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CORRELATION BETWEEN HISTAMINE CONTENT IN EXUDATE AND DEGREE OF EDEMA PRODUCED BY DEXTRAN

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Abstract—Edema was produced in the hind paws of rats by local subcutaneous injection of dextrans of four different molecular weights (7500, 28,000, 65,000 and 207,000), and the resultant exudate was assayed for histamine content 15–240 min after injury. Each dextran produced paw edema, the degree of which depended on the concentration (0.2, 1.0 and 4.0%, w/v). The maximum response was obtained 30–60 min after the injection. The highest concentration of histamine in the exudate collected from the inflamed paw was 3.7 to 6.9 μg/ml, which was obtained in the initial stage before the peak of edema. When the edema was at its peak the concentration of histamine had already declined. Statistical analysis showed a significant correlation between the concentration or total amount of histamine in the exudate and the degree of edema produced by most of the dextrans. These results suggest that release of histamine is not the result but rather the cause of the edema formation produced by dextrans.

A single intraperitoneal injection of dextran into rats produces an anaphylactoid reaction, including serious edema of the hind paws [1–3]. Harris and West [4, 5] have reported that dextrans of average molecular weight between 25,000 and 2,000,000 are the most effective in producing this reaction. Harris *et al.* [6] also showed that, when injected intradermally, dextrans of molecular weight above 10,000 and below 200,000 are more effective than those of lower molecular weight in increasing vascular permeability of the rat skin.

The mechanism of the anaphylactoid reaction has been analyzed by the use of pharmacological agents. Both histamine and 5-hydroxytryptamine (5-HT) have been suggested as possible mediators of paw edema [7] and of the increased vascular permeability [8] produced by local injection of dextran in rats. Parratt and West [9] showed that edema formation was inhibited significantly by pretreatment with the anti-5-HT compound, BOL 148, but was inhibited only slightly by H_1-receptor antagonists, and was prevented by depletion of skin 5-HT after treatment with reserpine or compound 48/80 but not by depletion of histamine alone after treatment with polymyxin B, and then concluded that the edema caused by dextran is mediated chiefly by 5-HT. However, the specificity of some of the agents employed is debatable.

Investigating the time course of release of biologically active substances during inflammation and edema formation could give useful information, by which the roles of these substances in inflammation might be clarified. It has been reported that at the peak of edema a 5-HT-like substance was detected in the exudate of paws treated with dextrans, but histamine was not [9]. In contrast, Mörsdorf [10] showed that maximum 5-HT release and maximum edema production by dextrans were attained simultaneously, and suggested that release of 5-HT is not the cause, but rather the result, of edema formation. Thus, a definite conclusion cannot be drawn, from these previous observations, concerning the contribution of histamine and/or 5-HT to paw edema production by dextrans. Furthermore, the possibility cannot be excluded that prostaglandins and the bradykinin system, which have been suggested as important factors in the process of other inflammations [11–15], may also be responsible for the edema produced by dextrans.

Our ongoing investigation has been designed to determine whether histamine, 5-HT and other putative mediators contribute to dextran-produced paw edema, by studying the relationship between the content of these substances in exudate and the degree of edema produced by local injection of dextrans of different molecular weights into rat paws: the present paper deals with histamine and 5-HT.

MATERIALS AND METHODS

The animals used were male Sprague–Dawley rats (Charles River Japan Inc., Atsugi, Japan) weighing 140–160 g. They were allowed free access to food and water. Dextran was kindly supplied from Meito Sangyo Co., Ltd., Nagoya, Japan. Histamine dihydrochloride and 5-hydroxytryptamine creatinine sulfate were purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan.

