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5-Hydroxytryptamine, Tissue Mast Cells and Skin Oedema

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It was *Selye* (12) who first noticed a peculiar reaction in the rat after the intraperitoneal injection of fresh eggwhite. This reaction is characterized by an extensive oedema of the face, tongue, paws and genitals, accompanied by hyperaemia. Since it occurs following the first injection of eggwhite, it may be considered as a natural hypersensitiveness of the rat and can therefore be classified as an anaphylactoid reaction. The reaction resembles allergic phenomena more closely than it does those of anaphylaxis, and provides the opportunity to evaluate experimentally the possible mechanism of certain allergies. It is well known that among the mammalian species the rat possesses naturally a certain degree of resistance to anaphylaxis, so much so that several authors have reported difficulty in interpreting the symptoms due to anaphylactic shock from those due to the toxicity of the foreign protein.

In the past, the anaphylactoid reaction has been ascribed to the release of histamine, evidence for which may be briefly summarized as follows:

1. Injections of eggwhite release histamine from rat tissues;

2. Injections of histamine releasers produce an extensive oedema of the extremities similar to that found after injections of eggwhite;

3. The reaction is inhibited in rats whose tissue histamine has been reduced by prolonged treatment with certain histamine liberators;

4. Large doses of certain antihistamine substances prevent or markedly reduce the reaction.

Although the symptoms are not reproduceable in full by injections of histamine, large doses of which must be used, it has been concluded that the effects seen result from the local action of histamine at the site of its release. The present paper describes experiments which show that 5-hydroxytryptamine (5-HT) is also involved in the anaphylactoid reaction in the rat. A similar conclusion has been reached recently by *Rowley* and *Benditt* (9) using less specific inhibitors of the actions of these amines than those reported here and without estimating the release of the amines from the tissues. It seems that 5-HT now takes its place alongside histamine as a mediator of the response of the rat to inflammation and injury (*Parratt* and *West*, 8). This is of particular interest since no single substance has yet been found to account for the hyperaemia and increased vascular permeability associated with many common reactions to injury.

Up to the present time, 5-HT has been found in all tissues containing cells belonging to the enterochromaffin systems (e.g. the gut), in platelets in the blood, in the spleen, and in some central nervous structures. Its physiological significance however still remains unsolved, though many possibilities exist; it may be responsible in part for normal intestinal motility, it may control vascular tone and therefore the systemic blood pressure, it may participate in the regulation of the function of the kidney, or it may play a role in brain function.

Recently 5-HT has been associated with tissue mast cells in the skin of the rat (*Benditt*, *Wong*, *Arase* and *Roeper*, 2). Such cells in the skin of many species including man are rich in two important physiologically potent tissue constituents – heparin and histamine – and it was obviously important to study the occurrence, distribution and reactivity of 5-HT as well as histamine in the skin. In this way, further insight into the possible physiological significance of each amine might be obtained.

Workers in the past have repeatedly shown that histamine is released during the antigen-antibody reaction of anaphylaxis but 5-HT may also be involved. Already it has been suggested that 5-HT may play a part in allergy (*Humphrey* and *Jaques*, 7) and in asthma (*Herxheimer*, 6). Eggwhite is frequently employed as a source

of protein antigen, yet it possesses a primary action both in the rat and in the cat which is similar to its action in other species after sensitization. This primary reaction is thus of paramount importance and attempts have been made to study the amines involved.

Methods of Investigation

The tissues of the various species were obtained from animals either at operation or immediately following death by a blow on the head. For extraction of 5-HT, the tissues were weighed, cut into small pieces and extracted with 5 parts (w/v) of acetone for 24 hours. After decanting the acetone, the tissues were re-extracted with five parts of 80% (v/v) acetone. The acetone from the combined filtrates was removed by evaporation in air below 30° C. The residue was brought to the desired volume (1—10 ml/g of original material) with 0.9% (w/v) NaCl solution. For extraction of histamine, similar pieces of tissue were weighed, cut into small pieces and extracted with 10% (w/v) trichloracetic acid (1–5 ml/g) for 12–24 hours. Excess acid was then removed by 4 shakings each using 4 volumes of ether. Application of gentle heat to the aqueous extract removed any dissolved ether.

