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INHIBITORY EFFECTS OF CARBOHYDRATES ON HISTAMINE RELEASE AND MAST CELL DISRUPTION BY DEXTRAN

BY

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Alloxan diabetic rats failed to show the skin reaction (blue spot) evoked by dextran, whereas the effects produced by histamine and compound 48/80 were not altered. When dextran and glucose were injected simultaneously into the skin the reaction was inhibited. *In vitro*, mast cell alterations produced by dextran occurred simultaneously with histamine release; both processes were inhibited by glucose, other carbohydrates related to glucose, and inhibitors of anaphylaxis. These experiments suggest that dextran releases histamine by a mechanism similar to that found with 48/80 and anaphylaxis in the rat. The inhibitory effect of carbohydrates may be understood on the basis of a competitive mechanism.

Dextran when injected in rats produces an anaphylactoid reaction (Voorhees, Baker & Pulaski, 1951) which is inhibited by antihistaminics and corticoids (Morrison, Richardson & Bloom, 1951). Halpern & Briot (1952) showed that dextran releases histamine from rat skin in *in vitro* experiments. Parratt & West (1957) extensively studied histamine release by dextran, and demonstrated the release of 5-hydroxytryptamine by dextran in the rat's paw. Goth, Nash, Nagler & Holman (1957) presented evidence that alloxan diabetic rats failed to show the characteristic oedema and elevated plasma histamine produced by dextran. Previous treatment of the normal rat with insulin increases the oedema caused by dextran (Adamkiewicz & Langlois, 1957). Recently Adamkiewicz & Adamkiewicz (1960) showed that rats simultaneously injected with dextran and glucose do not undergo anaphylactoid reaction.

In the present paper we studied the mechanism of the anaphylactoid reaction produced by dextran. In addition, the mechanism of inhibition of this reaction by alloxan diabetes was also investigated.

METHODS

Wistar rats of either sex, body weight 150 to 250 g, were used in all experiments. They were allowed to consume food and water *ad libitum*. Diabetes was produced by subcutaneous injection of alloxan 25 mg/100 g, dissolved in a constant volume of 0.9% sodium chloride solution (0.5 ml.). The animals were used 5 days later when all the injected rats presented glucosuria. The glucose in urine was detected by the qualitative method of Benedict (1911).

Skin reaction. The hair over the dorsal surface was gently clipped without irritating the skin. 0.5 ml. of a 0.5% solution of Evans blue in 0.9% sodium chloride solution was injected intravenously. Five min later, solutions containing the designated quantities of histamine or histamine liberator (dextran and compound 48/80) were prepared in 0.1 ml. 0.9% sodium chloride solution and injected intracutaneously with tuberculin-type syringes of 0.5 ml. capacity equipped with 27 gauge hypodermic needles. A maximum of four skin sites per rat was thus prepared. The rats were killed 15 min after the intracutaneous injection of histamine or histamine liberators and skin was carefully stripped from the subcutaneous tissue. The reactions were read by measuring the diameter of the blue area on the inner surface of the skin with a millimetre rule.

Preparation of tissues for in vitro *experiments.* The abdominal skin was shaved, cut in pieces of about 1 cm^2 and carefully removed from the abdominal wall; the mesentery was dissected away from the small intestine and both tissues were dipped into cold 0.9% sodium chloride solution.

Experiments on histamine release and mast cell damage. To study the histamine-releasing property of the dextran, samples of skin and mesentery were shaken with 3 ml. buffered solution (136 mM sodium chloride, 2.6 mM potassium chloride, 2.1 mM magnesium chloride, 1.8 mM calcium chloride in 10 mM phosphate buffer, *p*H 7.5), containing 0.75 mM dextran. The mixture was incubated in 50 ml. beakers, at 38° C for 15 min. The skin samples were transferred to 2 ml. N-hydrochloric acid for histamine extraction and mesentery samples to 4 ml. fixative solution for mast cell observation. As control, tissue samples of similar size, both of skin and mesentery, were incubated without dextran.

