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INHIBITION OF THE ANAPHYLACTOID REACTION IN RATS

BY

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The primary intraperitoneal or intravenous injection of egg-white (Selye, 1937), dextran (Voorhees, Baker & Pulaski, 1951 ; Morrison, Richardson & Bloom, 1951) or dextrin (Rowley, 1963 ; Veilleux, 1963) into rats produces an acute inflammatory response termed the anaphylactoid reaction. Some rats of the Wistar albino strain are genetically resistant to dextran and do not exhibit the reaction. Selective breeding experiments have since shown that the character of " non-reactivity " is associated with an autosomal recessive gene (Harris, Kalmus & West, 1963).

It was recently found that relatively high intravenous doses of egg-white elicit an anaphylactoid reaction in dextran-resistant rats (termed non-reactors) and that dextrin is equally active in both reactor (that is, sensitive) and non-reactor rats (Ankier & West, 1964a, b). In the present work, the effects of some antagonists of histamine and 5-hydroxytryptamine (5-HT) and of some carbohydrates on the anaphylactoid reaction elicited in both types of rat by egg-white and dextrin and in reactor rats by dextran have been studied. A preliminary note of part of this work has already been published (Ankier & West, 1965).

METHODS

Test for reactivity to dextran

Male Wistar albino rats (body weight 120–150 g) obtained from the Agricultural Research Council's Field Station (Compton) were injected with a single intraperitoneal dose of dextran 240 mg/kg (Intradex, Glaxo) once a week on three occasions. Animals showing peripheral oedema were classed as " reactors " and those failing to react to any of three injections were classed as " non-reactors." Rats were used subsequently after a period of time of not less than one week.

Measurement of the anaphylactoid reaction

Azovan blue dye (18 mg/kg) was injected intravenously immediately before the intraperitoneal or intravenous injection of fresh hens' egg-white (12 ml/kg), dextran (240 mg/kg) or dextrin (Astra, 1,250 mg/kg). The extent of oedema formation and colloidal dye accumulation in the extremities were estimated visually every half an hour over a period of 4 hr, using an arbitrary shock-score (with values of 0, 1, 2 or 3) for each of twelve areas (nose and bristle area, eyelids, lips, tongue, ear

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base, ear margin, front paws, hind paws, skin covering the penis, scrotal sac, and margin and tail). The maximum score therefore was 36 for each observation. The cumulative shock-scores after drug pretreatment have been expressed in Tables 1–3 as percentages of those obtained in untreated rats. In all experiments, differences of 20% or more in percentage inhibitions were found, using the *t*-test, to be statistically significant ($P=0.05$).

Intradermal injections

Rats were lightly anaesthetized with ether, and azovan blue dye (8 mg/kg) was injected intravenously. Each animal then was given six intradermal injections (dose-volume 0.1 ml.) into the shaved ventral abdominal skin, one of these always being isotonic neutralized saline (pH 7.4). After 30 min, the rats were killed and the injection sites were carefully excised and pinned, with the outer surface downwards, on to cork boards. The extent of the vascular permeability changes was estimated by measuring the mean diameter of each blue lesion. All drugs were dissolved in isotonic saline and the solutions were adjusted to pH 7.4 before injection.

Estimation of glucose in dextrin

A modification of a method described by Rédei & Nagy (1961) for the simultaneous determination of glucose and dextran was used. Samples of dextrin (5 ml., 25% w/v) were hydrolysed with hydrochloric acid (0.05 ml., 10 N). At different times, aliquots of 0.1 ml. were removed, neutralized and added to 8 ml. of freshly prepared *o*-toluidine reagent (6% *o*-toluidine in glacial acetic acid). After centrifugation at 3,000 rev/min for 20 min, the clear supernatant liquid was removed and heated in a boiling water bath for 8 min. The extinction value of the resultant green colour was read colorimetrically at 610 mμ and the amount of glucose present was calculated by reference to calibration-curves obtained with glucose standards. Using this method, recovery of added glucose is about 90%.

