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THE INFLUENCE OF MOLECULAR WEIGHT AND STRUCTURE ON THE VASCULAR PERMEABILITY RESPONSES INDUCED BY GLUCOSE POLYMERS IN RAT SKIN

BY

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An intraperitoneal injection of dextran into rats produces a generalized anaphylactoid reaction characterized by hyperaemia, pruritus and oedema of the extremities (Voorhees, Baker & Pulaski, 1951). Dextrans of average molecular weight between 25,000 and 2,000,000 are the most effective in producing this response, whereas low molecular weight fractions of 4,000 are ineffective (Halpern, Briot & Neveu, 1954; Harris & West, 1963, 1964). However, when injected intradermally, these low molecular weight polymers produce a slight increase in vascular permeability (Bonaccorsi & West, 1963).

In 1961 Harris and West found that some rats of the Wistar albino strain failed to react to an intraperitoneal dose of clinical dextran (Intradex) and these genetically resistant rats were subsequently designated as non-reactors (Harris & West, 1963; Harris, Kalmus & West, 1963). Intradermal administration of dextrans of various molecular weights also failed to increase vascular permeability in these rats (Bonaccorsi & West, 1963), unless given in very high dosage (Harris & Luscombe, 1965). The present work was undertaken in order to investigate in more detail the effects of molecular weight and chemical structure on the activity of dextrans and other polyglucoses in increasing vascular permeability in the skin of both reactor and non-reactor rats. Also, glucose and several other sugars have been tested as antagonists to the responses produced by these agents, as Poyser & West (1965) have shown that simple carbohydrates inhibit the local vascular changes produced by clinical dextran in rat skin.

METHODS

Preliminary screening

Male rats (body weight about 200 g) of the hooded Lister and Wistar strains were used. The hooded Listers were all known to be reactors but the Wistar rats were tested by the screening procedure described by Harris & West (1963) to separate them into reactors and non-reactors.

Tests on vascular permeability

These were carried out using the method of Bonaccorsi & West (1963). After the intravenous injection of azovan blue dye (7 mg/kg), each rat received eight to ten intradermal injections into the shaved ventral abdominal skin. Each agent was administered in a volume of 0.1 ml. 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. In each experiment three groups of three reactor or non-reactor rats were used and the results averaged for each type. The experimental design was similar to that described by Poyser & West (1965) and the activity of polysaccharides increasing vascular permeability in reactor rats was expressed on a percentage scale, clinical dextran (Intradex) of average molecular weight 140,000 being assigned a value of 100.

Agents used

Polyglucoses tested for their ability to increase vascular permeability included a range of dextran fractions of average molecular weight 4,000 to 2,000,000. These were prepared by acid hydrolysis and fractional precipitation of a native dextran produced by *Leuconostoc mesenteroides*, strain NRRL-B512, and were supplied by Fisons, Glaxo and Pharmacia. The sample of clinical dextran used (Intradex, Glaxo) was prepared in the same manner and from the same strain of organism. Other polymers of glucose tested were a synthetic polyglucose (Ricketts, 1954; Ricketts & Rowe, 1955), luteose prepared from a culture of *Penicillium luteum* (Anderson, Haworth, Raistrick & Stacey, 1939) and a sample of a highly branched dextran obtained from the Birmingham strain of *Betacoccus arabinosaceous* (Barker, Bourne, Bruce, Neely & Stacey, 1954; Rowe, 1957). These were kindly supplied by Dr. C. R. Ricketts of the M.R.C. Industrial Injuries and Burns Research Unit, Birmingham. A sample of dextrin (Astra) obtained from potato starch and of average molecular weight 6,000 was also used.

Inhibition by sugars

This was carried out using the method of Poyser & West (1965). Doses of glucose producing 50% inhibition (the ID50 values) of the responses produced by the polysaccharides were obtained. Each value shown is the mean and standard error of three experiments, each using three rats.

RESULTS

Activity of dextrans of various molecular weights

Dextrans of average molecular weight 10,000 to 200,000 were approximately equiactive in increasing vascular permeability in the skin of hooded Lister and Wistar reactor rats (Fig. 1). Above 200,000 activity slowly declined as molecular weight increased whereas below 10,000 there was a sharp fall in activity, dextrans of average molecular weight 4,000 to 6,000 being only one-tenth as active as Intradex (molecular weight 140,000).