Experiments to study inhibition of histamine release and mast cell damage by dextran. Tissue samples were first incubated for 15 min, at 38° C in buffered solution as described above, containing metabolic inhibitors or carbohydrates in required concentration. Dextran was added to make a 0.75 mM solution and the incubation was continued for a further 15 min. The tissues were then transferred to N-hydrochloric acid for histamine extraction or to fixative for mast cell observation.

Histamine extraction and biological assay. The method used was that previously described by Mota & Dias da Silva (1960). Histamine release was expressed as a percentage of the total tissue histamine and all values were given as base. In order to exclude any non-histamine component, the solutions were reassayed after the addition of 1 μg diphenhydramine hydrochloride into the perfusion bath containing the guinea-pig ileum.

Mast cell observation. The description of mast cell damage was based on observations in pieces of mesentery. This tissue was fixed and stained in 50% aqueous ethanol solution containing 10% formaldehyde, 5% acetic acid and 0.2% of toluidine blue. The mesentery was examined as a whole-mount preparation. Mast cell damage was assessed by counting the percentage of cells presenting granule extrusion, 300 cells being counted at $\times 200$.

Drugs used. Alloxan monohydrate (Merck). Dextran (M.W. 40,000, Abbott). Compound 48/80 was kindly given by Dr E. J. De Beer, Wellcome Laboratories, Tuckahoe, N.Y. N-Ethilmaleimide, iodoacetic acid, 2:4-dinitrophenol, nicotinamide, histamine diphosphate, and carbohydrates (Nutritional Biochemicals Corporation). Diphenhydramine hydrochloride ("Benadryl," Parke-Davis). Mepyramine hydrogen maleate ("Eulantinon," Laborterapica).

RESULTS

Effect of alloxan diabetes on the skin reaction (blue spot) produced by histamine, compound 48/80 and dextran. For these experiments, two groups of 8 rats each were used. The animals of one group were injected with alloxan and those of the other group with 0.9% sodium chloride solution. As soon as the diabetes was established, the skin test was performed with 10 μg histamine, 5 μg 48/80 and 3 mg dextran, in both groups. The results of these experiments, summarized in

TABLE 1

EFFECT OF ALLOXAN DIABETES ON SKIN REACTION PRODUCED BY HISTAMINE, COMPOUND 48/80 AND DEXTRAN

The results were recorded as: − (no reaction), ± (less than 5 mm), + (5–10 mm), ++ (10–15 mm) and +++ (15–20 mm)

Rat	Treatment	Histamine 10 μg	48/80 5 μg	Dextran 3 mg
1	Injected with alloxan	+++	++	+
2		+++	++	−
3		+++	++	−
4		+++	++	−
5		+++	++	−
6		+++	++	−
7		+++	++	−
8		++	+	−
9	Injected with 0·9% sodium chloride solution	+++	+++	++
10		+++	++	++
11		+++	++	++
12		+++	++	++
13		+	++	++
14		++	++	+
15		+++	++	+
16		++	+	++

Table 1, show that the alloxan diabetes inhibited the cutaneous reaction produced by dextran. Fig. 1 illustrates one of these experiments.

Effect of dextran on the mast cells of the lip skin. In order to investigate the participation of the mast cells in the skin reaction produced by dextran, 5 rats were