Drugs used

These included mepyramine maleate (May & Baker, 10 mg/kg), 2-bromo-*d*-lysergic acid diethylamide (Sandoz, BOL 148, 3 mg/kg), promethazine hydrochloride (May & Baker, 8 mg/kg) and cyproheptadine hydrochloride (Merck, Sharp & Dohme, 0.5 mg/kg); they were injected intravenously 30 min before the anaphylactoid agents. In other experiments, 2-deoxyglucose (B.D.H., 500 mg/kg) was injected intraperitoneally 30 min before the active agents, but glucose, galactose, mannose or *N*-acetylglucosamine (B.D.H.) was injected intraperitoneally (in two doses, each of 1,500 mg/kg) 30 min before the active agents as well as at the same time they were administered.

RESULTS

Effect of antagonists of histamine and 5-hydroxytryptamine

The antihistamine mepyramine did not reduce the egg-white and dextrin reactions in reactor and non-reactor rats or the dextran reaction in reactor rats, but BOL 148, a specific antagonist of 5-HT, was a potent antagonist of most of the responses (Table 1). Promethazine and cyproheptadine were also effective antagonists of most responses; both these drugs possess antihistamine and anti-5-HT activities (Stone, Wenger, Ludden, Starvorski & Ross, 1961). These results show that 5-HT is an important mediator of the anaphylactoid reaction produced in rats by egg-white, dextrin and dextran.

Inhibition by carbohydrates

Glucose reduced all the responses in reactor rats but was only weakly active against egg-white and completely inactive against dextrin in non-reactor animals (Table 2).

Galactose and mannose were about as effective as glucose as inhibitors. *N*-Acetylglucosamine was more potent in reactor rats and reduced the responses in non-reactor animals, whereas 2-deoxyglucose effectively blocked all the responses.

TABLE 1

EFFECT OF SOME ANTAGONISTS ON THE ANAPHYLACTOID REACTION PRODUCED BY INTRAPERITONEAL DOSES OF EGG-WHITE, DEXTRIN AND DEXTRAN IN REACTOR RATS (R) AND BY INTRAVENOUS DOSES OF EGG-WHITE AND INTRAPERITONEAL DOSES OF DEXTRIN IN NON-REACTOR RATS (NR)

Each value is the mean of results from a group of eight rats, and is expressed as a percentage inhibition of the control responses.

Antagonist	Dose (mg/kg)	Egg-white		Dextrin		Dextran
		R	NR	R	NR	R
Mepyramine	10	3	0	7	15	12
BOL 148	3	70	100	100	90	56
Promethazine	8	83	100	90	93	33
Cyproheptadine	0·5	89	100	97	65	52

TABLE 2

EFFECT OF SOME CARBOHYDRATES ON THE ANAPHYLACTOID REACTION PRODUCED BY INTRAPERITONEAL DOSES OF EGG-WHITE, DEXTRIN AND DEXTRAN IN REACTOR RATS (R) AND BY INTRAVENOUS DOSES OF EGG-WHITE AND INTRAPERITONEAL DOSES OF DEXTRIN IN NON-REACTOR RATS (NR)

Each value is the mean of results from a group of eight rats, and is expressed as a percentage inhibition of the control response.

Carbohydrate	Egg-white		Dextrin		Dextran
	R	NR	R	NR	R
Glucose	72	33	77	6	54
N-Acetylglucosamine	84	73	83	47	79
2-Deoxyglucose	93	99	98	80	98

Effect of hydrolysed dextrin

The anaphylactoid activity of intraperitoneal dextrin (25% w/v, 5 ml./kg) decreased as the time of hydrolysis increased when tests were made in both reactor and non-reactor rats (Fig. 1). However, the rates of loss of activity differed; for example, after 5 hr of hydrolysis no reaction was produced in reactor rats and yet a full response was obtained in non-reactor animals; at this time, 84% of the dextrin in the hydrolysate was present as glucose. Furthermore, after 7 hr of hydrolysis, the hydrolysate was inactive intravenously in reactor rats and yet a full response was obtained in non-reactor animals (Fig. 2); on assay, this sample contained 96% glucose. It is possible that glucose, released from the dextrin during the hydrolysis, inhibited the dextrin response in reactor rats and was ineffective in non-reactor animals (Table 2), but the possibility also exists that hydrolysis of dextrin yields a glucose polymer of low molecular weight which is a potent anaphylactoid agent only in non-reactor rats. Dextrin hydrolysed for 5 hr was therefore dialysed against running saline (flow rate, 2 l./hr) for different times to remove the glucose, and then tested in both types of rat. The concentration of glucose in the non-dialysable fraction as well as its biological activity in non-reactor rats decreased in parallel but reactivation did not occur in reactor rats. The biological activity of unhydrolysed dextrin in both types of rat, on the other hand, was unaffected by dialysis and

