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Abstract

Using the granuloma pouch technique of SELYE, effect of modification in local histamine on the inflammatory tissue reactions was examined in rats. The increase in the weight of pouch wall and histological inflammatory changes were distinctly inhibited in either case of histamine depletion by sinomenine and of desensitization to histamine by repeated injections of histamine. In rats injected with aminoguanidine, the skin and local histamine contents increased in similar degree as those in rats receiving histamine injection, but the inflammatory tissue reactions were severer than in the control. The total histamine of the pouch wall during inflammation reached the maximum four days after the injection of croton oil and decreased thereaftcr. The prcliferative processes indicating the recovery of injured tissues in later stages of the inflammation were the most vigorous in rats treated with histamine and this was in contrast to the extreme weakness of this tendency in animals in which the local histamine had been depleted. These observations not only suggests the fairly close relationship of histamine to carly reaction of inflammation but also indicates the role of histamine in its recovery processes.

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GRANULOMA POUCH AND SKIN HISTAMINE OF THE RAT[1,2]

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In the preceding paper[1] I have shown that the egg-white edema of the rat's hind paws was inhibited by substances which inhibit histamine release and that these substances also potentiate the edema-inhibiting effect of some histamine releasers. These findings may be explained as a result of restriction of the availability of local histamine to be released by egg white. Therefore, despite some recent suggestions that anaphylactoid edema is chiefly mediated by 5-hydroxytryptamine in this animal,[2–7] interest is still held to the rôle of histamine as the mediator of acute inflammation, including egg-white edema.

The granuloma pouch described by SELYE[8,9] seems to be another object suitable for the study of this problem. Croton oil used as an irritant for the development of this pouch should cause a strong release of local histamine because it was demonstrated that this oil disrupted mast cells in the skin of rat far more drastically than in the case of egg white[1].

The present paper describes some observations on the effects of modification of histamine content of the skin upon the development of granuloma pouch.

METHODS

Throughout the present experiments, male albino rats weighing 100-130g., fed on wheat, fresh vegetables, and water, were used.

The granuloma pouch was developed by the method of SELYE,[8,9] by the injection of 15c. c. of air into subcutaneous connective tissue between shoulder blades on the back, the hair being clippered, and followed by injection of 1 c. c. of 0.5 per cent croton oil dissolved in corn oil into the

1) Aided by a grant for Fundamental Scientific Research from the Ministry of Education.

2) Preliminary notes in *Folia pharmacol. japon.* 53, 207 § (1956).

resulting air space.

For the measurement of the intensity of inflammation, weight of the total pouch wall and its histological pictures were examined 2, 4, 6, and 8 days after injection of croton oil. After sacrificing by ether anesthesia, the skin surrounding the pouch was cut out in a large circular area around the pouch, the pouch wall was stripped off cautiously from the skin and back tissues that covered it so as not to injure the pouch wall, and the oval pouch containing an exudate was isolated. The exudate was removed by incision on the upper wall of the pouch, gross moisture was removed by pressing a filter paper, and the pouch was weighed. For pathohistological examination, a total of four tissue strips were obtained from the corresponding left and right positions of the upper and lower walls of the pouch. These strips were fixed in 10 per cent formalin solution and sections embedded in paraffin were stained with hematoxylin-eosine.

For the determination of histamine content, 0.2-0.5 g. of tissue specimens from the dome of the pouch and abdominal skin of the same animal were employed. Extraction and assay of histamine mainly followed the method described by SANUKI[10] of our laboratory. Histamine content was expressed as the weight of the base.

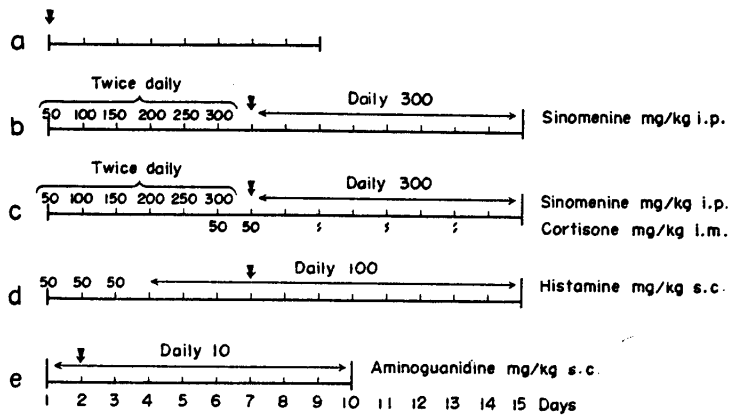

FIG. 1. Scheme of experimental design. a) Control, b) sinomenine-treated, c) sinomenine and cortisone-treated, d) histamine-treated, and e) aminoguanidine-treated group. At arrow granuloma pouch was formed.