Bioassay and identification of 5-HT. The acetone extracts were assayed on the isolated uterus of the oestrus rat. The tissue was suspended in an aerated organ bath containing 15 ml of de Jalon's fluid with atropine (10^{-7}) at 28–29° C. On many occasions, the extracts were also tested on the rat colon suspended in a similar bath at 20° C. Both preparations were usually sensitive to 10^{-9} 5-HT. The specificity of the action was checked by using one of the 5-HT antagonists, lysergic acid diethylamide (L.S.D.), bromo-L.S.D. (BOL 148), and dihydroergotamine, as well as by using the antagonistic action of large doses of 5-HT itself. On occasion, the 5-HT-like action was further checked by incubation with chymotrypsin, a procedure which hydrolyses polypeptides leaving 5-HT unchanged. In most of the tests, paper chromatography eliminated the possibility that the active substance in these extracts was tryptamine. The two solvent systems used were 10% (w/v) NaCl–1% (v/v) acetic acid mixture and N-butanol-acetic acid-water (4 : 1 : 5) mixture. The former system permitted separation of 5-HT from urea which always overlapped 5-HT in the

latter system. Chromatograms were developed with *p*-dimethylaminobenzaldehyde solution. Elution of the 5-HT areas enabled estimates to be made by this process. The standard 5-HT was used as the creatinine sulphate, but all values given in the text refer to the base.

Bioassay and identification of histamine. The trichloracetic acid extracts were assayed on the guinea-pig ileum suspended in an aerated organ bath containing 15 ml of Tyrode solution with atropine (10^{-7}) at 37° C. The specificity of the action was checked using mepyramine (10^{-8}). The histamine values given in the text refer to the base.

The value of each amine shown in the figures represents the mean of the results obtained from at least six extracts of samples of tissue obtained from different animals or groups of animals.

The anaphylactoid reaction in the rat. The anaphylactoid reaction was developed in groups of 5 or more albino rats (100-150 g) after the intraperitoneal injection of dextran (average molecular weight 145,000, 300 mg/kg as a 6% solution in N-saline) or of fresh eggwhite (24 ml native/kg as a 50% v/v solution in N-saline). The local reaction was studied after subcutaneous injections of these substances (as well as those of other oedema-producing compounds) into the dorsum of the foot. The local disturbances produced are essentially similar to those resulting from the systemic administration of these substances. Each rat was lightly anaesthetized with ether and injected intravenously with 2 mg of Evan's blue dye in 0.5 ml N-saline 30 minutes prior to the subcutaneous injection of the oedema-producing substances. Drugs used to inhibit oedema were dissolved in 0.5 ml N-saline and given intravenously with the Evan's blue dye. The oedema-producing substances were injected into the hindpaws of each animal, 0.10 ml into each paw. The following solutions were used: N-saline, dextran (60 μg/ml), eggwhite (0.5% v/v solution), histamine (1000 μg/ml), and 5-HT (5 μg/ml.)

Within five minutes of the local injection of the oedema-producing substances, blueing of the weal occurred. Maximal swelling and blueing of the dorsum of the foot was present in about 30 minutes and the results were read at this time. A second reading was always made at 2 hours. The swelling subsided in 3 hours but blueing persisted for more than 24 hours. Injections of saline alone

produced very slight blueing and swelling which subsided in about 1½ hours and never exceeded 10% of the maximal response shown by the other substances. The degree of swelling (oedema) and of blueing (leakage of plasma proteins into the tissue spaces) was recorded on a relative scale from 0 to +++. By allotting marks to each degree (+ = 2, ++ = 4, +++ = 6), the results were expressed as percentages of the maximal possible effect (+++) for the dose chosen for each substance. On this system of measurement, a difference of 20% is within the error of the test. The values shown in the tables are the mean of four different experiments, separate records of the oedema and blueing being assessed to each rat and the scores then averaged. Usually oedema and blueing go hand in hand, though the mechanisms involved may be different (*Gozsy* and *Kato*, 4).

Depletion of tissue amines. To deplete most of the tissues of the rat of their histamine, five intraperitoneal injections of polymyxin B were given over 3 days. 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. Twenty-four hour after the last dose of polymyxin B, less than 6% of the initial histamine concentration remained in those areas of the skin which are normally rich in mast cells. The anaphylactoid reaction was tested at this time.

To deplete most of the tissues of the rat of their 5-HT, two intraperitoneal injections of reserpine (10 mg/kg) were given 24 hours apart. Twenty-four hours after the last dose of reserpine, less than 10% of the initial concentration of 5-HT remained in the skin. The anaphylactoid reaction was tested at this time, and again at 8 and 19 days later when the tissue levels of 5-HT in the foot were respectively 47 and 87% of the initial control value.