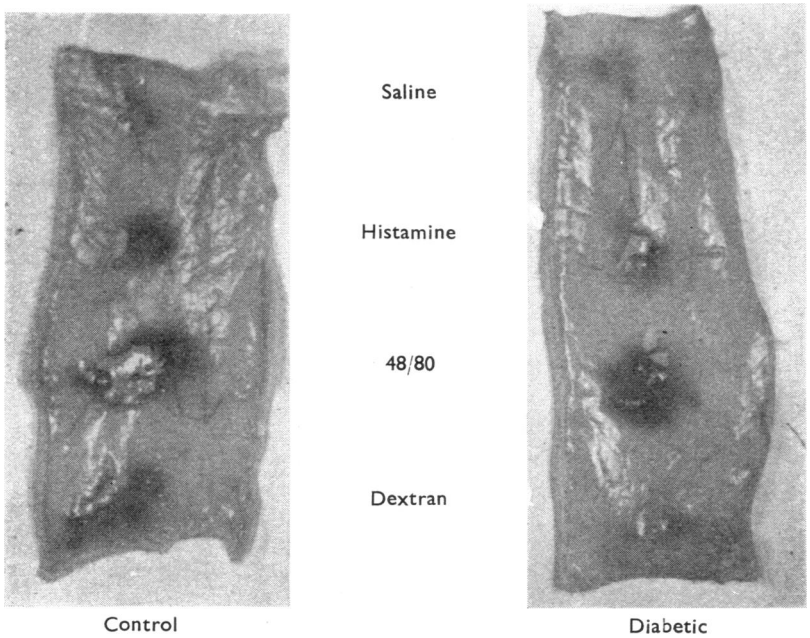

Fig. 1. Effect of alloxan diabetes on the skin reaction produced by histamine (10 μg), 48/80 (5 μg) and dextran (3 mg).

injected intradermally into the left side of the superior lip with 3 mg (0.1 ml.) dextran ; 0.1 ml. of 0.9% sodium chloride solution was injected into the right side (control). After 15 min the mast cells were fixed by injecting subcutaneously the fixative (without toluidine blue) in the areas previously injected with dextran and 0.9% sodium chloride solution. The skin was removed, fixation was continued overnight, and frozen sections, 50 μ thick, were then stained with 0.2% toluidine blue. The histological examination of the lip skin injected with dextran showed a few mast cells, exhibiting granule extrusion similar to that produced by 48/80 or antigen (Mota, Beraldo & Junqueira, 1953 ; Mota, 1957). Moreover, in a group of 9 rats, previously depleted of mast cells (Mota, 1957), 7 animals failed to show the skin reaction produced by dextran.

Inhibitory effect of glucose on the skin reaction produced by dextran. As in conditions of hyperglycaemia, dextran was ineffective in producing oedema (Goth, Nash, Nagler & Holman, 1957 ; Adamkiewicz & Adamkiewicz, 1959), it seemed interesting to investigate whether the hyperglycaemia acts *per se*, or whether a more complicated mechanism of blood sugar regulation was involved. To test this, the skin reaction was performed with dextran and dextran plus glucose, simultaneously injected into the skin. The optimum amount of glucose for producing a definite inhibition of the reaction was 3 mg (Fig. 2). Table 2 shows the results obtained in a group of 9 rats.

In vitro, *histamine release and mast cell disruption by dextran.* Skin samples were incubated in buffered solution containing 0.75 mM dextran and the percentage

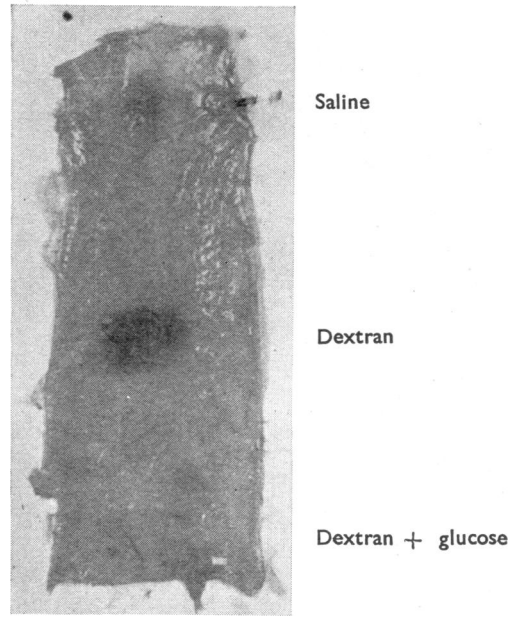

Fig. 2. Inhibitory effect of glucose (3 mg), simultaneously injected with dextran (3 mg). The same amount of dextran was used as control.