The experiment was carried out on 5 groups, each with 2-5 rats, divided into (a) non-treated control, (b) sinomenine-treated, (c) sinomenine and cortisone-treated, (d) histamine-treated, and (e) aminoguanidine-treated groups. The dosage of the drugs administered, period of adminis-

tration, and the time course of granuloma pouch development for each group are illustrated in Fig. 1. The prolonged treatment with sinomenine shown in this scheme is known to result in practical exhaustion of releasable histamine of the skin[1]. Aminoguanidine is the most powerful antihistaminase known to date[11,12]. Cortisone is known to effect on tissue histamine by two ways; by inhibition of reaccumulation of the depleted tissue histamine[13–16] and by inhibition of histamine release from the tissue[1,10].

The symptoms appearing after administration of sinomenine gradually decreased on repeated injection, even by the gradual increase in the dose, as had been pointed out in the preceding paper[1]. Subcutaneous injection of 50 mg./kg. of histamine caused reddening in the four limbs and around the ears and snout, and visible edema around the snout and dorsum of the paws. Inactive motion, and a slight dyspnea and prostration were also observable. These symptoms reached the maximum 5 minutes after injection and disappeared gradually. The acute tolerance on repeated injection of histamine was less marked than in the case of sinomenine.

The agents used were sinomenine hydrochloride (Shionogi), histamine dihydrochloride (Wako), cortisone acetate (Merck), and aminoguanidine bicarbonate (B. D. H.).

RESULTS

The variation in the weight of pouch wall in different groups is indicated in Fig. 2. In the control group, average weight of the granuloma pouch wall reached the maximum of 3.9 g. on the fourth day from 2.7 g. on the second day after formation, the weight was approximately the same until the sixth day, and fell to 3.2 g. on the eighth day.

In the group given repeated injections of sinomenine, the weight of the pouch wall was 1.0 g. on the second day, only about 1/2.7 of that of the control group. Later increase in weight was gradual and very slight. In this group, declination of the curve between sixth and eighth day could not be observed. In the group given cortisone every other day in addition to sinomenine treatment, the change in the weight of pouch wall was similar to that in the case of sinomenine alone except for the slightly smaller increase in the later course. In the histamine-treated group, increase in the weight of pouch wall was 1.8 g., 1/1.5 of that of the control, and later development was also slower. This is the reverse of the rate of increase in histamine content of the pouch wall, as will be described later. The weight increase in the aminoguanidine-treated group was generally

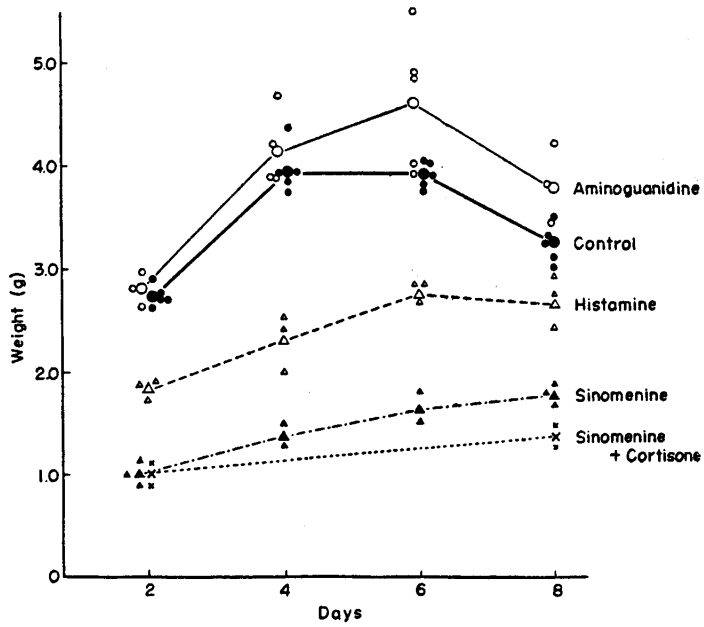

FIG. 2. Effects of the repeated daily administration of sinomenine, sinomenine plus cortisone, histamine and aminoguanidine on the development in weight of the granuloma pouch wall.

greater than that of the control, especially during the period from the sixth to the eighth day.

