound 48/80 is seen when the recovery of the amines after depletion is traced. Whereas in rats injected with compound 48/80 the depletion of histamine is made good in 28 days, a much longer recovery phase is needed following polymyxin B treatment. We do not know why this is so, but histological studies provide a possible explanation. Metachromatically stainable material from disrupted mast cells resides in the tissues for over a month following polymyxin B treatment, and these irregular blobs of material in macrophages may not be functional. The uptake of histamine by this material may be reduced from the normal rate, or even the production of fresh mast cells may be retarded. Nevertheless, there is a close relationship at all times between the histamine content of the tissue and its complement of new mast cells. It is probable that a stable complex forms between heparin and polymyxin B so that the heparin

which is retained by the tissue when the mast cells are initially disrupted is unable to remove histamine from the circulating blood. It would thus be valuable to estimate the heparin content of the tissues following polymyxin treatment, and work along these lines is in progress.

The mode of action of reserpine in depleting a tissue of its 5-HT appears to be different from that of compound 48/80. Whereas reserpine depletes all tissues of their 5-HT, compound 48/80 releases 5-HT from many tissues but fails to do so from the spleen, liver, lung and brain. The rates of return of 5-HT to the skin areas following depletion also differ considerably (compare Text-figs. 3 and 5). A side-effect which may be of importance in reserpine treatment is depletion of the catechol amines of the adrenal gland. The time course of this depletion and subsequent recovery of the catechol amines is remarkably similar to that of the depletion and subsequent recovery of the tissue 5-HT (Mann, M., Parratt, J. R. & West, G. B.; Oral Communication to British Pharmacological Society, July 1956). After large doses of reserpine, this recovery of tissue 5-HT is extremely slow in nearly every tissue studied, despite its rapid turnover. This may be taken as evidence in support of the hypothesis put forward by Brodie, Pletscher & Shore (1955) that reserpine acts by causing an alteration in the 5-HT-binding capacity of the cells—an effect persisting long after reserpine can be detected in the tissues. It is unlikely that 5-HT is released by reserpine *per se*, since intra-arterial injections of the drug do not release 5-HT from the perfused hind quarters of the rat (Bhattacharya & Lewis, 1956*b*).

That there are large amounts of releasable 5-HT in the skin of the rat tempts us to analyse its possible physiological function in this area. Both reserpine and histamine releasers (especially polymyxin) would serve as valuable tools in this elucidation. Feldberg & Smith (1953) showed that 5-HT is itself a histamine releaser and some of our own observations have indicated that this release can occur without visible effect on the mast cells. Both Feldberg & Smith (1953) and Bhattacharya & Lewis (1956*a*) have raised the question as to whether the release of histamine is dependent upon, or even influenced by, the release of 5-HT. These latter workers found that in perfused tissues the release of 5-HT by the so-called histamine releasers precedes that of histamine (also see Text-fig. 4). The present results show that rats treated with reserpine to deplete 5-HT stores in the body still release histamine when injected with the histamine releasers (Table 4). It is unlikely, therefore, that the release of histamine is dependent upon an initial release of 5-HT.

Using reserpine treatment, we have now a method of depleting the 5-HT content of a tissue, leaving the histamine content almost normal. This treatment therefore allows a study of the reactions of the 5-HT-depleted tissues of the rat in much the same way as Feldberg & Talesnik (1953) studied reactions in the histamine-depleted tissues using compound 48/80. Later work suggests

that the 5-HT release is the major cause of the oedema reaction occurring in rats following treatment with large-molecular-weight substances like dextran and egg-white, and the amine may be of more widespread importance in the reaction of the tissue to injury (Parratt & West, 1957*c*).

SUMMARY

1. The release of 5-HT and histamine in the rat by polymyxin B, compound 48/80, morphine and reserpine has been studied with particular reference to the tissue mast cells.

2. Results show that whereas histamine is chiefly contained in the mast cells of the skin of the rat, much of the 5-HT is not similarly held.

3. With polymyxin B, the rat can be depleted of its histamine with but little effect on its 5-HT content. With reserpine, the rat can be depleted of its 5-HT with but little effect on its histamine content. Both drugs provide valuable tools for the study of the physiological functions of histamine and 5-HT.

We wish to thank Upjohn Laboratories, Kalamazoo, for the gifts of 5-HT creatinine sulphate, Ciba Ltd., Horsham, for the reserpine, Burroughs Wellcome Ltd., London, for compound 48/80, and Sandoz Ltd., London, for the supply of L.S.D. and BOL 148. We also thank Mr D. King for valuable technical assistance.

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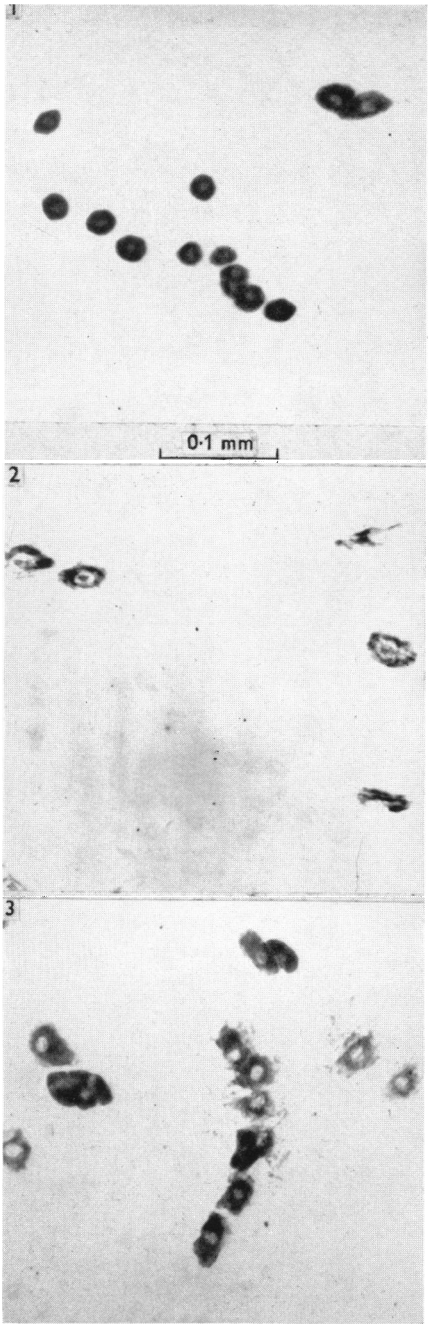
EXPLANATION OF PLATE

Mast cells in the subcutaneous connective tissue of the rat: toluidine blue.

Fig. 1. Normal rat.

Fig. 2. Eleven days after five doses of polymyxin B. This tissue contains only 25% of its histamine content but 88% of its 5-HT content. Note the reduced number of mast cells; those that remain are 'ghost cells', whilst other stainable debris is contained in macrophages.

Fig. 3. Twenty-four hours after a single dose of reserpine (10 mg/kg). This tissue contains 88% of its histamine content but only 25% of its 5-HT content. Note the swelling of some mast cells and slight spilling of the granules.



(*Facing p.* 192)