TABLE 2

INHIBITION BY GLUCOSE OF THE SKIN REACTION PRODUCED BY DEXTRAN

Glucose (3 mg) and dextran (3 mg) were simultaneously injected in the skin. The results were recorded as: — (no reaction), ± (less than 5 mm), + (5–10 mm), ++ (10–15 mm), and +++ (15–20 mm)

Rat	Dextran	Dextran plus glucose
1	++	—
2	++	—
3	+++	±
4	++	—
5	+++	+
6	+++	—
7	++	+
8	++	—
9	++	—

of histamine released determined. In 10 experiments the histamine released ranged from 8 to 12% of total skin histamine.

Microscopic examination of fragments of mesentery after contact with buffered solution containing 0.75 mM dextran showed alterations of mast cells, characterized by extrusion of granules similar to those already described in the lip skin. In 10 experiments about 48% of the mast cells were disrupted.

As glucose prevented the skin reaction induced by dextran, the possible inhibitory effect of glucose both on histamine release and mast cell disruption, *in vitro*, was examined. Pieces of skin and mesentery were incubated at 38° C with glucose

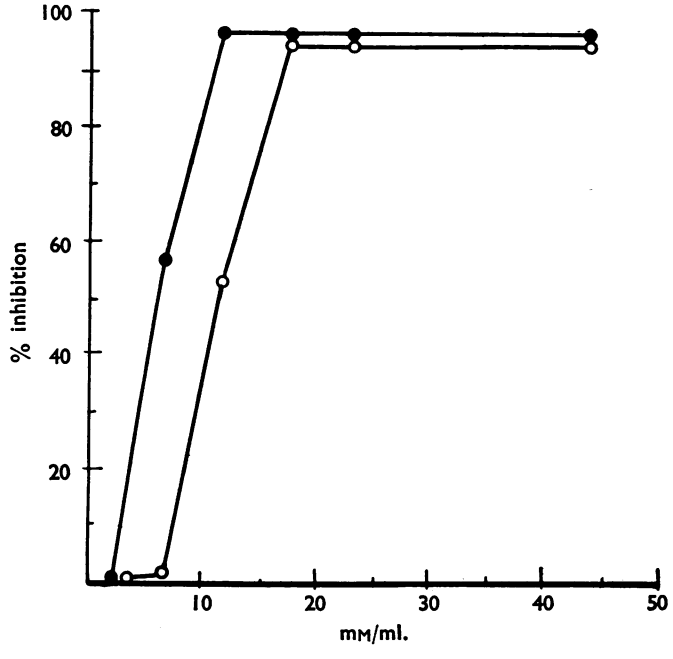

Fig. 3. Effect of D(+) glucose on histamine release (○——○) and mast cell disruption (●——●) induced by 0.75 mM dextran. Ordinates: % inhibition. Abscissae: concentrations of glucose. Each point is mean of 5 experiments.

in concentrations ranging from 3.4 mM to 44.4 mM. After 15 min dextran was added to give a concentration of 0.75 mM; the tissues were then incubated for a further period of 15 min. The results of 5 experiments are summarized in Fig. 3.

Other carbohydrates related to glucose were tested for possible inhibitory action on the effects of dextran, *in vitro*.

Samples of skin and mesentery were incubated at 38° C with either D(−) arabinose, D(+) xylose, D(−) ribose, D(−) lyxose, D(+) mannose, D(+) galactose, D(−) fructose, D(+) lactose, D(+) maltose or sucrose, in a concentration of 16.7 mM, which was the most suitable for demonstrating the inhibitory action of glucose. After 15 min the dextran was added and incubated as above. The results of these experiments are given in Tables 3 and 4.

