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The role of histamine and 5-hydroxytryptamine in inflammatory processes

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During aseptic inflammation there is a rise in the histamine and 5-hydroxytryptamine (5-HT) contents of the skin and several internal organs in mice and rats. The time course of this rise has been worked out. Depletion of histamine or 5-HT, adversely affects the exudative or reparative phases of inflammation in the rat. Reserpine, the agent used to deplete 5-HT, produces an effect which resembles the action of anti-inflammatory steroids like prednisolone.

THE occurrence of high levels of histamine at sites where the organism meets the external environment, led to the suggestion that histamine might be involved in a defence mechanism (Paton, 1955).

The close association of histamine with 5-hydroxytryptamine (5-HT) and the mast cell, and the demonstration that characteristic mast cell changes occur during aseptic inflammation (Sanyal, 1959), has strengthened the hypothesis of a defensive function for histamine and suggested a similar role for 5-hydroxytryptamine (5-HT).

The changes in histamine and 5-HT contents after aseptic inflammation have been studied and the effect of their depletion has been separately studied both for the exudative and reparative phases of inflammation. A preliminary communication was published earlier (Bhatt & Sanyal, 1963).

Materials and methods

Animals. Albino rats, 80–120 g, and albino mice 20–25 g, of either sex were obtained from local dealers and fed with Anidiet A, the composition of which has been previously described (Dhar & Sanyal, 1962). Animals were maintained in air conditioned rooms and water was allowed *ad lib.*

Production of aseptic inflammation. Rats or mice were anaesthetised with ether, and on the shaved dorsal skin an aseptic incision was made 2–3 cm long in mice and 3–5 cm in rats. The skin flaps were mobilised and then the wound was closed with suture clips. Similar animals anaesthetised and then allowed to recover served as controls. All were then transferred to sterilised cages. Animals, from control and test groups were killed at varying intervals, and tissues from 6 or more similarly-treated animals were collected and pooled for extraction and assay of histamine and 5-HT as described by Parratt & West (1957a). Mast cell examinations were made at the same time.

Production of granuloma pouch. The granuloma pouch technique of Selye, as described by Finney & Somers (1958) was used to examine the exudative phase of inflammation in rats (150–200 g, either sex). A pouch in the subcutaneous tissue of the dorsum was formed in anaesthetised animals by injecting 25 ml of air, 0·5 ml of a 2% solution of croton

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oil in arachis oil was subsequently injected into the pouch. The intense irritation thus set up leads to inflammatory changes in the pouch which can be assessed qualitatively while the amount of exudate can be measured.

Cotton wool pellet test. To study the phenomenon of fibrosis, the cotton wool pellet method of Meier, Schuler & De Soulles, as described by Finney & Somers (1958) was adopted. Sterilised cotton wool pellets (8/animal) each weighing 10 mg were implanted aseptically in the subcutaneous tissue of the back. They were removed after 7 days, cleared of extraneous tissues, and dehydrated in an oven to constant weight. The rise in weight denote the amount of fibrous tissue formed.

Depletion of tissue histamine and 5-HT. Tissue histamine or 5-HT was preferentially depleted by repeated injections of polymyxin B, or reserpine respectively, as described by Parratt & West (1957a).

Results

CHANGES IN MAST CELLS, TISSUE HISTAMINE AND 5-HT CONTENT DURING ASEPTIC INFLAMMATION

Changes in rats. Throughout the experiment, the operated animals were in apparent good health, their behaviour being indistinguishable from that of control animals. Animals were killed 1, 2, 3, 4, 6, 8, 12, 16 and 26 days after operation. Controls were killed at the same time.

A slight swelling and accumulation of serosanguineous fluid was noticed during the first 2–3 days, but this subsided later. The inflammatory changes were seen to be subsiding and strands of fibrous tissue had made their appearance 6 days after operation. The incised edges were united on day 12, and the union was quite firm after 26 days.

The mast cells in an adjacent site were swollen and degranulated after 24 hr, many had disappeared. The changes were more marked after 48 hr when very few cells were detected. In 4 days a large number of small mast cells made their appearance along with vascular strands, which were seen to invade the operated area. Some of these cells migrated away from blood vessels, and rapidly divided to form cell clumps.

After 8 days, mast cells were normal in appearance.