Dextran-produced paw edema and histamine content in the exudate. Dextran was dissolved in 0.9% saline in a concentration of 0.2, 1.0 and 4.0% (w/v), and 0.05 ml of this solution was injected into the subplantar region of one hind paw. The contralateral paw was injected with physiological saline alone as a control. The average molecular weights of the dextrans used were: 7500, 28,000, 65,000 and 207,000. Before any injections, the volume of each hind paw of

each rat was measured by a volume differential method. At various periods after the injection, changes in paw volume were determined and the animals were killed by decapitation. Immediately, an incision, about 1 cm long, was made on the plantar region of the skin. Without any squeezing, the exudate (0.01 to 0.08 ml) was then collected with hematocrit capillary tubes (Propper Manufacturing Co., Inc., Long Island, NY). The subplantar region of the contralateral paw was washed with saline of nearly the same volume as that of the exudate after the incision. The degree of paw edema is expressed as a percentage increase relative to the initial volume of the paw. The histamine content in the exudate and washings was assayed fluorometrically using the method of Shore *et al.* [16]. The concentration of histamine in the exudate is expressed as μg/ml. The total amount of histamine in the exudate (μg) was calculated as follows: concentration of histamine in exudate (μg/ml) $\times$ increase in paw volume (ml). All values for histamine in this paper refer to the base.

Histamine- and 5-HT-produced paw edema. Histamine or 5-HT dissolved in 0.05 ml saline was injected subcutaneously into the plantar region of one hind paw of rats. The method for measuring the paw volume and the expression of results are the same as described above. The doses of histamine and 5-HT are expressed in terms of the bases.

RESULTS

Degree of paw edema and molecular weight of dextran. Dextrans of different molecular weights produced paw edema, the degree of which depended on the concentration [between 0.2 and 4.0% (w/v)]. Maximal responses to most of the dextrans injected were obtained 30–60 min after injection, but the responses to 0.2 and 1.0% dextran of molecular weight 7500 were attained in 15–30 min. As indicated in Fig. 1, dextrans of molecular weights 28,000, 65,000 and 207,000 had approximately the same potency in producing edema, whereas the ability of dextran of molecular weight 7500 to produce edema was far lower than that of the others. The degree of edema, however, was directly proportional to the log-molar concentration of each dextran and also to the molecular weight of the dextrans injected (Fig. 2).

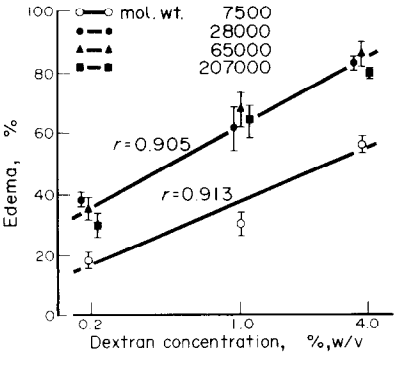

Fig. 1. Concentration–response curves for paw edema produced by dextrans of different molecular weights, dissolved in a concentration of 0.2, 1.0 and 4.0% (w/v). Degree of edema at its peak, 30–60 min after local injection of dextran, is expressed as a percentage increase in paw volume relative to the initial volume (1.32 $\pm$ 0.01 ml). Each point is the mean value of five to seven animals and the vertical bars indicate S. E. M.; r indicates correlation coefficient.

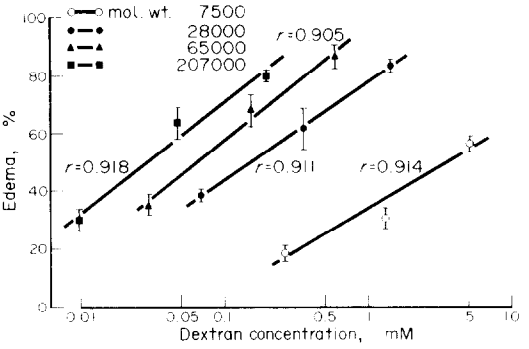

Fig. 2. Concentration response curves for paw edema produced by dextrans of different molecular weights. Degree of paw edema produced by different dextrans (shown in Fig. 1) is plotted against the log-molar concentration. Each point is the mean value of five to seven animals and the vertical bars indicate S. E. M.; r indicates correlation coefficient.

