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CHANGES IN VASCULAR PERMEABILITY PRODUCED IN RATS BY DEXTRAN, OVOMUCOID AND YEAST CELL WALL POLYSACCHARIDES

BY

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A single intradermal injection of dextran or ovomucoid into the ventral abdominal skin of rats produces a local increase in vascular permeability which is antagonized by glucose and other sugars (Beraldo, Dias Da Silva & Lemos Fernandes, 1962). The release of histamine and the partial disruption of tissue mast cells by dextran are also inhibited by these sugars. The present work was undertaken to determine the relative activities of different sugars and other simple carbohydrates in antagonizing the local vascular changes produced not only by dextran and ovomucoid but also by two polysaccharide preparations extracted from the yeast cell wall, namely yeast mannan and zymosan (Pillemer & Ecker, 1941; Peat, Whelan & Edwards, 1961). Whereas dextran is a polymer of glucose, yeast mannan is a polymer consisting entirely of mannose. Dextran, ovomucoid and yeast mannan have all been shown to produce the anaphylactoid reaction when injected intraperitoneally into rats (Voorhees, Baker & Pulaski, 1951; Bombara & Morabito, 1961).

METHODS

Tests on vascular permeability in hooded Lister rats (body weight about 200 g) were carried out using the method of Bonaccorsi & West (1963). After the intravenous injection of azovan blue dye (7 mg/kg), each rat received at least six intradermal injections into the shaved ventral abdominal skin. After 30 min, the rats were killed, and the reaction to each injection was assessed by measuring the mean diameter of the blue area on the inner surface of the skin.

Agents used

Polysaccharides tested for their ability to increase vascular permeability were dextran (Intradex, Glaxo), ovomucoid (L. Light, Colnbrook), yeast mannan prepared by the method of Peat *et al.* (1961b), and zymosan (Fleishmann's type A) *ex* fresh yeast (L. Light, Colnbrook).

Design of experiment

Doses of each polysaccharide producing graded responses were first obtained in groups of three rats. From the log dose/response curves the effective dose producing a 50% response (the ED50) was obtained,

taking the response to 100 μg of dextran as a maximum (100%). Each value shown in the Tables and Figures represents the mean and standard error of three experiments, each using three rats.

Inhibition by sugars

Each sugar under test was dissolved in different solutions of the polysaccharides and injected intradermally in volumes of 0.1 ml., as described above. The response given by the polysaccharide in the presence of each sugar was then expressed as a percentage of that in its absence. From the log dose/inhibition curves doses producing 50% inhibition (the ID50 values) were obtained. Each value shown is the mean and standard error of three experiments, each using three rats.

Antagonists of histamine and 5-hydroxytryptamine

Mepyramine, a specific antagonist of histamine, was used in a dose of 2 mg/kg; methysergide, a specific antagonist of 5-hydroxytryptamine, was tested in a dose of 50 μg/kg. Each antagonist was given intravenously 30 min before the intradermal injections of the four polysaccharides or of histamine (10 μg) or 5-hydroxytryptamine (0.1 μg) were made. In a few experiments both antagonists were given together.

RESULTS

Activity of the polysaccharides

Yeast mannan was about thirteen times more active than ovomucoid and twenty times more active than dextran in increasing vascular permeability in rat skin (see Fig. 1 and Table 1). With zymosan, a suspension in 0.9% saline was first used for the comparison, and this was about eighty times less active than yeast mannan. When the suspension was placed in a boiling-water bath for 30 min, cooled and filtered, the filtrate was as active as

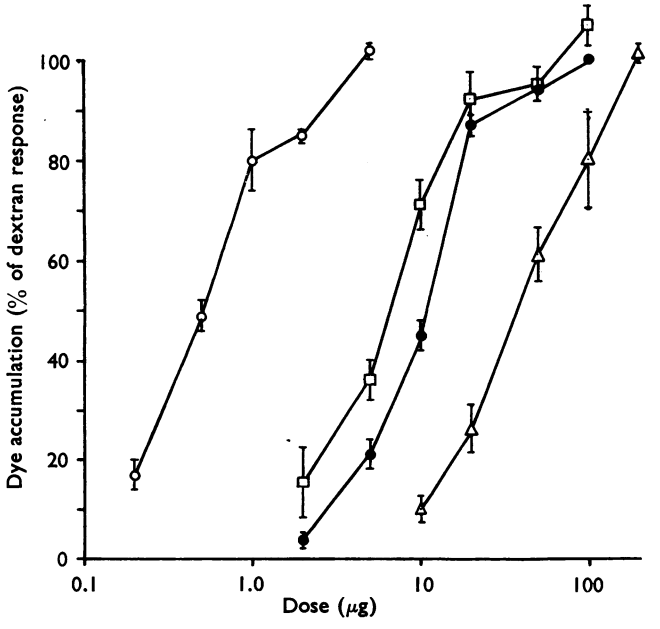


Fig. 1. The increase in vascular permeability produced in rats by yeast mannan (○), ovomucoid (□), dextran (●) and zymosan (△). Responses (ordinate) are expressed as the extent of dye accumulation in the skin after intradermal injection, as a percentage of the response to 100 μg of dextran. Abscissa with log scale.