The changes in histamine and 5-HT contents of skin from operated animals expressed as % of values obtained in control animals are shown in Figs. 1, 2 and 3 for the site of injury, an adjacent site and a distal area respectively. There was an initial lowering of histamine values at the injured site which was later followed by a rise. In other areas, there was a rise from the beginning, which, as in the injured site, was maximal in 8–12 days. The values in the injured area were subnormal in the 16–26 day period, but in other areas, by this time the contents were comparable with values obtained in control animals.

5-HT values from all the areas showed a rise, which was maximal in 6–8 days, and returned to basal levels after 16–26 days.

The changes in histamine and 5-HT contents of other tissues are shown in Table 1. The histamine content of the intestine was slightly raised, and returned to normal in 12 days; that of the spleen showed minor or no

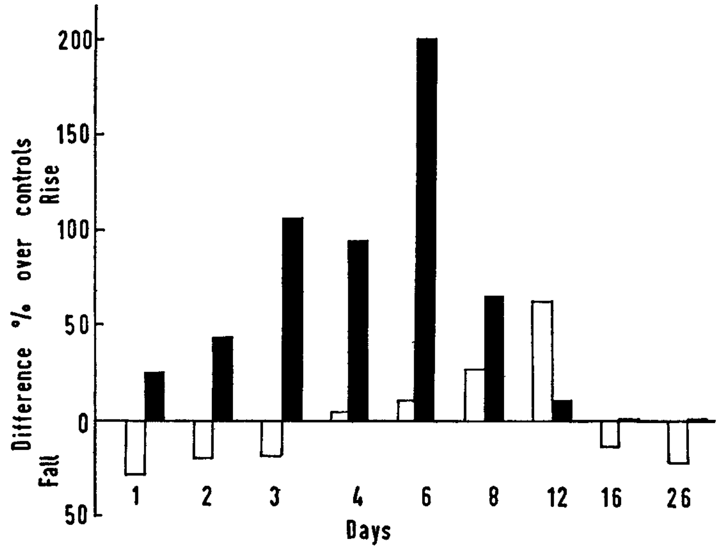

FIG. 1. Changes in histamine and 5-HT content of rat skin at the site of injury after production of aseptic inflammation. Results expressed as percentage differences over control values. Open columns = histamine; solid columns = 5-HT.

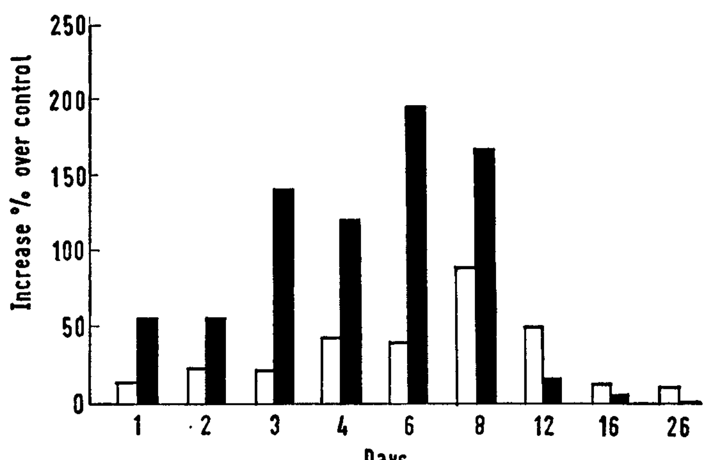

FIG. 2. Changes in histamine and 5-HT content of rat skin in an area adjacent to the site of injury after production of aseptic inflammation, expressed as percentage increase over control values. Open columns = histamine; solid columns = 5 HT.

changes; that of the lung showed erratic changes. Experiments were repeated several times, but no definite pattern could be established. The 5-HT values of tissues other than skin showed insignificant alterations.