Activity of other polyglucoses

The activity of a synthetic polyglucose of average molecular weight 3,000 was similar to that of dextran of molecular weight 4,000 (Table 1). Dextrin (average molecular weight 6,000) was three to four times more active than the dextran of the same molecular weight, whereas luteose of average molecular weight 14,000 was one-third to one-half as active as the equivalent dextran. The sample of highly branched dextran (Birmingham strain) was two to three times more active than Intradex (B512 strain).

Activity of dextrans in non-reactors

The responses produced by a dose of 1 mg of dextrans of average molecular weight 10,000 and above were much larger in reactors than non-reactors, whereas samples of average molecular weight 5,000 to 6,000 produced similar effects in both types of rat (Fig. 2). Therefore as molecular weight increased above the region of 6,000 to 10,000 there was increased activity in reactor rats but decreased activity in non-reactors.

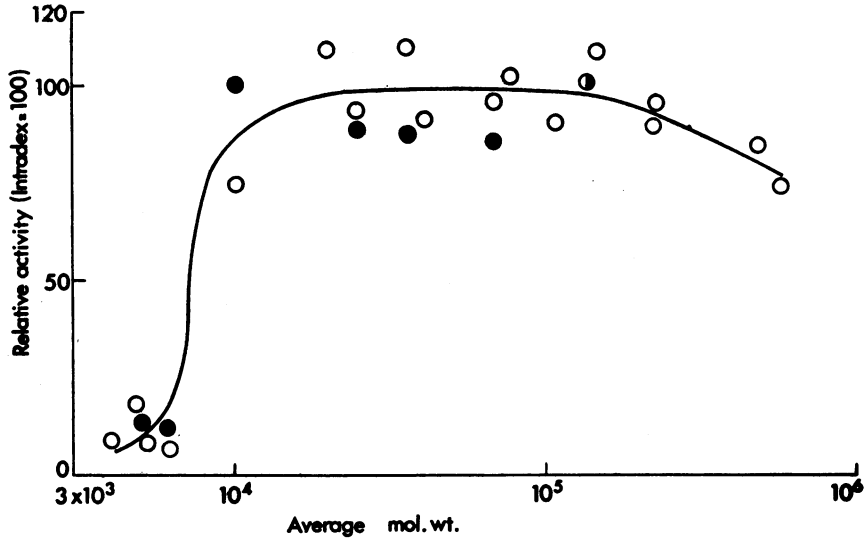

Fig. 1. Activities of dextrans of various molecular weight in increasing vascular permeability in the skin of hooded Lister (○), and Wistar reactor (●) rats. Activity (ordinate) is expressed on a percentage scale, clinical dextran (Intradex) of average molecular weight 140,000 being assigned a value of 100. Abscissa with log scale.

TABLE 1

ACTIVITIES OF POLYGLUCOSES OF VARIOUS MOLECULAR WEIGHTS IN INCREASING VASCULAR PERMEABILITY IN THE SKIN OF HOODED LISTER AND WISTAR REACTOR RATS

Activity is expressed on a percentage scale, clinical dextran (Intradex) of average molecular weight 140,000 being assigned a value of 100

Polyglucose	Average molecular weight	Activity	
		Hooded Lister	Wistar
Synthetic polyglucose	3,000	8	17
Dextran (B512)	4,000	8	11
Dextran (B512)	5,000	16	14
Dextran (B512	6,000	8	12
Dextrin	6,000	32	34
Dextran (B512)	10,000	74	100
Luteose	14,000	27	53
Dextran (B512)	20,000	110	90
Dextran (B512	40,000	91	87
Dextran (B512)	70,000	96	86
Dextran (B512, Intradex)	140,000	100	100
Dextran (Birmingham)	150,000	374	205
Dextran (B512)	250,000	91	94
Dextran (B512)	500,000	84	—
Dextran (B512)	2,000,000	32	—