Time course of paw edema and histamine content in exudate. The results of injection of 4.0% dextrans of different molecular weights are shown in Fig. 3. The results with 0.2 and 1.0% dextrans were similar to those with 4.0% dextrans. The highest concentration of histamine in the exudates of paws treated with dextran was 3.7 to 5.6 μg/ml for 0.2%, 4.2 to 5.7 μg/ml for 1.0% and 5.8 to 6.9 μg/ml for 4.0% dextrans of different molecular weights, obtained 15 min after local injection. At this time, the edema had not reached its peak. The highest total amount of histamine in the exudates was 1.8 to 2.2 μg for 0.2%, 2.2 to 3.4 μg for 1.0%, and 3.4 to 5.2 μg for 4.0% dextrans, obtained 15–30 min after the injection. When the edema was at its peak, at 30–60 min, the concentration of histamine had already declined. The total amount of histamine also declined 60 min after injection. The concentration and total amount of histamine in the washings of the contralateral paw were 0.6 μg/ml and 0.1 μg, respectively, 15 min after injection of saline. Statistical analysis was used to determine whether a significant correlation was present between the histamine content in the exudate and the degree of edema. The results are shown in Figs. 4 and 5, as the straight lines obtained by the method of least squares, together with the correlation coefficients and P values. A highly significant correlation was found to exist between the concentration of histamine in the 15 min exudate, and the degree of the 15 or 30 min edema, after local injection of dextrans of molecular weights 28,000 and 65,000 (Fig. 4). Moreover, there was a highly significant correlation between the total amount of histamine in the 15 min exudate and the degree of the 15 or 30 min edema, after local injection of dextrans of molecular weights 28,000, 65,000 and 207,000 (Fig. 5).

Histamine- and 5-HT-produced paw edema. The results are shown in Figs. 6 and 7. Histamine, even at a large dose, could not produce the same degree of

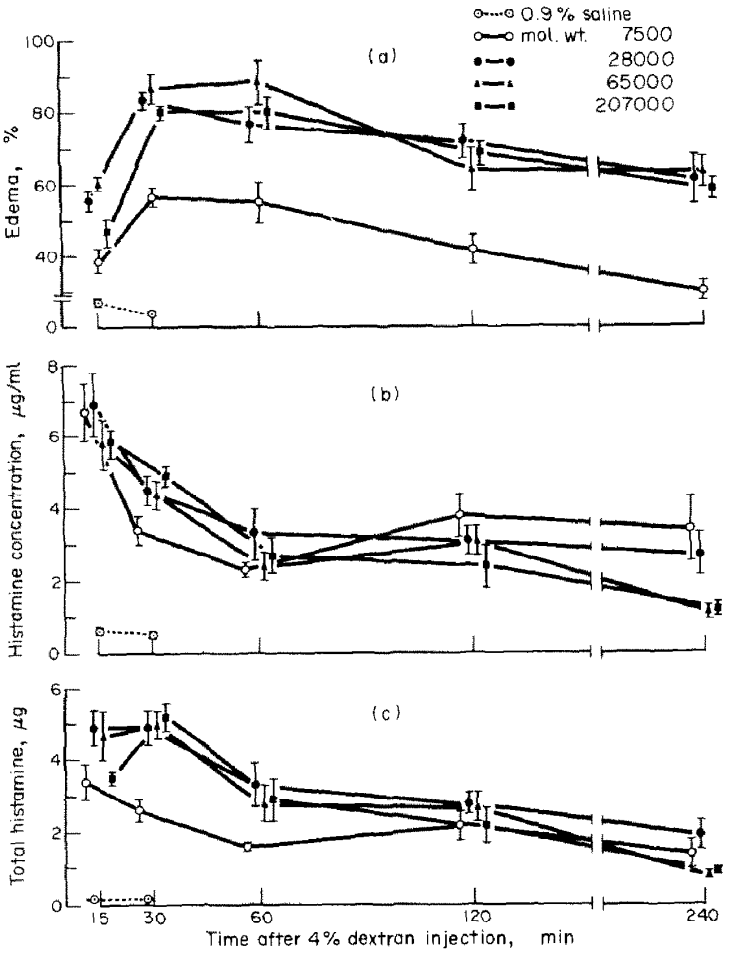

Fig. 3. Time course of paw edema (a), concentration of histamine (b) and total amount of histamine (c) in exudate collected from the rat paw after local injection of 4.0% (w/v) dextrans of different molecular weights and in washings from the saline-injected paw. Each point is the mean value of five to seven animals and the vertical bars indicate S. E. M.