Perfusion of rat tissues. Rats were anaesthetised with intraperitoneal injections of urethane (1.5 g/kg). The hindquarters were perfused through the abdominal aorta using oxygenated Locke solution and the venous effluent was collected from the vena cava. The venous effluent was tested for histamine on the atropinized guinea-pig ileum and for 5-HT on the atropinized rat uterus.

Histological examination of the mast cells. Fresh tissue spreads were fixed in absolute alcohol, stained for metachromatic material with toluidine blue (0.1% w/v aqueous), washed in 50% (v/v) alcohol and then taken through xylene before mounting.

Results

The presence of 5-HT in the skin and ears. The results of the assays of skin extracts are shown in figure 1. The 5-HT concentration in the ventral abdominal skin of most species is insignificant, and there appears to be no direct relationship between the 5-HT and histamine values. In fact, large amounts of 5-HT are found only in the rat. The mouse is the only other animal with a significant amount but in this case the quantity is quite small. On the other hand, there has always been a close relationship between the amount of histamine that can be extracted from the different specimens and their relative mast cell counts. For example, hamster skin contains much histamine and enormous numbers of mast cells, whereas that of the guinea-pig is very low in both.

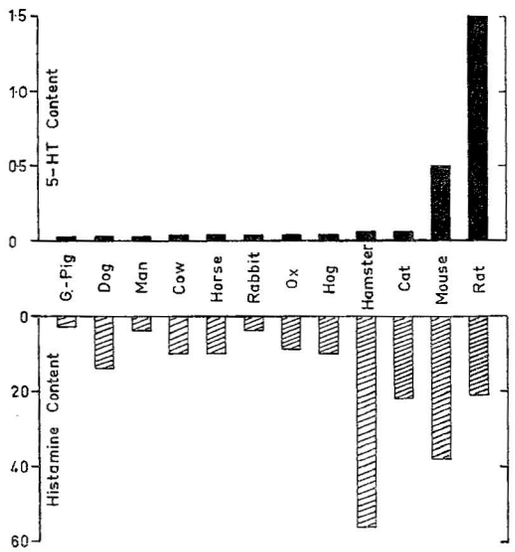

Fig. 1. Comparison of the 5-HT content (μg/g) of the ventral abdominal skin of different species with the histamine content (μg/g).

It is well known that regional variations not only of tissue mast cells but also of tissue histamine concentrations occur in the skin, the ears for example usually containing more of both. Yet the 5-HT values for the ears of different species again indicate that large amounts of this amine are present only in the rat and mouse (fig. 2). Ears of dog, hamster and cat are rich in histamine and mast cells but lack 5-HT. We do not yet know why the rat and

mouse should differ from the other species studied in respect of the amounts of skin 5-HT. It may be that the mast cells in these two species contain a heparin which differs in certain physical and chemical characteristics from the heparin of the other species, though other explanations of the differences suggest themselves – e.g. sensitivity of the blood vessels to 5-HT, or reactivity of the sensory nerve endings.

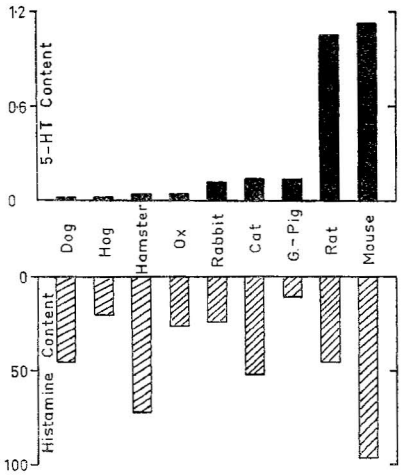

Fig. 2. Comparison of the 5-HT content (μg/g) of the ears of different species with the histamine content (μg/g).

Pathological tissues and 5-HT. Carcinoids are known to produce huge quantities of 5-HT, flooding both the blood and urine with 5-HT products. In such patients, however, tumour growth of the argentaffin cells in the gut occurs and mast cells are not involved. The rare subcutaneous mast cell tumour of the dog is rich in heparin and histamine and would seem ideal for studying the relationship between mast cells and 5-HT in the dog. Acetone extracts of these tumour tissues, however, have failed to reveal more than the normal very low concentration of 5-HT (table 1). Mast cells in the skin of the dog appear to be unable to store this amine.