Effect of inhibitors of anaphylaxis. It is known that various metabolic inhibitors, antihistamines, nicotinamide, calcium lack or previous heating of tissue at 45° C inhibit histamine release and mast cell disruption by compound 48/80 and antigen

TABLE 3

EFFECT OF CARBOHYDRATES ON THE HISTAMINE RELEASE BY DEXTRAN

Each value is the mean of 3 experiments. Carbohydrates and dextran were used in concentrations of 16·7 mM and 0·75 mM, respectively

Carbohydrates	% Histamine release by: Dextran alone	Dextran plus carbohydrate	% Inhibition
D(−) Arabinose	10·0	7·7	23
D(+) Xylose	10·0	4·3	57
D(−) Ribose	10·0	10·2	0
D(−) Lyxose	9·9	8·7	12
D(+) Glucose	10·2	0	100
D(+) Mannose	10·2	0·8	92
D(+) Galactose	10·2	13·0	0
D(−) Fructose	10·4	0·8	92
D(+) Lactose	9·9	10·0	0
D(+) Maltose	10·0	4·4	56
Sucrose	9·9	8·6	13

TABLE 4

EFFECT OF CARBOHYDRATES ON THE MAST CELL DISRUPTION INDUCED BY DEXTRAN

Each value is the mean of 3 experiments. Carbohydrates and dextran were used in concentrations of 16·7 mM and 0·75 mM, respectively

Carbohydrates	% Mast cell disruption by: Dextran alone	Dextran plus carbohydrate	% Inhibition
D(−) Arabinose	36·9	15·9	56·9
D(+) Xylose	46·0	12·6	72·8
D(−) Ribose	36·9	26·8	27·0
D(−) Lyxose	36·9	16·6	55·0
D(+) Glucose	46·0	0	100
D(+) Mannose	49·3	0	100
D(+) Galactose	49·3	44·8	9
D(−) Fructose	49·5	0	100
D(+) Lactose	36·9	32·3	12·5
D(+) Maltose	46·0	10·9	76·3
Sucrose	36·9	15·2	58·9

in rat (Mota & Ishii, 1960; Mota & Dias da Silva, 1960; Mota, Dias da Silva & Ferreira Fernandes, 1960). It was, therefore, of interest to investigate the effect of these treatments on the action of dextran. For this, samples of skin and mesentery were incubated for 15 min at 38° C with each of the following: 1 mM sodium iodoacetate, 1 mM N-ethylmaleimide, 40 mM nicotinamide, 0.62 mM mepyramine hydrogen maleate, 0.3 mM 2:4-dinitrophenol and calcium-free buffered solution containing 0.01% versene. For studying the effect of heating, the tissues were heated at 45° C for 5 min and then cooled to 38° C. Dextran was added to give a concentration of 0.75 mM and the tissues incubated for a further period of 15 min. As control, the tissues were only incubated with dextran without any further treatment. Fig. 4 shows that both histamine release and mast cell disruption were reduced or prevented by all these treatments.

Fig. 4. Effect of inhibitors of anaphylaxis on histamine release (■) and mast cell disruption (□) induced by 0.75 mM dextran.

It was demonstrated by Rothschild, Vugman & Rocha e Silva (1961) that the inhibitory action of several uncoupling agents on histamine release by 48/80 was reversed by glucose. In order to confirm whether the inhibitory action of 2:4 dinitrophenol on histamine release and mast cell disruption produced by dextran was also reversed by glucose, samples of skin and mesentery distributed in four flasks were incubated at 38° C in buffered solution containing: (*a*) buffered solution only (control); (*b*) 3.4 mM glucose; (*c*) 0.3 mM 2:4 dinitrophenol; (*d*) 0.3 mM

TABLE 5

REVERSAL BY GLUCOSE OF THE INHIBITORY EFFECT OF 2:4 DINITROPHENOL (DNP) ON HISTAMINE RELEASE AND MAST CELL DISRUPTION PRODUCED BY DEXTRAN

Each value is the mean of 3 experiments

Flask	Addition to medium	% Histamine released	% Mast cells disrupted
a	Control	9·9	53·1
b	3·4 mM glucose	12·5	70·9
c	0·3 mM DNP	0	1·0
d	0·3 mM DNP+3·4 mM glucose	8·5	45·6

2:4 dinitrophenol plus 3.4 mM glucose. After 15 min dextran was added to all samples to make a concentration of 0.75 mM and incubation continued as above. The results of these experiments are shown in Table 5.