TABLE 1

EQUIACTIVE INTRADERMAL DOSES (μG) OF FOUR POLYSACCHARIDES INCREASING VASCULAR PERMEABILITY IN RAT SKIN

For the dose ratios, yeast mannan was taken as unity. ED50s are means and standard errors of three experiments

Polysaccharide	ED50	Dose-ratio
Yeast mannan	0·5±0·02	1·0
Ovomucoid	6·6±0·7	13·3
Dextran	10·2±0·4	20·4
Zymosan suspension	40·0±4·0	80·0
Filtrate from boiled zymosan	40·2±4·2	80·4
Filtrate from unboiled zymosan	112·0±8·7	224·0

the original suspension; but the filtrate obtained from an unboiled zymosan suspension was nearly three times less active. In all further experiments the filtrate after boiling the zymosan was used.

Inhibition by glucose and mannose

Equiactive doses of the polysaccharides were used in these experiments and the log dose/response curves were plotted for the inhibitory actions of glucose and mannose (see Fig. 2 for the glucose results). Whereas 100 μg of glucose exerted little inhibitory effect, 400 μg produced almost complete inhibition of the responses. The dose producing 50% inhibition (the ID50) was about 200 μg when tested against each of the four polysaccharides. Therefore glucose was equally effective ($P<0.05$) in antagonizing the responses of yeast mannan, ovomucoid, dextran and zymosan (Table 2). Mannose was equally effective

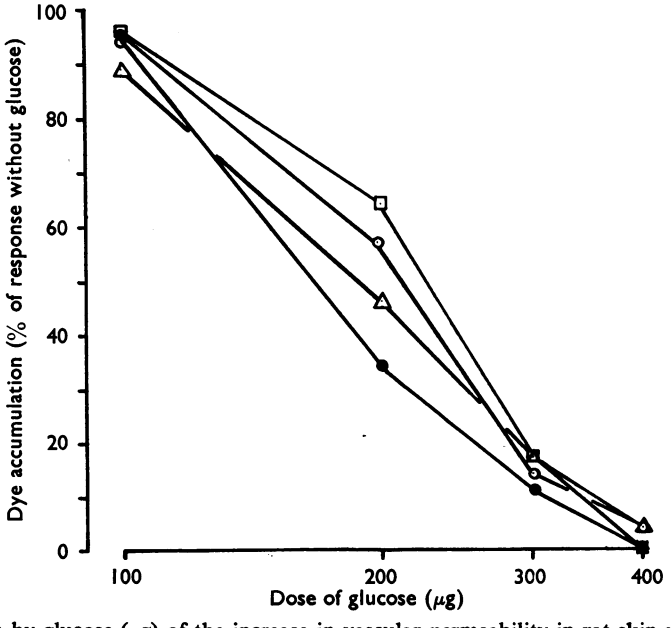


Fig. 2. Inhibition by glucose (μg) of the increase in vascular permeability in rat skin produced by yeast mannan (5 μg, ○), ovomucoid (65 μg, □), dextran (100 μg, ●) and filtrate from boiled zymosan (400 μg, △). Responses (ordinate) are expressed as the extent of dye accumulation in the skin after intradermal injection, as a percentage of the response without glucose. Abscissa with log scale.

TABLE 2

INHIBITION BY INTRADERMAL GLUCOSE AND MANNOSE OF THE INCREASE IN VASCULAR PERMEABILITY IN RAT SKIN PRODUCED BY EQUIACTIVE INTRADERMAL DOSES OF FOUR POLYSACCHARIDES

Inhibition was measured as the dose required to produce 50% reduction in response. ID50s are means and standard errors of three experiments

Polysaccharide	Dose (μg)	ID50 for Glucose	ID50 for Mannose
Yeast mannan	5	208±13·0	193±12·0
Ovomucoid	65	225± 2·9	—
Dextran	100	183±24·5	155± 2·9
Filtrate from boiled zymosan	400	190±15·3	—

($P<0.05$) against yeast mannan and dextran and the results were not significantly different from those of glucose.

