Brit. J. Pharmacol. (1963), **20**, 550–562.

RATS RESISTANT TO THE DEXTRAN ANAPHYLACTOID REACTION

BY

J. M. HARRIS AND G. B. WEST

From the Departments of Pharmacology, School of Pharmacy, College of Technology, Brighton, and School of Pharmacy, University of London, Brunswick Square, London, W.C.1

(*Received February* 16, 1963)

In one colony of Wistar albino rats 23% were resistant to dextran and to egg-white, these animals (non-reactors) failing to show an anaphylactoid reaction after the intraperitoneal or intravenous injection of either of the two substances. Non-reactors were also found in a few other colonies of Wistar rats but not in colonies of other strains. Procedures which potentiated the anaphylactoid reaction in sensitive rats (reactors) did not alter the response of non-reactors. Dextran failed to release histamine from the perfused hind-quarters of non-reactors although other chemical liberators of histamine were effective. Lack of anaphylactoid reaction was not due to a deficiency of skin histamine or 5-hydroxytryptamine, and the blood sugar and serum protein levels of non-reactors were also normal. Non-reactors could be sensitized to antigens and their serum was antigenic to guinea-pigs. It is suggested that non-reactors lack a blood or tissue component with which dextran normally combines to produce an intermediate substance active in releasing amines.

The term "anaphylactoid reaction" was used by Selye in 1937 to describe the hyperaemia, pruritus and oedema of the extremities produced by the primary intraperitoneal injection of fresh egg-white into rats. Many years later, a similar reaction was shown to be produced by dextran, a carbohydrate polymer (Voorhees, Baker & Pulaski, 1951; Morrison, Bloom & Richardson, 1951; Morrison, Richardson & Bloom, 1951). Both reactions are enhanced by treatment with thyroxine and by adrenalectomy (Léger & Masson, 1948; Parratt, 1957; Parratt & West, 1960; Spencer & West, 1962); treatment with insulin also enhances the dextran reaction (Adamkiewicz & Langlois, 1957) whereas alloxan-diabetic rats fail to respond (Edlund, Lofgren & Vali, 1952; Goth, Nash, Nagler & Holman, 1957; Adamkiewicz & Adamkiewicz, 1959).

There are reports in the literature that a very small number of rats occasionally fail to respond fully to dextran or egg-white (Léger, Masson & Prado, 1947; Léger & Masson, 1948; Kátó & Gözsy, 1960a, b; Levy & Vaillancourt, 1960), but few details are given. The chance discovery (Harris & West, 1961) that more than one-fifth of the rats secured from one colony failed to react to dextran was the starting point of the present study. Selective breeding experiments have since shown that this character is inherited, non-reactivity being due to an autosomal recessive gene, dx (Harris, Kalmus & West, 1963). In the present paper, a comparison is

made between some biochemical properties of rats which react to dextran and those of rats which do not, but no biochemical property associated with non-reactivity has so far been found.

METHODS

Groups of six Wistar albino rats (body weight 60 to 300 g) from the closed-population colony of the Agricultural Research Council's Field Station at Compton were used in most experiments. These random-bred animals were fed on diet 41B (Associated London Flour Millers), allowed drinking water *ad libitum*, and kept at 21±2° C. A few experiments were carried out with Wistar rats from other colonies and with rats of the August, hooded Lister and Sprague Dawley strains.

Agents used to study the anaphylactoid reaction. Two brands of commercial dextran (Intradex, Glaxo, and Dextraven, Bengers), each with an average molecular weight of 140,000, were injected intravenously or intraperitoneally in doses ranging from 30 to 6,000 mg/kg. Eight other dextrans of different molecular weights were also used in doses of 180 mg/kg. Fresh hen or duck egg-white (50% solution in saline) was given in doses of 10 to 20 ml./kg, ovomucoid in doses of 12.5 to 200 mg/kg, crude egg albumen (British Drug Houses) in doses of 100 to 400 mg/kg, and purified ovalbumin (L. Light) in doses of 100 to 400 mg/kg.