so the active material in the hydrolysed dextrin eliciting the anaphylactoid reaction in non-reactor animals may be of a relatively low molecular weight. This glucose polymer has not so far been identified.

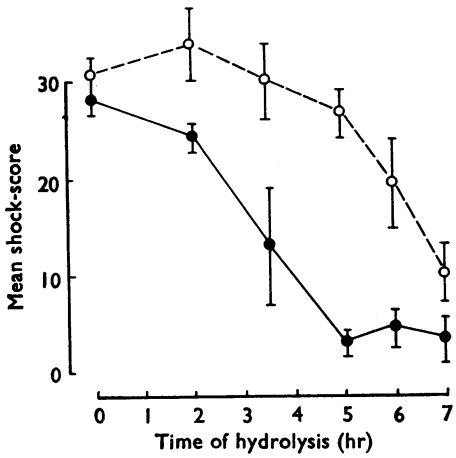
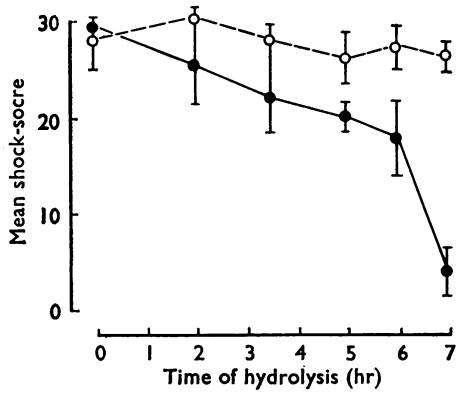


Fig. 1. Anaphylactoid reaction produced by hydrolysed dextrin after intraperitoneal injection into reactor (●——●) and non-reactor (○- - - -○) rats. Shock-scores (±S.E.) are measured on an arbitrary scale (see text).

Fig. 2. Anaphylactoid reaction produced by hydrolysed dextrin after intravenous injection into reactor (●——●) and non-reactor (○- - - -○) rats. Shock-scores (±S.E.) are measured on an arbitrary scale (see text).



Intradermal studies

The increase in vascular permeability induced by intradermal dextrin in both reactor and non-reactor rats was similar in threshold and intensity, a dose of 60 μg producing a maximal response. The concomitant intradermal injection of glucose inhibited the reaction equally well in both types of animal, 50% inhibition of the response to 60 μg dextrin being produced by about 500 μg glucose. When egg-white was injected intradermally, the threshold of activity in non-reactors was relatively higher (by a factor of 8–16) than that in reactors and glucose, injected together with the egg-white, was inhibitory at the doses indicated in Table 3 ; the extent of the inhibition corresponded with the difference in threshold. For example, 360 μg glucose completely inhibited the response of 0.25% egg-white in reactors and 1% in non-reactors, and 720 μg glucose inhibited 0.5% egg-white in reactors and 2% in non-reactors.

TABLE 3

EFFECT OF GLUCOSE ON THE INCREASE IN VASCULAR PERMEABILITY PRODUCED BY THE INTRADERMAL INJECTION OF DIFFERENT CONCENTRATIONS OF EGG-WHITE IN REACTOR (R) AND NON-REACTOR (NR) RATS

Each value is the mean of results from a group of twelve rats, and is expressed as a percentage inhibition of the control response.