Inhibition by other sugars

Among the disaccharides tested, only lactose failed to inhibit the responses of the four polysaccharides (Table 3). D-Galactose, a component of lactose, was the only hexose monosaccharide to exert a poor inhibitory action. In the pentose group, only D-ribose did not possess an inhibitory action. Of the two trioses, DL-glyceraldehyde was active, whereas dihydroxyacetone had no activity in this test. Lastly, whereas 6-deoxy-L-mannose was without activity, 2-deoxy-D-glucose was a very effective antagonist. In all instances where inhibition occurred it was always of a similar order against each of the four polysaccharides tested. It appears that inhibition is in some way related to the stereochemistry of the sugar molecule.

TABLE 3

PERCENTAGE INHIBITION BY VARIOUS SUGARS OF THE INCREASE IN VASCULAR PERMEABILITY IN RAT SKIN PRODUCED BY EQUIACTIVE DOSES OF FOUR POLYSACCHARIDES

Dose of each sugar = 500 μg

Sugar	Inhibition (%) of response due to polysaccharide: Yeast mannan	Ovomucoid	Dextran	Filtrate from boiled zymosan
Trehalose	100	100	100	96
Maltose	90	88	100	93
Cellobiose	90	100	96	100
Gentiobiose	96	92	100	100
Lactose	6	18	11	0
Sucrose	51	35	40	49
D-Glucose	100	100	100	100
D-Mannose	100	100	100	100
D-Galactose	4	14	8	8
D-Fructose	100	100	100	81
L-Sorbose	100	100	100	95
D-Ribose	8	7	12	10
D-Arabinose	100	100	95	91
D-Xylose	99	100	100	84
D-Lyxose	86	99	91	92
Rhamnose (6-Deoxy-L-mannose)	9	0	7	4
2-Deoxy-D-glucose	96	88	91	100
DL-Glyceraldehyde	90	100	100	80
Dihydroxyacetone	6	0	7	2

Although the chemistry of ovomucoid has not yet been fully elucidated, the compound is known to contain the three sugars, mannose, *n*-acetyl-D-glucosamine and galactose (Stacey & Wooley, 1940, 1942). Mannose (ID50=172±13.5 μg, mean and standard error) was a powerful inhibitor of ovomucoid whereas galactose (ID50=1,075±71.4 μg) was only a weak antagonist. The ID50 value for *n*-acetyl-D-glucosamine (210±12.5 μg) was not significantly different from that of glucose (see Table 2), and was at least five times that of galactose.

Antagonists of histamine and 5-hydroxytryptamine

Mepyramine prevented the increase in vascular permeability produced by histamine but did not alter that due to 5-hydroxytryptamine or the four polysaccharides. Similarly, methysergide only prevented the response to 5-hydroxytryptamine (see Table 4). However, when both mepyramine and methysergide were injected together, the responses of all the active agents, including that to the polysaccharides, were prevented.

TABLE 4

PERCENTAGE INHIBITION BY INTRAVENOUS MEPYRAMINE (2 MG/KG) AND METHYSERGIDE (50 μG/KG) OF THE INCREASE IN VASCULAR PERMEABILITY IN RAT SKIN PRODUCED BY EQUIACTIVE INTRADERMAL DOSES OF FOUR POLYSACCHARIDES, HISTAMINE AND 5-HYDROXYTRYPTAMINE

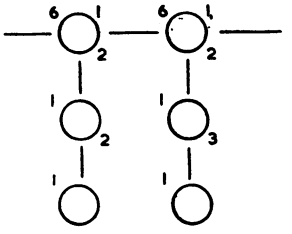
Active agent	Dose (μg)	Inhibition (%) by		
		Mepyramine	Methysergide	Mepyramine and Methysergide
Yeast mannan	5	0	0	100
Ovomucoid	65	0	38	100
Dextran	100	7	27	100
Filtrate from boiled zymosan	400	0	0	100
Histamine	10	96	0	93
5-Hydroxytryptamine	0·1	0	98	100

DISCUSSION

Dextran, ovomucoid and the two polysaccharides extracted from yeast cell wall, mannan and zymosan, increase the vascular permeability in rat skin. The dose/response curves of the four polysaccharides are parallel and many sugars are equally effective in inhibiting the responses of each. This suggests that a similar mechanism is involved for each agent, the same common receptor being occupied before the response is produced. This receptor appears to be stereospecific.