Measurement of oedema formation. In preliminary experiments, the plethysmographic method as described by Buttle, D'Arcy, Howard & Kellett (1957) and modified by Harris & Spencer (1962) was used. Changes in paw volume are detected by this method before they are visible to the naked eye but, as rats have to be anaesthetized before each measurement, it is difficult to make readings frequently.

In later experiments, the degree of swelling in the paws was determined visually and recorded on an arbitrary scale from 0 to +++ (Parratt & West, 1957b). Usually, readings were made every 30 min for 4.5 hr and marks were allotted to each degree of swelling (+=2, ++=4, +++=6). These marks were then added to give the cumulative "shock-score" over this period. The maximum possible score per rat is therefore 54 (9 scores each of 6 marks); on this system, a difference of 20% is within the error of the test.

Test for non-reactivity to dextran. Rats received an intraperitoneal injection of dextran (Intradex, 180 mg/kg), and any failing to respond to this dose in 4.5 hr were tested again with the same dose on two further occasions at weekly intervals. Rats failing to respond to all three doses were designated "non-reactors."

Procedures to potentiate the anaphylactoid reaction. Soluble insulin (4 units/kg intraperitoneally) was used in some experiments together with the dextran. Sodium chlorpropamide (100 mg/kg) was given intraperitoneally. Tri-iodothyronine (Glaxo, 5 mg/kg daily) was injected subcutaneously, usually for 8 days before the dextran.

Adrenalectomy. Bilateral adrenalectomy was performed on rats anaesthetized with ether, using the dorsal approach. Animals were then maintained on 0.9% saline. Sham-adrenalectomized rats were similarly treated.

Sensitivity to histamine. The intravenous toxicity of histamine was determined for groups of ten rats by a slow injection technique using a solution of 20 mg/ml. in saline injected at a constant rate of 0.88 ml./min. The dose required to stop respiration was recorded.

Perfusion of hind-quarters. This was carried out using a modification of the method of Feldberg & Mongar (1954). The rats were killed by a blow on the head and polyethylene cannulae were placed in the abdominal aorta and the inferior vena cava. The anterior part of the body was then removed and the hind-quarters were placed on a wire grid in the mouth of a large funnel. Oxygenated Locke solution maintained at room temperature was used to wash out the blood from the preparation and then samples of perfusate were collected every 10 min. These were assayed immediately for their histamine content using the guinea-pig isolated ileum.

Extraction of blood and tissue histamine. Blood samples taken from the abdominal aorta of rats were mixed with an equal volume of trichloroacetic acid solution (10% w/v) and left in the refrigerator for 18 hr. After centrifugation, aliquots of the supernatant fluid were shaken four times with four volumes of ether. The ether layers were discarded and the aqueous extracts were warmed, neutralized and assayed for histamine using the guinea-pig isolated ileum. Tissue samples were extracted with trichloroacetic acid solution (5 ml./g of tissue) and treated similarly.

Extraction of tissue 5-hydroxytryptamine. Tissues were extracted with acetone using the method of Parratt & West (1957a).

Assay procedures for histamine and 5-hydroxytryptamine. Histamine was assayed on the isolated ileum of the guinea-pig, and 5-hydroxytryptamine on the isolated uterus of the rat (Parratt & West, 1957a). Each value of histamine and 5-hydroxytryptamine refers to the base. Statistical analysis showed that values differing from the means by more than 25% were significant ($P<0.05$).

Estimation of blood sugar. This was estimated by the method of Hagedorn & Jensen (1923) using blood from the abdominal aorta.

Serum proteins. Electrophoretic patterns of serum samples were prepared using cellulose acetate strips at *p*H 8.6 (0.05 M-barbitone buffer). A current of 0.5 mA/cm width of strip was used. The amount of serum applied was 3 to 5 μl., and the running time was 90 min. The strips were stained with Ponceau S and washed with 2% acetic acid. After drying and clearing with Dekalin, the strips were evaluated by scanning.