Specimens of skin from two human cases of urticaria pigmentosa and from two cases of human mast cell tumours have also failed to yield raised 5-HT levels, despite grossly raised histamine values. It is not known if patients with other allergic disorders such as dermatoses possess raised skin 5-HT levels.

Two biopsy specimens of skin from a Shorthorn heifer with multiple nodules rich in mast cells and histamine also did not show raised 5-HT values.

Thus, in dog, man and cow, mast cell proliferation is not accompanied by any appreciable increase in the skin 5-HT content.

Table I

The amine contents (μg/g) of mast cell tumours in the skin of different species

Species	Number of samples	Histamine	5-HT
Cow	2	150	0.03
Man	2	233	0.05
Dog	6	788	0.04

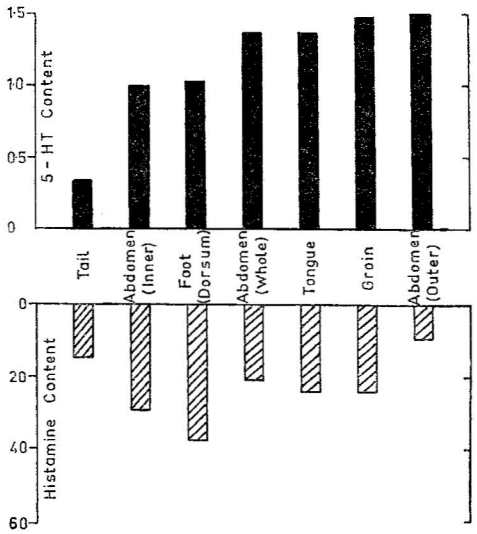

Fig. 3. Comparison of the 5-HT content (μg/g) of the skin from different locations of the rat with the histamine content (μg/g).

The location of 5-HT in the skin of the rat. Having established that 5-HT resides in the skin of certain species, it is important next to locate the area of the skin where it is concentrated. The adult rat was used for this purpose since calculations indicated that over half of the total 5-HT in this animal (53 μg) can be found in the skin (27 μg). A study was made of the skin from various specialized regions of the rat. The results shown in figure 3 demonstrate that whereas the histamine content is highest in the

dorsal skin of the rat, groin skin contains the most 5-HT. The skin of the tail contains the least of both amines. All areas contain appreciable quantities of 5-HT and it is most probable that to the rat the 5-HT in the skin is of physiological significance.

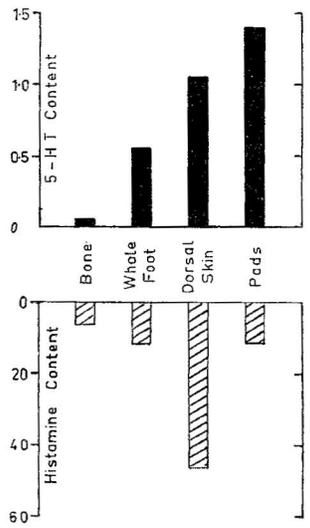

Fig. 4. Comparison of the 5-HT content (μg/g) of the skin from different areas of the the foot of the rat with the histamine content (μg/g).

Most of the densely-staining mast cells in the areolar connective tissue of the skin of the rat can be scraped off from an excised strip simply by pinning the strip out on a board (fur-side downwards) and using an ordinary scalpel. Some mast cells remain in the corium but they are smaller in size and less densely stainable than those removed with the scrapings (called the inner layer). The histamine is concentrated in this inner layer of the skin of the rat (see figure 3). But the reverse is true for 5-HT, since assay shows that the outer layer contains nearly twice as much 5-HT as does the inner layer. Thus tissue mast cells may not be the only source of 5-HT in rat skin and other tissue components may have the faculty of storing this compound. Further support for this hypothesis is found when the structures in the feet are extracted and assayed separately. Mast cells and histamine are concentrated in the dorsal skin of the foot but this is not so for 5-HT. The pads are rich in 5-HT but are relatively poor in histamine and mast cells (figure 4).