Changes in mice. Groups of animals were killed 1, 3, 7 and 15 days after operation for mast cell study and extraction and assay of histamine

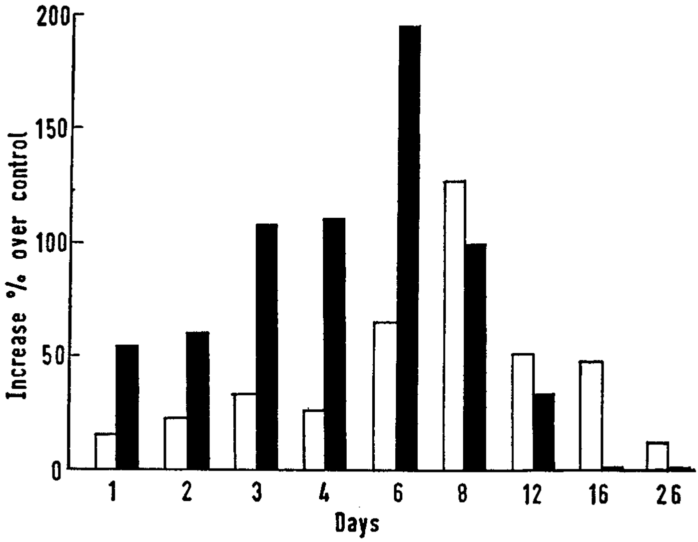

FIG. 3. Changes in histamine and 5-HT content of rat skin in an area distal to the site of injury after production of aseptic inflammation, expressed as percentage increase over control values. Open columns = histamine; solid columns = 5-HT.

TABLE 1. HISTAMINE AND 5-HT CONTENT OF SPLEEN AND INTESTINE OF RAT, AFTER PRODUCTION OF ASEPTIC INJURY ON THE SKIN EXPRESSED AS % OF CONTROL

	Spleen		Intestine	
Days after injury	Histamine	5-HT	Histamine	5-HT
1	105	112	146	82
2	105	85	135	76
3	105	102	108	82
4	110	80	116	100
6	97	100	114	100
8	91	120	136	100
12	92	89	108	100
16	100	100	108	100
26	100	100	100	87

and 5-HT from various tissues. Control animals were killed at similar times.

No animals developed obvious sepsis, and a minimal amount of serosanguineous discharge was seen only in the first 24 hr. By day 7 inflammation had subsided, and the wound edges were uniting. The union was firm by day 15.

Mast cell changes were similar to those seen in the rats, a later near-normal state being obtained by day 15.

The changes in the histamine and 5-HT contents are shown in Table 2. In an area adjacent to the operated site, there was an initial lowering, followed by a rise of histamine content which returned to near normal values in 15 days. In distal areas, high values were seen from the beginning and these returned to normal in 15 days. 5-HT values were raised from

TABLE 2. CHANGES IN THE HISTAMINE AND 5-HT CONTENT OF THE AREA ADJACENT AND DISTAL TO THE SITE OF INJURY OF MOUSE SKIN AND INTESTINE AFTER PRODUCTION OF ASEPTIC INFLAMMATION ON THE SKIN EXPRESSED AS % OF CONTROL VALUES

Days after production of injury	Area adjacent to site of injury		Area distal to site of injury		Intestine	
	Histamine	5-HT	Histamine	5-HT	Histamine	5-HT
1	73·00	116·66	150·00	125·00	333	320
3	131·62	140·62	154·28	150·00	100	90
7	156·66	200·00	200·00	275·00	100	100
15	100·00	100·00	115·00	112·50	106	104

the beginning and were maximal in 7 days, normal values were obtained in 15 days.

CHANGES IN HISTAMINE CONTENTS OF SKIN DURING SEPTIC INFECTION

As studies were in progress, rats received in a batch from a local dealer were seen to have multiple abscesses in the skin, and it was thought that it might afford an opportunity of studying the histamine content during natural infections. A smear of the pus, on examination, showed clumps of organisms resembling staphylococci. The clinically non-infected animals were separated and served as control. There was a 50% rise in the histamine content of the skin of infected animals as compared with controls.

EFFECT OF DEPLETIONS OF HISTAMINE OR 5-HT ON GRANULOMA POUCH PRODUCTION IN RATS

Granuloma pouch in control animals. Granuloma pouches were produced in a number of rats, and animals were killed after 3, 5, 8, 13 and 18 days. Intense inflammation and serosanguineous discharge was seen in 3 days, between 5–8 days, the pouch became adherent to adjacent structures with much thickening of the wall, and the discharge also became seropurulent. Later, there was greater thickening of the wall and the pus became thick and viscid. Changes were most marked between 5 and 8 days and in all further experiments pouches were examined after 6 days.