DISCUSSION

These results show that dextran did not produce an increase in the capillary permeability of the skin in diabetic rats, and the skin reactivity for histamine and 48/80 in these animals remained unaltered.

Goth, Nash, Nagler & Holman (1957) and Adamkiewicz & Adamkiewicz (1959) employed the appearance of oedema as an indication of the dextran effect. However, an important point is that the increase in the capillary permeability may be detected before the appearance of the oedema. When a colloidal dye, as the Evans blue used in our experiments, is intravenously injected, the increase in capillary permeability produced by a histamine releaser can be detected before the appearance of oedema (Feldberg, 1954).

It is known that mast cells contain histamine (Riley & West, 1953) and 5-hydroxytryptamine (Benditt, Wong, Arase & Roeper, 1955 ; Bhattacharya & Lewis, 1956). Dextran, when injected intracutaneously, produces mast cell disruption at the site of skin reaction. Similar alterations which correlated well with histamine release were observed in *in vitro* experiments. Our findings suggest the participation not only of histamine but possibly also of 5-hydroxytryptamine in the mechanism of increase of the capillary permeability produced by dextran. Mast cells contain 5-hydroxytryptamine. When mast cells are disrupted, as, for example, by dextran, 5-hydroxytryptamine may be released, as occurs with 48/80 (Bhattacharya & Lewis, 1956). The failure of the dextran to produce the skin reaction in rats depleted of mast cells reinforces the hypothesis that these cells participate in the mode of action of dextran.

The inhibition of the skin reaction observed when glucose and dextran were simultaneously injected suggests that the inhibitory effect of glucose occurs at the site of the reaction, blocking the disruption of mast cells. This hypothesis is supported by the results obtained from *in vitro* experiments in which both mast cell disruption and histamine release were inhibited by glucose. Since dextrans are polymers of glucose the mechanism of the inhibition is probably competitive in nature.

Xylose, mannose, fructose and maltose also reduce both histamine release and mast cell disruption by dextran, whereas the other carbohydrates do not inhibit or produce only a small inhibition. In addition, an increase (about 30%) in histamine release by dextran was observed with galactose. It is interesting to note that the more powerful inhibitors are those hexoses more related to glucose. This suggests that configuration of the carbon atom 4 of the hexoses is important in the inhibitory process. Such observations stress the hypothesis that dextran competes with glucose probably in the mast cells.

The inhibitory action of the uncoupling agent 2:4-dinitrophenol on the histamine-releasing activity of dextran is remarkably reversed by glucose, when used in lower concentrations. In these concentrations glucose alone increases the histamine-

releasing activity of dextran. Barry (1952) and Maxwell, Kalckar & Burton (1955) demonstrated that galactose can be converted into glucose by animal tissues. Our results, on effects of galactose, perhaps could be explained on the basis of the conversion of galactose into glucose. The results agree with those obtained by Rothschild, Vugman & Rocha e Silva (1961) for histamine release and mast cell disruption with compound 48/80. Our results, as in the case of 48/80, may be interpreted as an indication that metabolic intermediates necessary for the activity of dextran can be generated by a mechanism, possibly glucolytic, functioning independently of the Krebs cycle. The experiments with inhibitors of anaphylaxis suggest that dextran releases histamine by a mechanism presenting similar steps to those found with 48/80 and anaphylaxis in the rat.

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