During the course of experiments designed to confirm early observations (*Hardwick*, 1954) that histamine levels in the skin of the rat are raised in the few days following weaning (at day 21), it has been noted that a similar change occurs in the 5-HT values. Two days after weaning, the histamine and 5-HT levels for abdominal skin, ears and dorsal skin of the foot are about twice those found in unweaned littermate rats, yet mast cells in these regions are not increased in number or altered in appearance. The exact cause of these increases is still unknown though it may be simply related to the stress involved in weaning, since the return to normal levels is complete in the next six days. It is not due to an increase in ingested amines, nor to a redistribution of the amines, since the histamine and 5-HT contents of the whole animal are increased at this time. However, changes in glandular secretion rates and in intestinal flora may be involved.

The release of 5-HT and histamine from the skin of the rat. An extensive amount of research work has been carried out in recent years with histamine-releasing substances. It has been thought that the histamine released from tissue mast cells under the action of some specific agent may be responsible in part for the capillary hyperaemia, increased permeability and oedema found in the acute inflammatory response. It now seems possible that some of the 5-HT in the skin of the rat, and possibly of the mouse also, is released together with the histamine and the two amines may account for most of the vascular changes seen in injury.

Experiments were therefore designed to study the changes in the 5-HT and histamine contents of the tissues of the rat following treatment with drugs. The two drugs chosen for extensive observations were polymyxin B and reserpine (figure 5). The first drug releases a maximal amount of histamine from most areas (about 90%) with complete disruption of tissue mast cells, yet has little effect on the tissue 5-HT from the same areas (release about 20%). The second drug releases a maximal amount of 5-HT from most areas of the skin of the rat (about 85%), yet has little effect on tissue mast cells or on tissue histamine (release about 20%). This finding alone shows that only a small part of the 5-HT content of the skin is likely to be associated with tissue mast cells and therefore with histamine. When both 5-HT and histamine are released (as for example after doses of compound 48/80), it is concluded

that they may not originate from the same cell, and further that the release of one amine is not dependent upon the release of the other. Thus, treatment with polymyxin B or reserpine allows a study of the reactions of the histamine-depleted or 5-HT-depleted rat.

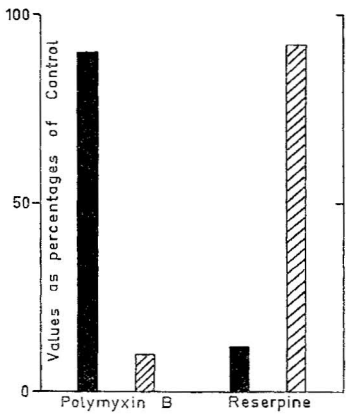

Fig. 5. The effects of repeated intraperitoneal injections of polymyxin B and reserpine on the 5-HT (blackened blocks) and the histamine (striped blocks) contents of rat skin. Values are expressed as percentages of the control levels.

Oedema-producing substances in the rat. The visible effects of single intraperitoneal injections of either dextran or eggwhite have been extensively described by other workers and briefly consist of violent scratching, oedema of the extremities and marked erythema. It is in those areas which are rich in histamine and mast cells that pruritis and oedema are most marked. These areas also contain much 5-HT. When the rats are killed and their tissues are assayed, the major changes are found in these areas – feet skin, pads and ears. Losses of both amines have taken place, in certain cases less than 50% of the amine remaining. Dextran and eggwhite in the doses used usually release similar relative amounts of the amines from similar tissues. Tissue mast cells in the feet, ears and abdominal skin are only slightly affected, exhibiting degranulation rather than disruption.

Further proof that dextran and eggwhite release both 5-HT and histamine from rat tissues is provided by results of intra-arterial injections of these substances into the perfused hindquarters. Both substances release greater amounts of histamine than 5-HT, the ratio being about 30.

Tests were then made to determine if the oedema fluid contained a 5-HT-like substance. Rats were given a single intraperitoneal injection of dextran or eggwhite and killed when the oedema was at its peak (1½ hours). The dorsal skin of the foot was removed and the fluid in the subcutaneous regions absorbed on to small strips of filter paper. These strips when placed in isolated organ baths produced contraction of both atropinized rat uterus and colon (test preparations sensitive to 10^{-9} 5-HT). These contractions as well as those of the standard 5-HT were prevented by BOL 148 and recovery of the responses followed removal of the anti-5-HT compound from the bath fluid. Thus there can be little doubt that the oedema fluid contained a 5-HT-like substance.