Egg-white (%)	Glucose (μg)					
	180		360		720	
	R	NR	R	NR	R	NR
0·125	100	—	100	—	100	—
0·25	31	—	100	—	100	—
0·5	18	—	46	—	100	—
1·0	9	70	17	100	48	100
2·0	0	19	8	35	19	100
4·0	0	8	0	16	0	74

Other studies showed that 720 μg glucose did not inhibit the increases in vascular permeability induced by histamine (20 μg), 5-HT (0.4 μg) or bradykinin (0.2 μg) in both reactor and non-reactor rats.

DISCUSSION

The results of the present study using specific antagonists of histamine and 5-HT show that 5-HT has an important role as a mediator of the anaphylactoid reactions elicited by egg-white and dextrin in reactor and non-reactor rats and by dextran in reactor rats. Inhibition of the dextran response by antagonists of 5-HT was more difficult to achieve although higher doses of the antagonists have been shown in other experiments to be completely effective.

Ovomucoid, the active anaphylactoid-inducing portion of egg-white (Léger, Masson & Prado, 1947; Benditt, Bader & Arase, 1953; Ankier & West, 1964b), is a glycoprotein containing galactose, mannose and *N*-acetylglucosamine (Dixon, 1955; Bragg & Hough, 1961; Hartley & Jevons, 1962; Montgomery & Wu, 1963), whereas both dextrin and dextran are polymers of glucose. When these carbohydrates were used to antagonize the anaphylactoid reaction, a striking difference between the reactivity of reactor and that of non-reactor rats was found. For example, doses of glucose, galactose and mannose which prevent the anaphylactoid reaction produced by dextrin in reactor rats fail to modify the dextrin response in non-reactor rats. This suggests that an initial or intermediary stage in the dextrin reaction is different in the two types of animals. Furthermore, doses of *N*-acetylglucosamine which prevent the dextrin response in reactor rats are only partially effective against dextrin in non-reactor rats. In this respect, several monosaccharides and disaccharides share a similar inhibitory activity in reactor rats, as parenteral injections of equimolar concentrations of glucose, galactose, maltose, lactose and sucrose have been shown to produce an analogous inhibition of the generalized anaphylactoid reaction elicited by dextran (Adamkiewicz, 1965; Adamkiewicz & Sacra, 1967). The mechanism by which these carbohydrates inhibit a generalized anaphylactoid reaction is unknown. It seems unlikely that a biochemical carrier system is involved, because the configurational specificity of these carbohydrates for known biological trans-

port systems does not parallel their ability to inhibit the anaphylactoid reaction in reactor rats. Moreover, a direct influence on the intracellular metabolism of carbohydrates is not necessarily implicated for non-utilizable, as well as utilizable, carbohydrates are capable of inhibiting the reaction. The reports showing that a correlation exists between glucosuria or hyperglycaemia and an inhibition of the anaphylactoid reaction (Adamkiewicz & Adamkiewicz, 1960; Adamkiewicz & Sacra, 1967) suggest that the extracellular concentration of glucose or a structurally similar carbohydrate is related to antagonism of the reaction.

In the present study, 2-deoxyglucose was the most effective carbohydrate antagonist of the anaphylactoid reaction elicited by egg-white and dextrin in reactor and non-reactor rats and by dextran in reactor rats. 2-Deoxyglucose raises the blood glucose (Landau & Lubs, 1958) and inhibits glucose utilization (Kipnis & Cori, 1959); however, hyperglycaemia does not explain the relative activity of 2-deoxyglucose in inhibiting the egg-white and dextrin reactions in both reactor and non-reactor rats, because glucose is only effective in reactor animals. Görög & Szporny (1965) showed that suppression of glucolysis inhibits the anaphylactoid reaction, and 2-deoxyglucose is active in this respect (Sols & Crane, 1954; Wick, Drury & Morita, 1955; Wick, Drury, Nakado & Wolfe, 1957); such a mechanism may block an energy-dependent process, possibly involving adenosine triphosphate, which normally leads to the release of mediators of the anaphylactoid reaction. The high activity of the amino-sugar *N*-acetylglucosamine as an antagonist of the egg-white and dextrin reaction in non-reactor rats may also depend on such a mechanism, because *N*-acetylglucosamine is a potent inhibitor of glucokinase (Harpur & Quastel, 1949). Recently, 2-deoxyglucose has been found to be a potent diuretic and removal of oedema fluid from the reactive sites represents a possible inhibitory mechanism; this is unlikely, however, because other potent diuretics such as hydrochlorothiazide and theophylline do not inhibit the dextran reaction (Ankier, unpublished results). 2-Deoxyglucose may be potent in inhibiting the anaphylactoid response, as it is an efficient releaser of catecholamines from the adrenal medulla (Brown & Bachrach, 1959; Hökfelt & Bydgeman, 1961), and catecholamines are known to be active in antagonizing the reactions of rats to egg-white (Clark & MacKay, 1949) and dextran (Briot & Halpern, 1952).