Production and testing of anaphylatoxin. Serum samples were incubated with powdered dextran (molecular weight, 180,000; 5 mg/ml. of serum) for 1 hr at 37° C (Giertz, Hahn, Opferkuch & Schmutzler, 1961). Aliquots of 1 ml. were then injected intracardially into male guinea-pigs weighing about 500 g.

Production of anaphylaxis in guinea-pigs. Guinea-pigs were sensitized with 1 ml. of rat serum given intracardially, and after 21 days were challenged with 1 ml. of the same serum given intracardially.

Production of anaphylaxis in rats. Groups of ten rats were sensitized with 1 ml. of horse serum and 0.25 ml. of *Haemophilus pertussis* vaccine (80,000 million organisms/ml.). Seven to ten days later, they were challenged with either 0.5 or 1.0 ml. of horse serum given intravenously.

RESULTS

Testing for non-reactivity to dextran. The results of injecting twenty-four male rats with single intraperitoneal doses of 30, 120 and 480 mg/kg of dextran (Intradex) once a week for three consecutive weeks are shown in Table 1. The doses were

TABLE 1

INDIVIDUAL "SHOCK-SCORES" OF TWENTY-FOUR RATS (THREE GROUPS OF EIGHT) GIVEN DIFFERENT DOSES OF DEXTRAN EACH WEEK

Group	Week	Dose (mg/kg)	Shock-score of rat 1	2	3	4	5	6	7	8	Number of rats failing to react
A	1	480	40	44	40	0	0	0	34	0	4
	2	120	36	46	28	0	0	0	26	0	4
	3	30	0	12	0	0	0	0	0	0	7
B	1	30	0	0	0	19	28	0	0	10	5
	2	480	0	0	42	27	46	0	30	32	3
	3	120	0	0	38	42	32	0	32	24	3
C	1	120	24	30	36	48	46	0	40	4	1
	2	30	0	32	0	38	0	0	0	0	6
	3	480	16	34	38	38	42	0	48	42	1

arranged in a randomized fashion using three groups each of eight rats. In group A, four failed to react to any dose ; in group B, three failed and in group C one rat failed to react. The total of non-reactors was therefore eight, although eighteen had failed to respond to the lowest dose of dextran. In general, the shock-scores of reactor rats receiving 120 mg/kg were lower than those of the same rats receiving 480 mg/kg, and the dose/response curve for dextran was next determined (Fig. 1). The optimal dose was 180 mg/kg, and doses higher than this did not produce an increased response. Therefore, in subsequent work, intraperitoneal doses of 180 mg/kg were used for testing rats for non-reactivity to dextran.

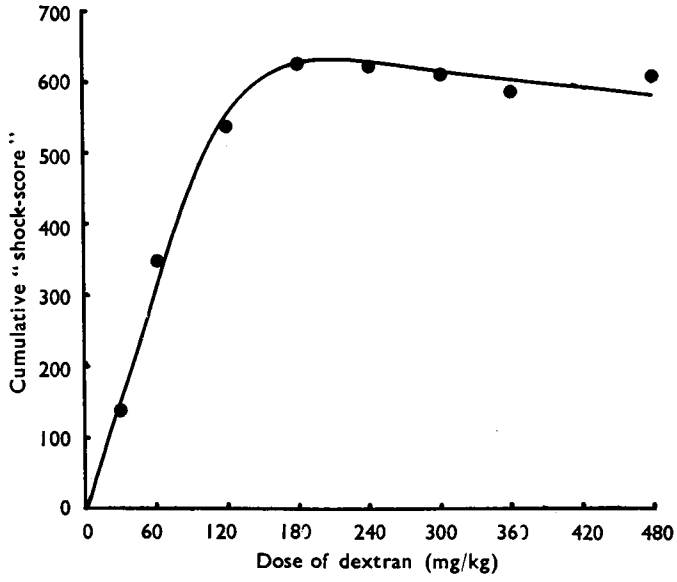

Fig. 1. Dose/response curve for intraperitoneal dextran in male rats. Each point gives the cumulative " shock-score " (ordinate) of eighteen animals. Note that the maximal " shock-score " is obtained by using doses (abscissa) of about 180 mg/kg.