Repeated injections of gradually increasing doses of dextran (maximal dose 1.2 g/kg) produced a refractory state in rats when massive doses failed to elicit the anaphylactoid reaction. When the tissues were analysed at this point, only the histamine levels in the pads and ears were lower than after a single dose of dextran, and the 5-HT levels showed little change. Thus, when the anaphylactoid reaction is absent, as a result of repeated doses of dextran, histamine and 5-HT stores are not entirely depleted. The damage to mast cells is no more than that seen after a single injection of dextran. Rats refractory to dextran were also refractory to eggwhite, but if left for 3 or more days without injection the anaphylactoid response to both substances was restored.

The next step was to administer polymyxin B to rats to deplete the tissues of their histamine (leaving the 5-HT content almost unchanged) and then test the anaphylactoid response. Twenty-four hours after the last dose of polymyxin B, a single intraperitoneal injection of dextran or eggwhite gave the full response with gross oedema of the feet, erythema and pruritis, despite the histamine content of the tissues being only 10% of the control levels. Assay of tissue extracts after the challenge with dextran or eggwhite indicated that these substances released 5-HT from most skin regions. Thus depletion of histamine alone is not sufficient to prevent the production of oedema since the 5-HT that remains can be released.

When rats received reserpine to deplete the tissues of their 5-HT (leaving the histamine content almost unchanged), the single intraperitoneal injection of dextran or eggwhite failed to

produce oedema, and pruritis was greatly reduced. Thus depletion of 5-HT alone is sufficient to prevent the production of oedema by dextran or eggwhite, and 5-HT appears to be the amine largely responsible for the effects of the anaphylactoid reaction in rats.

Local injections of dextran and eggwhite in the rat. In a series of preliminary experiments, concentrations of each oedema-producing agent were found which produced nearly maximal oedema of approximately equal severity. The doses used for this response in each hindpaw were 6 μg dextran, 50 μg native eggwhite, 100 μg histamine and 0.5 μg 5-HT. Saline injected into the foot of the opposite side always served as a control. Two methods were used to study the mechanism of this local response – a) depletion of tissue amines, and b) the use of specific antagonists.

a) *Depletion of tissue amines.* Groups of rats were injected intraperitoneally with repeated doses of polymyxin B before being challenged 24 hours later with local injections of the oedema-producing agents. Despite the low histamine level in the skin, the oedema was almost maximal in all cases (table II). When however groups of rats were injected with reserpine, the oedema produced by dextran or eggwhite was regularly inhibited, although 10 ml of saline was injected intraperitoneally 30 minutes previously to increase body fluid. After reserpine, 5-HT and histamine still produced their full responses. When the 5-HT stores in the tissues were partly replenished 8 days later (e.g. 5-HT content of feet skin 47% of control value), the oedema produced by dextran or eggwhite was present but not severe. After a further 11 days of recovery from the reserpine treatment the responses of all agents were nearly maximal. Thus recovery of the 5-HT levels in the tissues is necessary before the anaphylactoid reaction is regained.

Table II

The effect of prolonged treatment with polymyxin B or reserpine on the local oedema reaction produced in rats by four different agents. Action tested 1 day after polymyxin B treatment, and 1, 8 and 19 days after reserpine treatment. Responses measured in terms of the maximal (100%) found in saline-treated rats.

Agent	1 day after polymyxin	Days after reserpine		
		1	8	19
Dextran	85	7	34	85
Eggwhite	87	12	42	85
5-HT	100	85	100	100
Histamine	100	85	96	100

b) *The use of specific antagonists.* Having established that the chief mediator of the anaphylactoid response was most likely 5-HT, it seemed highly desirable to determine if one of the most specific antagonists of 5-HT modified the response. The most active substance in this series is lysergic acid diethylamide, but this is too toxic to administer intravenously into rats. The brominated derivative, BOL 148, lacks the toxic side-effects of L.S.D. but retains the specific antagonistic action on 5-HT. Doses of BOL 148 (4 mg/kg) were therefore injected intravenously 30 minutes before the local injections of the oedema-producing substances. The effects of 5-HT, eggwhite and dextran were completely prevented, whilst that of histamine remained unchanged (table III). On the other hand, the specific antihistamine substance, mepyramine, did not modify the responses of dextran, eggwhite or 5-HT, but slightly reduced that of histamine. Further, promethazine greatly reduced the reactions of dextran, eggwhite and histamine without modifying that of 5-HT. This last result is to be expected since promethazine possesses marked anti-5-HT as well as marked antihistamine properties.