Dextrin is equally active in eliciting the anaphylactoid response in both reactor and non-reactor rats (Ankier & West, 1964b) and yet when this glucose polymer is partially hydrolysed anaphylactoid activity remains in non-reactor rats but is lost in reactor rats.

The active component of hydrolysed dextrin is freely dialysable and is therefore of a relatively lower molecular weight than unhydrolysed dextrin (average molecular weight 6,000). This anaphylactoid material may be active only in non-reactor rats, although the possibility exists that hydrolysis of dextrin yields glucose which is an effective inhibitor of the reaction in reactor rats.

Because both dextrin and dextran are branched polymers of glucose and because ovomucoid contains several small carbohydrate side-chains linked to a peptide main-chain (Neuberger & Papkoff, 1963), one of the structural requirements of a molecule for intrinsic anaphylactoid activity may be the presence of side-chain branching. The importance of the molecular weight has been shown with regard to the reactivity of dextran in reactor rats (Harris & West, 1963), and this structural feature may govern distribution

to reactive sites. Dextran of a molecular weight of 4,000, but not of 10,000 or greater, is active in non-reactor rats (Ankier, Starr & West, 1967) so important restrictions in the distribution of injected polysaccharide to active sites in the extracellular spaces, lymphatic system, cell wall membrane or within the cell, may exist in this type of animal.

As on intraperitoneal injection, dextrin when given intradermally is equally active in the two types of rat but glucose locally applied is also equally active in inhibiting this response. This suggests that a similar mechanism of action of intradermal dextrin is involved in both types of rat, a result which is in contrast with that found when the agents are injected intraperitoneally. Furthermore, galactose on intraperitoneal injection is about as active as glucose as an inhibitor of the dextrin reaction in reactor rats and yet on intradermal injection it is much weaker (Poyser & West, 1965). These results show how the processes involved at the different tissue levels after intraperitoneal and intradermal injection may not be similar. It may, however, be easier to apply data obtained from experiments involving intraperitoneal injections to those obtained after intravenous injections; for example, it has been found that both intravenously and intraperitoneally injected glucose inhibit the intravenous dextrin reaction in reactor rats and have no effect on the reaction in non-reactor rats. The character which originally distinguished non-reactor animals was that they did not react to intraperitoneal or intravenous dextran, and yet recent work (Ankier, Starr & West, 1967; Harris, Luscombe & Poyser, 1967) shows that dextrans of low molecular weight are equally active in both types of rat when injected parenterally as well as topically.

SUMMARY

1. 5-Hydroxytryptamine is an important mediator of the anaphylactoid reactions elicited both by egg-white and dextrin in reactor and non-reactor rats and by dextran in reactor rats.

2. Glucose, galactose and mannose prevent the anaphylactoid reactions elicited by egg-white, dextrin and dextran in reactor rats and reduce that produced by egg-white in non-reactor rats; the reaction produced by dextrin in non-reactor animals, however, is not altered. *N*-acetylglucosamine prevents the reactions except that produced by dextrin in non-reactor rats, whereas 2-deoxyglucose is a potent inhibitor of all these anaphylactoid reactions.

3. When dextrin is hydrolysed under certain conditions, the product produces the anaphylactoid reaction in non-reactor rats but not in reactor rats. A polysaccharide of low molecular weight may be formed during the hydrolysis and this is only active in non-reactor rats.

4. The increase in vascular permeability produced by intradermal injections of dextrin in both types of rat is equally prevented by intradermal glucose.

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