Microscopic pictures of the second days' control pouch wall revealed coarseness and dissociation of the connective tissue fibers together with congestion of the arteriols, capillary stasis, slight hemorrhage, inflammatory edema, and marked infiltration of polymorphonuclear leucocytes, mixed with a few eosinophyllic leucocytes, and lymphocytes (Plate 1). In the back muscles constituting the pouch fundus exudation and cellular infiltration were also observed, although slighter than in the connective tissue layers. These changes later became more intense and progressed toward a deeper portion. On the fourth day, a wide regressive changes, partial necrosis of infiltrated cells and tissues, were observed in portions near the internal cavity, i. e. in older inflammation, and demarcation was also noticed. On the sixth day, general observations were the same as those on the fourth day but with stronger leucocyte infiltration. From about this time, granulation was seen to be progressing around the pouch wall (Plate 2, 1). By the eighth day, proliferative changes suggesting organization became more marked. Vascular formation, appearance of

fibroblasts, connective tissue cells, monocytes, and histiocytes, as well as proliferation of connective tissue fibers were observed.

In the sinomenine-treated group these changes were much more slight and their progress into deeper portion was also of slight degree. By the sixth day, only a very slight cellular infiltration remained but reparative process indicated by proliferation was less marked than that of the control group (Plate 2, 3). In the group treated with sinomenine and cortisone, proliferative changes of connective tissues was somewhat less than that of the group treated by sinomenine alone but otherwise similar observations were noted.

In the histamine-treated group also, the intensity of exudation and cellular infiltration on the second day was much more slight than the control group. In this case, degenerative changes were almost not observable which were evident in the control around the fourth day and the proliferation and other regenerative changes of connective tissue were much more marked than in the control and the sinomenine-treated groups, suggesting activation of the loose mesenchyma. By the sixth day, granulation had already progressed accompanying new formation of capillaries. There was no necrotic layer in the portion adjacent to the pouch cavity (Plate 2, 4).

Histological changes in the aminoguanidine-treated group were in contrast to the groups treated with other drugs. There was no great difference from that of the control on the second day but on the fourth and sixth days, exudation and cellular infiltration were much more severe, and necrosis and hemorrhage were observed in some places (Plate 2, 2).

From the histological findings, it is seen that the intensity of congestion, exudation, and cellular infiltration was in parallel with the degree of increase in the weight of the pouch in all the groups while regenerative changes, such as vascular formation and proliferation of connective tissue cells and fibers, were the most marked in the histamine-treated group and very slight in the sinomenine-treated and the sinomenine and cortisone-treated groups.

Histamine content in the abdominal skin after pouch formation is shown in Fig. 3. In the control group, this was approximately constant throughout the whole course with a mean value ranging 37-50 μg. /g. This value agrees with that obtained from normal intact rats[1], indicating that the skin histamine does not change with the formation of the pouch. In the sinomenine-treated and the sinomenine and cortisone-treated groups, the value was quite markedly smaller than that of the control, the second-day value being only 10 μg./g. or less. The slight increase in the later

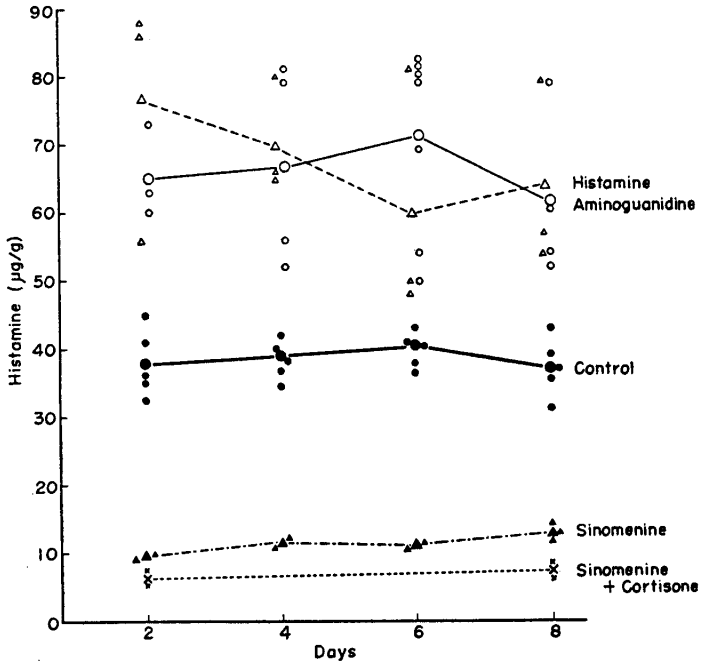

FIG. 3. Effects of the repeated daily administration of sinomenine, sinomenine plus cortisone, histamine and aminoguanidine on the histamine content in μg./g. of the abdominal skin.

course in the sinomenine-treated group was inhibited by the concurrent use of cortisone. This suggests the inhibitory action of cortisone on the new binding of histamine in the tissues[13–16]. In the aminoguainidine-treated and the histamine-treated groups, the result was the reverse of the above; the skin histamine content was almost twice the normal value. These values seemed not to differ greatly during the second to the eighth day although they showed a wide range of individuals.