Table III

The effect of certain antagonists (4 mg/kg) on the local oedema reaction produced in rats by four different agents. Responses measured in terms of the maximal (100%) found in saline-treated rats.

Agent	Antagonist		
	BOL	Promethazine	Mepyramine
Dextran	13	29	85
Eggwhite	12	65	85
5-HT	7	93	100
Histamine	98	39	66

Discussion and Conclusions

The work of *Benditt, Wong, Arase* and *Roeper* (2) indicated that 5-HT is associated with mast cells in the subcutaneous areolar tissue of the rat. The results of the present experiments, however, show that it is most unlikely that the mast cells contain any considerable quantity of 5-HT. The evidence for this is as follows:

1. The ventral abdominal skin of various species is usually rich in mast cells and histamine but lacks 5-HT. There appears to be no relationship between the mast cell population of the skin and its 5-HT content, since it is only in the rat and mouse that much 5-HT can be found in this area.

2. In the rat, the 5-HT can be found in high concentration in skin areas where mast cells (and histamine) are scarce. For example, the outer layers contain nearly twice as much 5-HT as do the inner layers where the large densely-staining mast cells reside, and the pads of the feet are rich in 5-HT but are relatively poor in mast cells (and histamine).

3. Both 5-HT and histamine levels in the skin of the rat are raised at weaning time though mast cells in this region are not increased in number or altered in appearance. However, one can find a raised histamine value for skin but not a raised 5-HT value (as at birth), and a raised 5-HT value but not a raised histamine value (as after thyroid treatment). Thus it is not possible to postulate a single physiological mechanism which controls the levels of these amines.

4. Polymyxin B releases a maximal amount of histamine from many areas of the skin of the rat with complete disruption of tissue mast cells, yet it has little effect on the tissue 5-HT in the same areas. A loss of about 20% of the 5-HT occurs and this relative amount could be contained in mast cells.

5. On the other hand, reserpine releases a maximal amount of 5-HT from most areas of the skin of the rat yet it has little effect on tissue mast cells or on tissue histamine. If polymyxin B is then injected, release of histamine occurs and the mast cells disrupt.

6. It is relatively easy to obtain a histamine-heparin complex *in vitro*, but extremely difficult to do so with 5-HT (*Sanyal* and *West*, 10). It must be stressed, however, that work of this nature is usually carried out using ox heparin and a different result might be obtained if rat, or even mouse, heparin was employed.

The results of the present experiments also show that the anaphylactoid reaction produced in rats by injections of dextran and eggwhite is mediated chiefly through the release of 5-HT. The facts may be summarized as follows:

1. 5-HT is present in rat skin.

2. Oedema-producing substances release 5-HT as well as histamine from rat skin.

3. Oedema fluid contains a 5-HT-like substance.

4. No oedema occurs if the skin is first depleted of its 5-HT content by reserpine treatment.

5. Oedema can be produced in rats whose skin has been depleted of its histamine content by polymyxin B treatment.

6. Minute amounts of 5-HT produce the phenomenon both in the normal rat and in the animal whose skin histamine or 5-HT has been depleted.

7. Whereas a specific antagonist of 5-HT (BOL 148) prevents the anaphylactoid reaction, antihistamine substances are only weakly antagonistic in this respect.

Although the anaphylactoid reaction has been noted chiefly in rats, it is possible to show a reaction in mice by injecting dextran or eggwhite into animals which have previously received an intravenous injection of Evan's blue dye. Oedema is difficult to judge but blueing of the extremities (i.e. leakage of plasma proteins into the tissue spaces) is easily seen. The anaphylactoid reaction has not been noted in the guinea-pig, rabbit, hamster or cat, and thus can directly be related to the presence or absence of 5-HT in the skin. Injections of 5-HT into the skin of the different species likewise give similar results, only the rat and mouse exhibiting full oedema reactions after minute doses of the amine. If 5-HT is injected into human skin, a very mild degree of oedema is sometimes produced, whereas only minute doses of histamine are needed for the triple response.