Granuloma pouch in histamine-depleted animals. Groups of animals received repeated injections of polymyxin B beginning with 0·5 mg/kg and increasing to 5·0 mg/kg over 4 days. Control animals received injections of normal saline at similar times. One group each of polymyxin B-treated, and control animals were killed for extraction and assay of tissue histamine and 5-HT. The histamine content of the skin and lung was much reduced, the residual amount being approximately 10% of control values. Spleen values were not significantly altered, and the intestinal contents were depleted by about 50%. Changes in 5-HT values were insignificant.

In another set of polymyxin B-treated and control animals, granuloma pouches were induced. On the sixth day, a mild to moderate degree of inflammation was seen in 50% of animals and in the rest, inflammation was patchy and mild; the amount of exudate was small (Fig. 4).

Granuloma pouch in 5-HT *depleted rats.* Injections of reserpine, 10 mg/kg, were given on two consecutive days. This resulted in a 75–95% depletion of 5-HT from most organs, leaving the histamine values unaffected. The granuloma pouches in this group, in contrast to the control animals, were not warm to the touch. The skin was thin and parchment-like. Mild inflammation was present in only 25% of animals. In the rest there was no sign of inflammation. The amount of exudate was insignificant (Fig. 4).

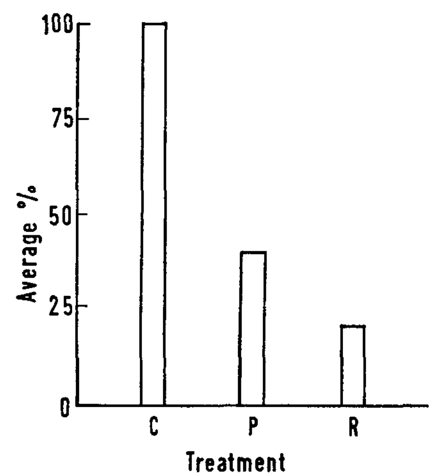

FIG. 4. Amount of exudate in granuloma pouch after various treatments expressed as average % (control value = 100%). C = control; P = polymixin; R = reserpine.

Granuloma pouch in prednisolone-treated animals. Groups of rats were given intraperitoneal injections of prednisolone hemisuccinate, 20 mg/kg, for 3–4 days. There was a 80% reduction in the 5-HT content of skin and lungs, whereas the amounts in the spleen and intestine were approximately halved.

Histamine content of skin and intestine was reduced by approximately 30%, the lung and spleen values being unaffected. The changes in the granuloma pouch after prednisolone treatment were similar to those seen in reserpine-treated animals. The exudate was insignificant, and inflammation was minimal compared with control animals.

GRANULATION TISSUE FORMATION AFTER DEPLETION OF TISSUE AMINES

One group of 6 rats received injections of polymyxin B, as in the previous experiment, another group of 6 received injections of reserpine, a further 6, the control group, received injections of normal saline. Cotton wool pellets were then inserted in all the animals as described. The amount of fibrous tissue developed in 7 days is shown in Fig. 5.

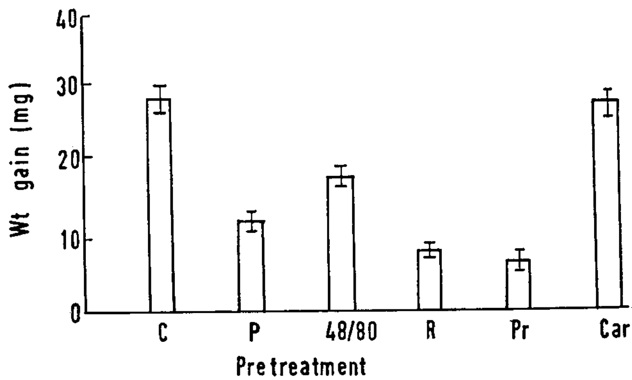

FIG. 5. Illustrating the gain in weight of implanted cotton wool pellets in rats after various pretreatments. Data based on average of 48 observations for each. C = control; P = polymixin; 48/80 = compound 48/80; R = reserpine; Pr = Prednisolone; Car = carbachol.

Development of granulation tissue was minimal in reserpine-treated animals; the amount in polymyxin B-treated animals was also lower than that of the controls. The differences were statistically significant.