The results of testing 897 male and 688 female random-bred rats with dextran (Intradex) are shown in Table 2. The percentage of non-reactors was similar in both sexes (23%) and did not vary much from year to year, although the results in different tests using small groups of rats ranged from 12.3 to 37.9%. The second brand of dextran (Dextraven) was also ineffective in the non-reactors.

When the dextrans were given intravenously, oedema developed more rapidly in reactor rats than after an intraperitoneal dose, and conspicuous blueing developed when the rats were given Evan's blue dye simultaneously with the intravenous dextran. When non-reactors were similarly treated, neither oedema nor blueing developed, although conspicuous reddening of the extremities was occasionally noted. Doses of dextran as high as 6 g/kg were also ineffective in producing oedema and blueing in non-reactors.

TABLE 2

THE PERCENTAGES OF NON-REACTOR RATS IN GROUPS OF ANIMALS FROM THE CLOSED-POPULATION WISTAR COLONY AT COMPTON

Year	Month	Male rats		Female rats	
		Number tested	Non-reactors (%)	Number tested	Non-reactors (%)
1959	Dec.	24	33·3	12	25·0
1960	Feb.	18	16·6	—	—
	Mar.	86	18·7	—	—
	Apr.	80	18·8	—	—
	May	—	—	48	20·8
	June	194	27·8	—	—
	July	48	18·8	—	—
	Sep.	140	12·3	—	—
	Oct.	64	15·6	—	—
	Nov.	131	31·2	119	14·2
1961	Feb.	5	20·0	108	23·0
	Mar.	—	—	100	23·0
	May	—	—	100	31·0
	June	34	23·5	—	—
	Dec.	—	—	20	25·0
1962	Feb.	—	—	29	37·9
	June	—	—	54	25·9
	July	49	26·0	50	28·0
	Sep.	—	—	48	20·8
	Oct.	24	25·0	—	—
Totals		897	23·3	688	23·4

Experiments with dextrans of different molecular weights. The results are shown in Table 3. Most samples gave some oedema formation in reactor rats but all were ineffective in non-reactors.

Experiments with egg-white. Rats which reacted to dextran always gave the anaphylactoid reaction to fresh hen or duck egg-white, whereas rats in which there

TABLE 3

MEAN " SHOCK-SCORES " OF GROUPS OF SIX REACTOR AND NON-REACTOR RATS GIVEN DEXTRANS OF DIFFERENT MOLECULAR WEIGHTS

Standard dose was 180 mg/kg, given intraperitoneally

Dextran sample		Mean " shock-score "	
Intrinsic viscosity (η)	Approximate molecular weight	Reactors	Non-reactors
0·05	4,000	0	0
0·09	25,000	21	0
0·18	40,000	20	0
0·26	97,000	41	0
0·33	180,000	37	0
0·43	258,000	32	0
0·68	1,780,000	34	0
1 to 2	20,000,000	6	0

was no reaction to dextran never responded to egg-white. Similarly the fractions of egg-white, ovomucoid and crude albumen, injected either intravenously or intraperitoneally, were effective only in reactor rats (Table 4). In addition, purified ovalbumin caused no oedema either in reactors or in non-reactors.