When dextran or eggwhite are added to the perfusion fluid, both 5-HT and histamine are released from the rat's hindquarters in relative amounts which correspond well with those found after intraperitoneal injections into the whole animal. Thus there can be no doubt that both amines are released. If, however, repeated doses of dextran are given to rats, a refractory state is reached when further doses of dextran fail to elicit the anaphylactoid reaction. Eggwhite also fails to give a reaction at this time, so animals refractory to one oedema-producing substance (dextran) are refractory to another (eggwhite). This result obviously suggests a similar mode of action of these two substances. Yet estimates of tissue amine levels at this refractory state show that there is still much 5-HT as well as histamine remaining in the tissues and on the whole tissue mast cells are intact. In fact, other oedema-producing agents

such as compound 48/80 react maximally at this stage and we are unable to explain as yet why the refractory state with dextran results.

Besides dextran and eggwhite, a study has also been made of the oedema reaction produced by the local injection of the well-known potent histamine releaser, compound 48/80. This substance produces oedema of the extremities in a manner similar to that of eggwhite and dextran. This oedema is prevented by pretreatment with repeated doses of reserpine or single doses of BOL 148, but almost unaffected by single doses of antihistamine substances. It is well known that compound 48/80 releases both 5-HT and histamine and disrupts mast cells. After repeated doses of polymyxin B (when tissue histamine levels are only about 10% of the control values), compound 48/80 is fully effective in producing oedema and in fact releases only 5-HT. Thus there is a similarity in the mode of action of dextran, eggwhite and compound 48/80.

The effects of other antagonists of 5-HT and of histamine (e.g. antimetabolites of 5-HT, phenothiazine derivatives, and sympathomimetic amines) upon the oedema reaction in the rat are now in progress.

The evidence presented in this paper shows that 5-HT now takes its place alongside histamine as a mediator of the response to inflammation or injury in certain areas in the rat. Human skin is deficient in 5-HT, even when abnormally large amounts of histamine are present, and it is not known whether 5-HT appears in detectable amounts in human skin in various allergic states. It may be that in normal life 5-HT is too toxic to the sensory organs in human skin for it to remain there in concentrations which can easily be detected. It is important to remember that the concentration of 5-HT in wasp venom exceeds the pain-producing threshold concentration of this substance (10^{-9}) when applied to human skin as at the base of a blister (*Armstrong*, *Dry*, *Keele* and *Markham*, 1), and may therefore account for some of the features of the human skin reaction following a wasp (but not a bee) sting. Another point of interest is that 5-HT occurs with histamine and acethylcholine in the sting of the nettle and may be responsible for, or augment, some of the side effects of stings from these plants.

Eggwhite is freqently used as a source of protein antigen and is generally considered to have no primary toxicity. In the rat, however, we have seen that eggwhite releases both 5-HT and

histamine, and evidence from other workers shows that it can release histamine in the non-sensitized cat, but not in the dog. The histamine-releasing constituent of eggwhite nevertheless is different for the cat and rat, since in the former it is heat-labile and in the latter it is heat-stable (*Schachter* and *Talesnik*, 11). In a similar manner, it can be shown that horse serum (another frequent source of protein antigen) is able to release histamine both from rats and from isolated cat skin preparations, and to raise the plasma histamine level in non-sensitized cats. As with eggwhite, it loses this property in cats on boiling for a short time. These findings are relevant to the well-known facts that specific antigens which have no effect on blood from normal patients, release histamine from blood of patients with the specific sensitivities. On the other hand, in the dog horse serum only releases histamine if the animal has been sensitized to horse serum, i.e. during the anaphylactic shock. The release of histamine from dog skin preparations by either antigen or anaphylatoxin is always greater in the presence of saline perfusions than when supplied with the natural circulation, but this is not so for dog liver. It is not certain why such a difference in the release occurs in the same animal.

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Summary

1. 5-Hydroxytryptamine is not concentrated in tissue mast cells in the guinea-pig, dog, man, cow, horse, rabbit, ox, hog, hamster or cat. It may be partly associated with tissue mast cells in the rat and mouse.

2. The skin of the rat contains more than half of the total 5-HT in the body. This can be depleted by treatment with reserpine. On the other hand, the rat can be depleted of its histamine by treatment with polymyxin B.

3. Substances which produce the anaphylactoid reaction in rats (e.g. dextran and eggwhite) release 5-HT as well as histamine from skin tissues. The reaction is prevented by depletion of the

skin 5-HT using reserpine but it is not prevented by depletion of skin histamine alone. The reaction is prevented by previous administration of the specific anti-5-HT compound, BOL 148, but it is only slightly modified by antihistamine substances.

4. It is concluded that the anaphylactoid reaction in rats is mediated chiefly through the release of 5-HT.

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