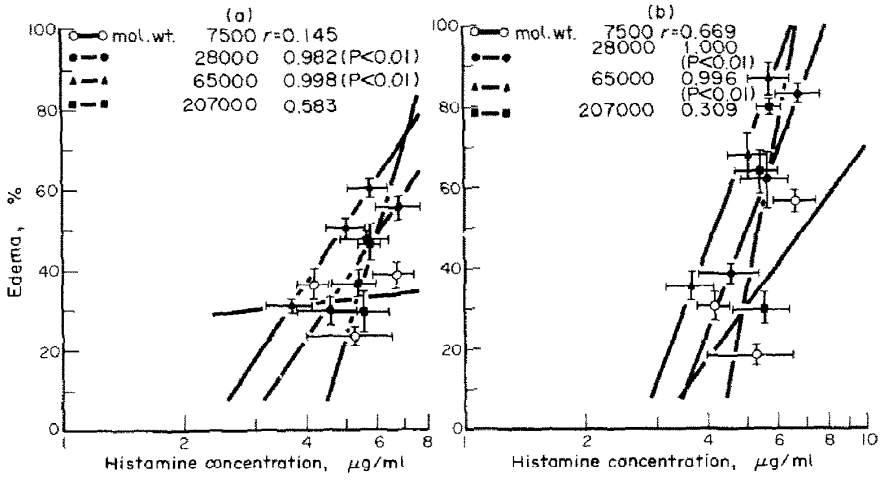

Fig. 4. Correlation between concentration of histamine in exudate collected from the dextran-injected paw at 15 min (a and b) and degree of paw edema at 15 min (a) or at 30 min (b) after local injection of dextrans of different molecular weights dissolved in a concentration of 0.2, 1.0 or 4.0% (w/v). Each point is the mean value of five to seven animals and the bars indicate S. E. M.; r indicates correlation coefficient.

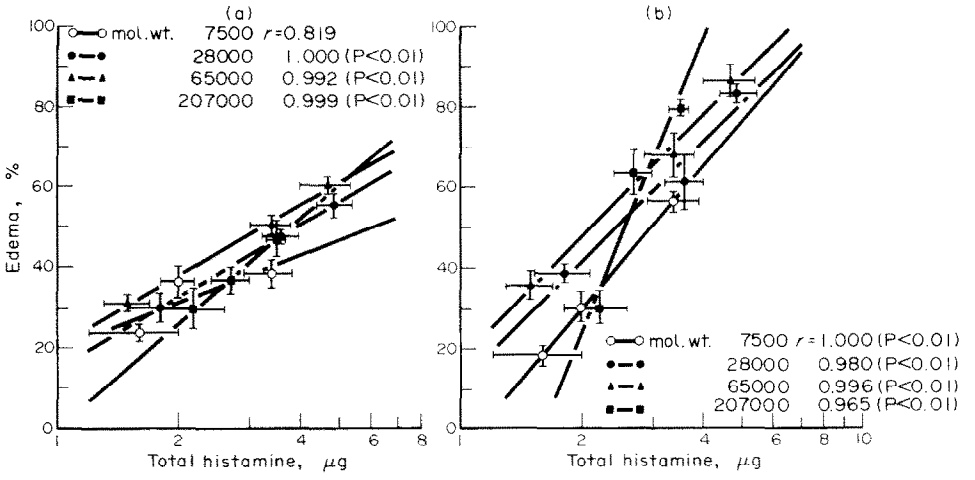

Fig. 5. Correlation between total amount of histamine in exudate collected from dextran-injected paw at 15 min (a and b) and degree of paw edema at 15 min (a) or at 30 min (b) after local injection of dextrans of different molecular weights dissolved in a concentration of 0.2, 1.0 or 4.0% (w/v). Each point is the mean value of five to seven animals and the bars indicate S. E. M.; r indicates correlation coefficient.