The histamine content per unit weight of pouch wall was one-third to one-quarter of that of skin histamine for each group on the second day. But, the values of four groups were distributed approximately in the same order as the skin histamine content on the second day as indicated in Fig. 4. After the fourth day these values declined gradually, with the exception of two sinomenine groups. However, such declination in histamine content may be possibly caused by edematous swelling without any change in the total amount of pouch wall histamine. In order to ascertain this point, the total amount of histamine contained in the whole pouch wall was calculated from the histamine content per unit

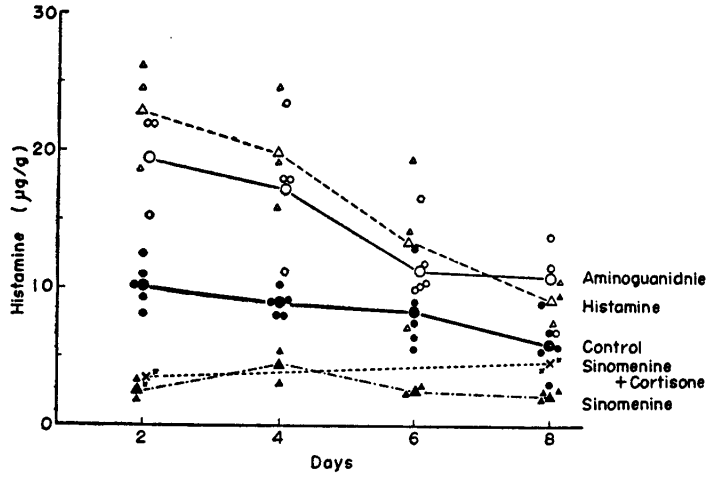

FIG. 4. Effects of the repeated daily administration of sinomenine, sinomenine plus cortisone, histamine and aminoguanidine on the histamine content in μg./g. of the granuloma pouch wall.

TABLE 1. Total histamine of the wall tissue of granuloma pouch after various treatments. Figures showing mean values with range.

Experimental groups	Total histamine of the pouch wall (μg.) on days :			
	2	4	6	8
Control	28.3 (22.1—34.0)	36.5 (32.7—45.1)	32.4 (19.1—53.2)	19.2 (10.3—26.8)
Sinomenine-treated	3.0 (2.4—3.5)	6.2 (4.0—8.3)	4.7 (4.4—4.9)	4.0 (3.5—3.9)
Sinomenine and cortisone-treated	3.6 (3.3—3.9)	—	—	6.0 (5.9—6.0)
Histamine-treated	42.3 (32.7—50.9)	46.8 (32.4—62.5)	37.2 (19.8—54.6)	24.7 (20.1—31.3)
Aminoguanidine-treated	55.5 (43.0—65.6)	72.6 (41.5—89.9)	55.9 (38.4—81.3)	42.4 (23.4—54.0)

weight and weight of the pouch. The result is shown in Table 1. The values remained unchanged in the sinomenine-treated and the sinomenine and cortisone-treated groups but in other groups, total histamine content of the pouch wall showed a distinct increase on the fourth day.

DISCUSSION

The granuloma pouch technique introduced by SELYE[8,9] has been utilized as a means for many studies on acute inflammation. In the wall of this pouch, some typical course of acute inflammation such as vasodi-

latation, exudation, leucocyte infiltration, hemorrhage, and subsequent regressive or reproductive changes was observable. This sample thus offered a qualitative indication of inflammation and also a quantitative expression of changes in the pouch wall weight as an approximate indication of plasma seepage through the capillaries.

The current concept that the inflammatory tissue responses are produced by the release or activation of chemical mediators initiated by cell injury is in part connected to the discovery of some active principles other than histamine which can simulate some of these reactions. The possibility has been suggested that the activation of hyarulonidase is caused by a definite phlogogenic stimuli and facilitates plasma seepage into interstitial tissue[17]. MENKIN[18] has isolated leucotaxine from inflammatory exudate, a substance capable of inducing increased capillary permeability and leucocyte emigration. Exudin is also a polypeptide which promotes exudation in later stages of inflammation[19]. Appearance of activated globulin that promotes leucocytosis[19,20], permeability factor of diluted serum[21] and of bradykinine or bradykinine-like substance which is a dilatator principle[22] has also been suggested. Destruction of mast cells in a rat may also cause liberation of 5-HT, a permeability increasing principle, besides histamine[23]. It is possible that these factors mutually reinforce the inflammatory reactions. To what extent would histamine be responsible for the inflammatory tissue reactions ?