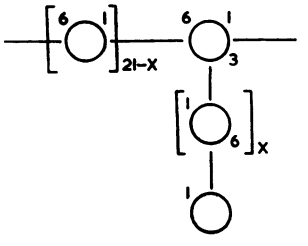
Dextran is a polymer of glucose, whereas yeast mannan consists entirely of mannose. Comparison of the inhibitory properties of glucose and mannose on the vascular reaction being studied indicates that these two sugars have the same affinity for the receptor. It is of interest that the repeating units of the main chain in both dextran and yeast mannan are of the α-pyranosyl configuration and are 1,6-linked (Van Cleeve, Schaefer & Rist, 1956; Peat *et al.*, 1961b; Peat, Turvey & Doyle, 1961). This type of linkage may therefore be of great importance. Yeast mannan has been found to be much more active than dextran and this may be due to a high degree of branching in the mannan structure. A unique

structure for yeast mannan has not yet been postulated but Peat *et al.* (1961a) have proposed the following for the simplest repeating unit:



The α-mannopyranosyl residue is represented by O

The structure of dextran is represented by the following repeating unit (Van Cleeve *et al.*, 1956):



The α-glucopyranosyl residue is represented by O

The value of $\times$ has been suggested to be zero, and if this is so, then dextran has only one side chain per twenty-two main chain units, whereas yeast mannan has a side chain on every main chain unit. As dextran is about twenty times less active than yeast mannan, it is tempting to suggest that the activity of the molecule depends on the ratio of side chain to main chain units. If this proves to be correct, then it may be the monosaccharide residue or residues on the side chain which fit the active centres on the receptor surface.

Whereas yeast mannan and dextran are composed entirely of sugar residues, the carbohydrate residue of ovomucoid constitutes only 20% of the parent compound (Stacey & Wooley, 1942). The monosaccharides in ovomucoid have been identified as *n*-acetyl-D-glucosamine, mannose and galactose, present in molar ratios of 7 : 3 : 1 (Stacey & Wooley, 1942; Bragg & Hough, 1961). As galactose is present in a relatively small amount, and has a low affinity for the receptor, it probably does not contribute much to the activity of the ovomucoid molecule. Therefore *n*-acetyl-D-glucosamine and mannose probably represent the active material. The terminal residues in ovomucoid have been reported by Stacey & Wooley (1942) to consist of *n*-acetyl-D-glucosamine units. However, the full structure of ovomucoid has not yet been fully elucidated; it cannot be classified as a linear molecule, and peptides are linked to the carbohydrate units.

According to Pillemer & Ecker (1941), zymosan is insoluble both in cold and in hot water. During its preparation (Pillemer, Blum, Lepow, Wurz & Todd, 1956) various procedures are carried out to free the substance from soluble carbohydrate. The membranes of the yeast cell wall consist in part of glucan and mannan (Northcote & Horne, 1952), but on

isolation only the mannan is soluble in water (Falcone & Nickerson, 1956; Peat, Whelan & Edwards, 1958, 1961). Hence the activity of the sample of zymosan used in the present experiments was probably due to the presence of soluble mannan. As yeast mannan was eighty times as active as zymosan, only just over 1% of zymosan needs to be mannan to account for its activity. The difference in activity between boiled and unboiled zymosan may be due to the mannan in the zymosan sample being partly in combination with other inert materials.

Bonaccorsi & West (1963) showed that mepyramine and methysergide had little effect by themselves on the intradermal responses to dextran and ovomucoid but together they produced complete inhibition. In the present work the responses to yeast mannan and zymosan were also antagonized by the combination of mepyramine and methysergide but not by each separately. Since the combination effectively prevented the responses of exogenous histamine and 5-hydroxytryptamine, it may be that yeast mannan and zymosan, like dextran and ovomucoid, increase vascular permeability by a mechanism which involves the release of histamine and/or 5-hydroxytryptamine. However, there may be major differences between endogenously released histamine and 5-hydroxytryptamine and exogenously applied amine, and another mediator may be involved, as suggested by Bonaccorsi & West (1963).

SUMMARY

1. Yeast mannan, a polymer of mannose, was found to be about thirteen times as active as ovomucoid, twenty times as active as dextran, and eighty times as active as zymosan, in increasing vascular permeability in rat skin.

2. The responses to the four polysaccharides were each antagonized by similar concentrations of glucose and mannose, other hexoses, pentoses, trioses and disaccharides. Lactose, galactose, ribose and rhamnose were inactive in the doses used against all four agents.

3. The four polysaccharides may have a common site of action, and their relative activities may be determined by the extent of branching within the molecule.

4. The combination of mepyramine and methysergide prevented the increase in vascular permeability produced by yeast mannan, dextran, ovomucoid, and zymosan, suggesting that histamine and 5-hydroxytryptamine are involved at some stage in the response.

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