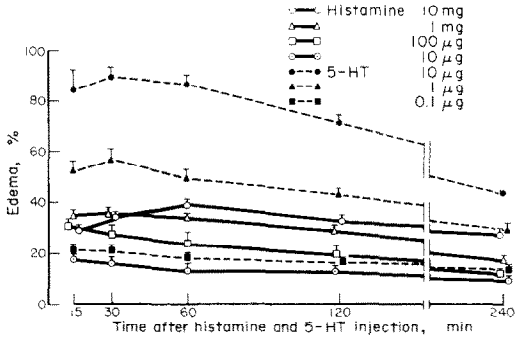

Fig. 6. Time course of paw edema after local injection of histamine (——) and 5-HT (– – –) in rats. Initial paw volume was 1.26 ± 0.01 ml. Each point is the mean value of five to six animals and the vertical bars indicate S. E. M.

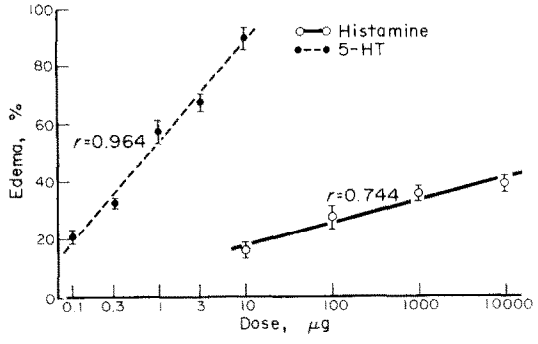

Fig. 7. Dose–response curves for paw edema produced by histamine (——) and 5-HT (– – –) in rats. The degree of edema at 30 min after injection of these amines was plotted. Each point is the mean value of five to six animals and the vertical bars indicate S.E.M.; r indicates correlation coefficient.

edema as dextran: 10 mg resulted in a 39 per cent increase in paw volume. A marked paw edema was produced by local injection of 1 μg 5-HT. These findings indicate that the exogenous dose required to produce a marked paw edema is in the order of 10 mg or more for histamine and 1 μg for 5-HT.

DISCUSSION

It has been proposed that histamine contributes to the development of some inflammations [15, 17–20]. In the present experiment, histamine was detected in the exudate produced by dextran, one of the experimentally produced inflammations. The highest concentration of histamine (4.7 μg/ml) was obtained prior to the peak of edema.

It is improbable that the amount of histamine recovered from the inflamed paws in the present experiment is the amount of histamine released into the inflammatory locus, owing to the enzymatic inactivation of released histamine and diffusion into the circulation. Histamine is known to be metabolized mainly by diamine oxidase and imidazole-*N*-methyl transferase in rats [21]; the metabolism differs by species and tissues [21–24]. It has been reported that, in rats, exogenous [21] and newly formed (endogenous) histamine [24] are metabolized mainly by diamine oxidase and by imidazole-*N*-methyl transferase respectively. However, the enzyme activity of the latter enzyme in the rat skin was not studied in these experiments; this tissue may have a lower activity of this enzyme than other tissues *in vitro* [25]. No, or little, activity of diamine oxidase has been found in this tissue [26]. Thus, the histamine released into the inflammatory site is probably not metabolized rapidly by these enzymes. However, it may be that the activities of these enzymes vary with the development of inflammation. We do not know what factor is primarily involved in the disappearance of histamine from the inflammatory site. Thus, it is impossible to

determine what proportion of the liberated histamine was recovered from the exudate in the present experiment.