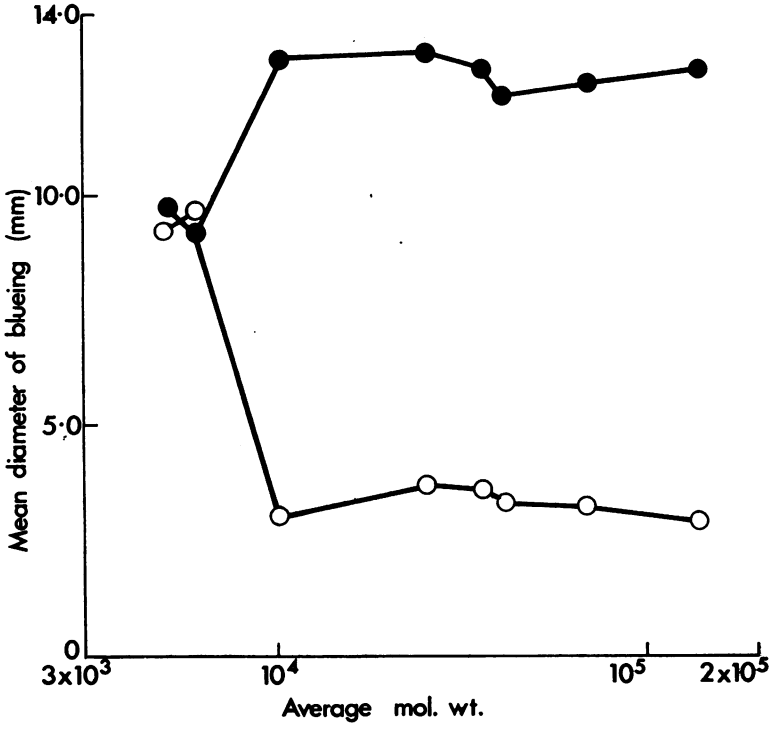

Fig. 2. The increase in vascular permeability produced by a 1 mg dose of various molecular weight dextrans in reactors (●) and non-reactors (○). Responses (ordinate) are expressed as the mean diameter of blueing in excess of that produced by the saline control. Abscissa with log scale.

Activity of other polyglucoses in non-reactors

Molecular weight also appeared to be a determinant factor regarding the activity of other polyglucoses in non-reactor rats. Low molecular weight polymers (synthetic polyglucose and dextrin) were equiactive in both types of rat whereas polyglucoses of higher molecular weight such as luteose and dextran (Birmingham strain) were much less effective in non-reactors (Table 2), the change again occurring in the 6,000 to 10,000 region.

TABLE 2

ACTIVITIES OF POLYGLUCOSES IN INCREASING VASCULAR PERMEABILITY IN THE SKIN OF WISTAR NON-REACTOR RATS

Values are quoted as the dose in non-reactors divided by the dose to produce a similar response in reactors

Polyglucose	Average molecular weight	Dose in non-reactors / Dose in reactors
Synthetic polyglucose	3,000	1·13
Dextran (B512)	6,000	0·78
Dextrin	6,000	1·75
Dextran (B512)	10,000	450
Luteose	14,000	395
Dextran (B512, Intradex)	140,000	450
Dextran (Birmingham)	150,000	435

Inhibition by glucose

Equiactive doses of the polysaccharides were used and it was observed that the doses of glucose required to produce 50% inhibition of the response (the ID50 values) were not significantly different ($P<0.05$) when tested against each of the dextrans and other polyglucoses of average molecular weight 10,000 to 2,000,000 (Table 3). However, larger amounts of glucose were required to antagonize the responses produced by low molecular weight polymers such as dextrans of average molecular weight 4,000 to 6,000, synthetic polyglucose and dextrin. When tested against polysaccharides active in non-reactor rats, the ID50 values for glucose did not significantly differ ($P<0.05$) from those obtained in reactors (Table 4).

TABLE 3

INHIBITION BY INTRADERMAL GLUCOSE OF THE INCREASE IN VASCULAR PERMEABILITY IN RAT SKIN PRODUCED BY EQUIACTIVE INTRADERMAL DOSES OF POLYGLUCOSES OF VARIOUS MOLECULAR WEIGHTS