The prevention of granulation tissue formation after reserpine treatment could be due to depletion of 5-HT, or depletion of catecholamines (Muscholl & Vogt, 1957) or to its own pharmacological actions. To test these possibilities the experiments were repeated after the following pretreatments.

(i) Compound 48/80, graded doses 1 to 5 mg/kg over 4 days, a substance depleting both histamine and 5-HT (Parratt & West, 1957b).

(ii) Carbachol, 0·5 mg/kg twice daily for two days, a substance depleting catecholamine stores (Butterworth & Mann, 1958).

(iii) Prednisolone, a substance with known anti-inflammatory actions, and known to deplete 5-HT from several tissues.

The results are in Fig. 5. Prednisolone prevented development of grannulation tissue to a marked extent; carbachol had no action. Compound 48/80 pretreatment reduced the amount of fibrosis around the implanted pellets.

The differences were statistically significant for prednisolone and compound 48/80.

Discussion

During aseptic inflammation in rats and mice there is a rise in tissue histamine and 5-HT, the process being most marked in the skin. Depletions of histamine or 5-HT adversely affected the exudative as well as the reparative phases of the inflammation. A rise also occurred in septic infections. Depletion of histamine or 5-HT has been shown previously by Mishra & Sanyal (1959) to increase the invasiveness of pathogenic bacteria in rats, to which in natural state they are relatively immune.

There is some parallelism between the changes in histamine and 5-HT levels with those occuring in mast cells, but the association is not very close. An interesting feature of the rise is that it is not localised but is found over all the skin in sites distal to injury and in some internal organs. This suggests the possibility of some general mechanism which it is hoped to consider at a later date.

The anti-inflammatory action of reserpine is most likely to be related to its 5-HT depleting properties, as this is shared with other substances having similar actions.

The reserpine action resembles that of prednisolone. Both substances affect the actions of 5-HT more than they do those of histamine, though there are qualitative and quantitative differences. It is debatable whether reserpine action is mediated through the cortical hormones, or that both substances have a similar site of action.

Much evidence has now accumulated to suggest that histamine and more particularly 5-HT may be involved in defensive functions in response to injury, infection, inflammation and repair.

This may be summarised as follows:

(i) There is a rise in skin histamine after aseptic injury as well as in naturally infected states (see also Geiringer & Hardwick, 1953).

(ii) The inflammatory response in the granuloma pouch method, and development of fibrous tissue round implanted cotton wool pellets are reduced after histamine depletion, and more particularly after depletion of 5-HT. Stern & Nikulin (1960) also noted that histological changes in the granuloma pouch were less marked after histamine or 5-HT depletion.

(iii) The resistance of rats to pathogenic organisms is reduced after histamine or 5-HT depletion (Mishra & Sanyal, 1959).

(iv) Inflammatory exudates contain both histamine and 5-HT and each substance is capable of increasing capillary permeability (Dale & Richards, 1918; Spector & Willoughby, 1957).

(v) Depletion of histamine or the use of antihistamine substances reduces the amount of pleural exudate produced by injection of turpentine (Spector & Willoughby, 1958).

(vi) Histamine and 5-HT stimulate phagocytosis (Kato & Gozsy, 1956; Buttle & Northover, cit. Mishra & Sanyal, 1959).

(vii) Tensile strength of healing skin incisions is increased when the formation of histamine is accelerated and decreased when it is inhibited (Kahlson, 1960).

Spector and Willoughby (1958) did not find any reduction in exudate of turpentine pleurisy in reserpine-treated animals or in animals pretreated with 2-bromolysergic acid diethylamide, an antagonist of 5-HT. However, the dosage of reserpine used was only 20% of the amount we gave.

The exact mechanism of action of histamine or 5-HT in these processes is not very clear. Recently it has been suggested that histamine liberated from mast cells may prepare many more connective tissue cells than are normally available, to receive heparin or heparin-containing granules, and these may be used in preparing ground substances; 5-HT may possibly, particularly in the rat and mouse, act similarly (Riley, 1962; West, 1962).

Most of the experiments reported were in rats and mice, and it may be a fair conclusion that at least in these two species, histamine as well as 5-HT participate in tissue defence and repair during inflammation.

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