The amount of histamine detected in the exudate appears to be proportional to that released into the inflammatory site. Statistical analysis shows that a significant correlation exists between the concentration or total amount of histamine in the exudate and the degree of edema produced by most of the dextrans. The highest concentration of histamine detected in the exudate was found before the peak of edema. When the edema reached its peak, 30–60 min after local injection of four dextrans, the concentration of histamine had already declined. The total amount of histamine declined 60 min after the injection. These results, in the present investigation, suggest strongly that release of histamine is not the result but rather the cause of the edema formation.

A similar result has been obtained after thermal injury by Horakova and Beaven [20], who showed that histamine levels in the rat paw edema fluid, after scalding, paralleled the development of edema, but that once the edema was fully developed there was little release of histamine. Accordingly, they have emphasized the important role of histamine in the development of thermal injury. In their experiments, a concentration of about 2 μg/ml of histamine was detected in the edema fluid, using a specific enzymatic method. The difference between the histamine content recovered from the inflammatory site in this study, and that of Horakova and Beaven may depend on the type of experimental inflammation, the method for histamine assay and the procedure for collection of the edema fluid.

A relationship was found to exist between the molecular weights of dextrans and their abilities to produce anaphylactoid reactions in rats. When injected intravenously, five different dextrans, ranging from 25,000 to 170,000 in molecular weight, had approximately the same potency in producing the reaction [3]. Harris and West [4, 5] have reported that dextrans with average molecular weights between 25,000 and 2,000,000 are the most effective, in this respect, when injected intraperitoneally, whereas a low molecular weight dextran of 4000 is ineffective. Furthermore, Harris *et al.* [6] have shown that dextrans with average molecular weights between 10,000 and 200,000 are the most effective and are approximately equally active in increasing vascular permeability of the rat skin after intradermal injection, whereas for those of molecular weight below 10,000 there is a sharp fall in activity. They concluded, therefore, that a dextran molecular weight of 10,000 is a critical value for local vascular changes in the rat skin.

In the present investigation, we used four dextrans with average molecular weights of 7500, 28,000, 65,000 and 207,000, and produced a concentration-dependent paw edema after subcutaneous injection into the rat paw. No significant difference was found among dextrans of four different molecular weights, except for the lowest molecular weight dextran, in the degree, onset and diminution of the edema when used in a concentration between 0.2 and 4.0% (w/v). The lowest molecular weight dextran was found to have far lower activity (about one fifth) than the others in producing paw edema. In addition, it has been observed that dextran of molecular weight 4300 produced only a slight increase in paw volume (one tenth of that produced by dextrans of molecular weights above 28,000) 30 min after injection. These findings are not incompatible with the idea that a molecular weight of 10,000 may be a critical value in the ability of subcutaneously injected dextran to produce paw edema.

It has been suggested that histamine has a lower ability than 5-HT to increase vascular permeability of rat skin after intradermal injection [27, 28] and to produce rat paw edema after subcutaneous injection [7, 28]. In the present experiment, the local injection of histamine failed to produce a marked paw edema at a large dose of 10 mg. Therefore, it cannot be concluded that histamine alone participates in the development of edema produced by dextrans. However, it is possible that access to active sites may differ, depending on catabolism and diffusion, between released histamine and injected histamine. This may explain, in part, the failure to produce marked responses to exogenous histamine in the present experiment. Furthermore, it is possible that other mediators may be responsible for the edema produced by dextrans, since several mediators may be involved in inflammation [14, 15, 19]. It has been suggested that 5-HT is an exceedingly potent substance in increasing vascular permeability of the rat skin [27, 28] and in producing rat paw edema [7, 28]. In fact, the results in the present experiments show that the exogenous dose required to produce marked paw edema (60%) is in the order of 10 mg or more for histamine and 1 μg for 5-HT. Therefore, it should be kept in mind that, because of its potency, 5-HT may be responsible for the edema production by dextrans.

The results obtained in the present investigation suggest that histamine release may play a role in initiating paw edema by dextrans and may influence the later stages of inflammation.

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