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

Polyglucose	Average molecular weight	Polyglucose dose (μg)	ID50 Glucose (μg)
Synthetic polyglucose	3,000	1,000	1,317 ± 92·8
Dextran (B512)	4,000	1,200	418 ± 38·0
Dextran (B512)	5,000	1,000	577 ± 46·3
Dextran (B512)	6,000	1,250	400 ± 42·3
Dextrin	6,000	300	355 ± 13·2
Dextran (B512)	10,000	100	172 ± 2·3
Luteose	14,000	375	173 ± 3·5
Dextran (B512)	20,000	100	182 ± 3·7
Dextran (B512)	40,000	100	193 ± 8·8
Dextran (B512)	70,000	100	178 ± 5·2
Dextran (B512, Intradex)	140,000	100	183 ± 24·5
Dextran (Birmingham)	150,000	27	213 ± 6·0
Dextran (B512)	250,000	100	196 ± 12·5
Dextran (B512)	500,000	100	207 ± 6·7
Dextran (B512)	2,000,000	300	132 ± 23·9

TABLE 4

INHIBITION BY INTRADERMAL GLUCOSE OF THE INCREASE IN VASCULAR PERMEABILITY PRODUCED BY EQUIACTIVE INTRADERMAL DOSES OF LOW MOLECULAR WEIGHT POLYGLUCOSES IN REACTOR AND NON-REACTOR WISTAR RATS

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

Polyglucose	Average molecular weight	Polyglucose dose (μg)	ID50 Glucose (μg)	
			Reactors	Non-reactors
Synthetic polyglucose	3,000	1,000	1,400 ± 150·3	1,375 ± 125·3
Dextran	6,000	1,000	310 ± 28·1	320 ± 10·1
Dextrin	6,000	300	315 ± 14·0	310 ± 25·1

Inhibition by other sugars

The range of sugars used was as previously described by Poyser & West (1965). A dose of 500 μg of each sugar was tested against Intradex (dextran B512), dextran (Birmingham strain) and luteose. For the two low molecular weight polymers, synthetic

polyglucose and dextrin, the dose of sugar was increased in proportion to the ID50 values for glucose. Varying degrees of inhibition were obtained by the different sugars under test and the results were similar to those reported by Poyser & West (1965) for other carbohydrate polymers (Table 5). In all instances where inhibition occurred it was of a similar order against each of the polysaccharides tested.

TABLE 5

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

The dose of each sugar used was 500 μg against luteose and the two dextrans, whereas 970 μg were used against dextrin and 3,500 μg against synthetic polyglucose (these values being in proportion to the ID50 values for glucose). Hooded Lister rats were used

	Percentage inhibition of response due to polyglucose				
Sugar	Synthetic polyglucose	Dextrin	Luteose	Dextran (Birmingham)	Dextran (Intradex)
Trehalose	100	96	93	100	100
Maltose	100	90	98	92	100
Cellobiose	95	85	100	100	96
Gentiobiose	87	100	100	90	100
Lactose	12	7	0	0	11
Sucrose	64	55	45	35	40
D-Glucose	100	100	100	100	100
D-Mannose	100	92	100	100	100
D-Galactose	30	5	11	7	8
D-Fructose	100	88	100	100	100
L-Sorbose	100	97	100	100	100
D-Ribose	39	18	14	22	12
D-Arabinose	100	97	90	96	95
D-Xylose	94	88	98	100	100
D-Lyxose	100	99	100	94	91
6-Deoxy-L-mannose	0	0	2	0	7
2-Deoxy-D-glucose	98	96	100	100	91
DL-Glyceraldehyde	96	85	78	82	100
Dihydroxyacetone	0	1	0	3	7

DISCUSSION

A molecular weight of 10,000 appears to be a critical value regarding the activity of polyglucoses in increasing vascular permeability in rat skin. For example, dextrans (B512 strain) of average molecular weight 4,000 to 6,000 are equiactive in both reactor and non-reactor rats, but when the molecular weight increases to 10,000 and above there is increase in activity in reactors but a decrease in activity in non-reactors. Two other polyglucoses of average molecular weight below 10,000—namely, synthetic polyglucose and dextrin—also produce similar effects in both types of rat, whereas luteose and dextran (Birmingham strain) of higher molecular weight are much more active in reactors than non-reactors.

Harris & West (1963, 1964) reported that low molecular weight fractions of dextran failed to produce an anaphylactoid response when injected intraperitoneally into reactor rats and that all the fractions of dextran that they tested were ineffective in non-reactors. However, Ankier & West (1964a and b) observed that large doses of dextrin will produce

an anaphylactoid reaction when injected intraperitoneally or intravenously in both types of rat. The use of local injections in the present study has revealed that, whereas dextrin is three to four times more active than the same molecular weight dextran (6,000), each is equally effective in increasing vascular permeability in the skin of reactor and non-reactor rats. The higher activity of dextrin may be due to a difference in molecular structure or to the fact that the activity curve for dextrans is very steep at the molecular weight range under consideration, and therefore any slight variance in weight average or distribution will greatly affect activity.