In the present experiments, it has been revealed that under the marked depletion of histamine in the skin and pouch wall caused by prolonged treatment with sinomenine, vasodilatation and exudation are strongly inhibited in the pouch wall, and other inflammatory reactions including leucocytic emigration are also weak. This fact suggests the importance of histamine in producing these reactions. But sinomenine may have caused depletion of 5-HT as well as histamine, because the compound 48/80, which is also a basic histamine liberator, is known to liberate both amines at the same time[23–25]. However, according to the unpublished observations of SAITO[26] of our laboratory, seepage of the circulating dye on the site of intracutaneous injection of sinomenine was found in the same degree even after depletion of 5-HT by reserpine in rats, while it was weakened on the site of injection of 48/80 or dextran. This finding suggests that sinomenine is a histamine releaser which can not liberate 5-HT, differing from other two substances. On the other hand, these inflammatory reactions were fairly weakened in the group given repeated injection of histamine. Provided that such a treatment is almost without effect on the occurrence of other phlogogenic principles,

the foregoing observations will give a greater value to the rôle of histamine in this kind of inflammation because the susceptibility of the tissue is thought to have become low only against histamine. The action of 5-HT and of leucotaxine seems to be partly related to their histamine-releasing activity[27–30], while histamine itself promotes emigration of leucocytes in a definite concentration[31].

In the aminoguanidine-treated group, histamine content of the skin and of pouch wall increased similarly as that in the histamine-treated group but inflammatory reactions became marked. A possible cause of such an increased reaction in this group may be due to the interference of histamine desensitization, since this diamine-oxidase inhibitor fortifies histamine reaction in the body[32–35]. Another possible explanation is that, because this drug was administered only from the day prior to pouch formation in this group, there had not been a high level of skin histamine for a sufficient period to cause the histamine desensitization[10].

In spite of the fact that the skin histamine level had remained fairly constant during the period of treatment, total amount of pouch wall histamine varied markedly. In the groups showing distinct inflammatory reactions, the maximum was attained at around the fourth day after the injection of croton oil. This fact suggests that histamine is closely related to early inflammatory reactions.

Reparative changes in later course was the weakest in the sinomenine-treated groups and strongest in the histamine-treated group. These findings are reminiscent of the experiments of RILEY and WEST[36], who observed temporary mobilization of loose mesenchyma thought to have been caused by the flooding of tissues with protein-rich edema fluid produced by histamine. This fact probably signifies the important rôle of histamine also in the recovery of injuries.

SUMMARY

Using the granuloma pouch technique of SELYE, effect of modification in local histamine on the inflammatory tissue reactions was examined in rats.

The increase in the weight of pouch wall and histological inflammatory changes were distinctly inhibited in either case of histamine depletion by sinomenine and of desensitization to histamine by repeated injections of histamine. In rats injected with aminoguanidine, the skin and local histamine contents increased in similar degree as those in rats receiving histamine injection, but the inflammatory tissue reactions were severer

than in the control. The total histamine of the pouch wall during inflammation reached the maximum four days after the injection of croton oil and decreased thereafter.

The proliferative processes indicating the recovery of injured tissues in later stages of the inflammation were the most vigorous in rats treated with histamine and this was in contrast to the extreme weakness of this tendency in animals in which the local histamine had been depleted.

These observations not only suggests the fairly close relationship of histamine to early reaction of inflammation but also indicates the rôle of histamine in its recovery processes.

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LEGENDS FOR PLATES

Plate 1. Granuloma pouch wall stained with hematoxylin-eosine, 2 days after the injection of croton oil. Cellular infiltration, inflammatory edema, congestion of the arterioles and capillary stasis. ×180.

Plate 2. Granuloma pouch wall stained with hematoxylin-eosine, 6 days after the injection of croton oil. ×145. (1) Non-treated control : marked cellular infiltration and partial necrosis with demarcation in portions near the pouch cavity. Slight formation of granulation tissues. (2) Aminoguanidine-treated : severe hemorrhages in addition to the marked cellular infiltration and necrosis with demarcation. (3) Sinomenine-treated : only a very slight cellular infiltration and less marked granulation tissues. (4) Histamine-treated : marked granulation tissues accompanying new formation of capillaries. No necrotic layer in the portion adjacent to the pouch cavity.

Plate 1

Plate 2