Recently we have been able to show that large doses of low molecular weight dextran (5,000 to 6,000) will produce an anaphylactoid reaction in both reactor and non-reactor rats when injected intraperitoneally or intravenously (unpublished observations). Therefore rats designated by Harris & West (1963) as non-reactors because of their failure to respond to intraperitoneal injections of clinical dextran will react to low molecular weight polymers of glucose in the same way as reactors.

All the polyglucoses found to increase vascular permeability in the present work were branched chain structures. Furthermore, the highly branched dextran obtained from the Birmingham strain of *Betacoccus arabinosaceous* was observed to be two to three times more active than the less branched clinical dextran of the same molecular weight (Intradex). These findings provide further evidence to support the suggestion made by Poyser & West (1965) that activity may be related to the extent of branching within the molecule. This suggestion was based on the observation that yeast mannan, a highly branched polymer of the sugar mannose, was several times more active than dextran (Intradex) of similar molecular weight. However, the two polymers used for this comparison were composed of different sugars, whereas in the present study a similar conclusion has been reached using two polyglucoses. These effects of branching on the activity of polysaccharides in the rat agree with the clinical observations of Tarrow & Pulaski (1953), who found that the more highly branched dextrans were associated with a greater incidence of allergic reactions in man than the less branched dextran that they tested. This suggests a similarity between these effects in the rat and allergic reactions to dextran in man.

Senti (personal communication to Wales, Marshall & Weissberg, 1953) reported that the degree of branching in NRRL-B512 dextrans was independent of the molecular weight, whereas Granath (1958) indicated that branching diminishes rapidly as molecular weight approached 10,000. This may explain the rapid fall off in activity of dextrans of molecular weight below this figure and also why dextrans of average molecular weight 10,000 to 200,000 are equiactive. The decrease in activity with high molecular weight polymers may be due to a failure to penetrate to the site of action or to structural factors such as an increase in the size of branch chains associated with high molecular weight (Wales, Marshall & Weissberg, 1953).

Luteose has a fairly high degree of branching but it is less active than dextran (B512) of the equivalent molecular weight. Unlike dextran the predominant glucosidic link in luteose is β-1:6 and also chemical evidence suggests that the branch chains are interconnected to form a closed chain structure (Anderson *et al.*, 1939). Luteose will, therefore, tend to have a more rigid configuration compared with the flexible structure of dextran (Granath, 1958). These properties may account for the low activity of this polymer of glucose.

Several sugars are equally effective in inhibiting the responses of all the polyglucoses under test. Also, the amount of glucose required to antagonize the effects of each of the low molecular weight polysaccharides in non-reactor rats is similar to the amount required in reactors. This suggests that a similar mechanism is involved for each agent in both types of rat. The stereospecificity of the receptor system involved in the action of these polyglucoses is similar to that described by Poyser & West (1965) for other carbohydrate polymers such as ovomucoid and yeast mannan. It is interesting to note that larger amounts of glucose are required to antagonize the responses produced by equiactive doses of low molecular weight polysaccharides. This would suggest that decreased activity of these polyglucoses is not due to a decreased affinity for the receptor site.

SUMMARY

1. Dextran fractions of average molecular weight 10,000 to 200,000 are more effective in increasing vascular permeability in rat skin than those of lower molecular weight.

2. Whereas a synthetic polyglucose was equiactive, dextrin was more active and luteose less active than the corresponding molecular weight dextrans. A highly branched dextran was two to three times more active than the less branched clinical sample.

3. Dextrin, synthetic polyglucose and dextrans of average molecular weight below 10,000 were equiactive in both rats which react (reactors) and those that fail to react (non-reactors) to intraperitoneal injections of clinical dextran. However, luteose and dextrans of higher molecular weight were much more active in reactors than non-reactors.

4. Although structural factors such as branching are important, a molecular weight of 10,000 appears to be a critical value regarding the local vascular changes produced by polyglucoses in rat skin.

5. The responses to all the polyglucoses were each antagonized by glucose and several other sugars suggesting that a common site of action may be involved.

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