TABLE 4

MEAN " SHOCK-SCORES " OF GROUPS OF SIX REACTOR AND NON-REACTOR RATS GIVEN VARIOUS FRACTIONS OF EGG-WHITE

Fraction	Route of injection	Dose (mg/kg)	Mean " shock-score "	
			Reactors	Non-reactors
Crude egg albumen	Intravenous	100	23	0
	Intraperitoneal	400	13	0
Purified ovalbumin	Intravenous	100	0	0
	Intraperitoneal	400	0	0
Ovomucoid	Intravenous	12·5	21	0
		25	28	0
		50	37	0
	Intraperitoneal	50	18	0
		100	26	0
		200	32	0

Procedures which potentiate the anaphylactoid reaction. Insulin, chlorpropamide and tri-iodothyronine each potentiated the response to dextran in reactor rats, markedly increasing the rate of onset of oedema and sometimes producing death, but non-reactors still failed to respond to dextran after any of these treatments. The magnitude of the potentiation brought about by insulin is shown in Fig. 2.

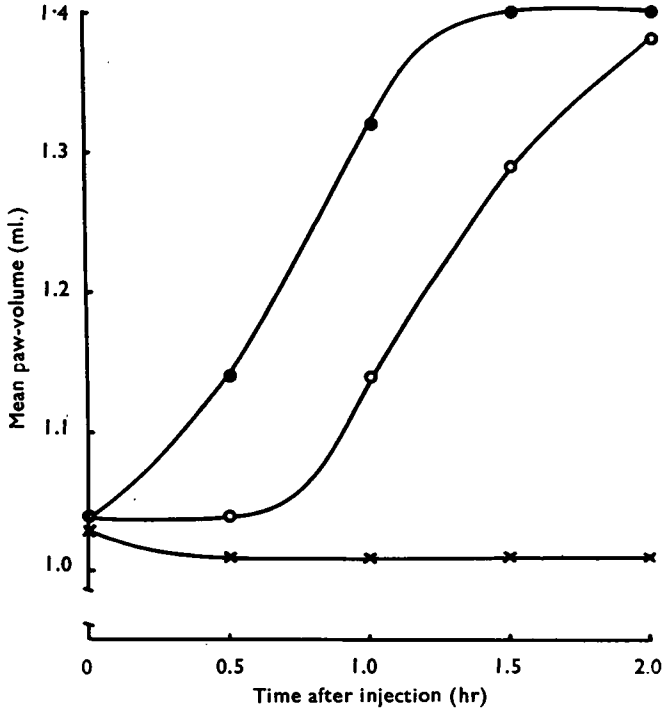

Fig. 2. The mean paw-volume of rats. Comparison of the effects of insulin (4 U/kg) and dextran (180 mg/kg) in reactor rats (● —— ●) and non-reactor rats (x——x). The effect of saline and dextran (180 mg/kg) in reactor rats is also shown (o —— o).

Adrenalectomy increased the severity of the anaphylactoid reaction to dextran in reactor rats and many died after the injection (Table 5). Adrenalectomized non-reactors did not respond to dextran, and also failed to respond when insulin was given with the dextran 13 days after the operation. There was no significant difference between the adrenal weights of reactor rats (34.1 ± 1.9 mg, for both adrenals) and those of non-reactors (38.2 ± 2.9 mg).

TABLE 5

EFFECT OF ADRENALECTOMY ON THE DEXTRAN REACTION AS MEASURED BY MEAN "SHOCK-SCORES" AND PERCENTAGE MORTALITIES IN THE TWO TYPES OF RAT

Groups of six rats were used for each observation

Type of rat	Treatment	Time after operation (days)	Mean "shock-score"	Mortality (%)
Reactors	Adrenalectomy	6	54	66
		13	54	33
	Sham-adrenalectomy	6	38	0
		13	32	0
Non-reactors	Adrenalectomy	6	0	0
		13	0	0

Sensitivity to histamine. The lethal dose of histamine in reactor rats was 399.7 ± 33.1 mg/kg (mean $\pm$ s.e.) and in non-reactors it was 407.0 ± 34.7 mg/kg.

Perfusion of hindquarters. Dextran (60 mg) injected into the abdominal aorta of four reactor rats released an average of 2.6 μg of histamine from the hindquarters in 30 min. Polymyxin B (100 μg) released 8.9 μg from these rats in a similar time when given after the dextran. In four non-reactors, dextran failed to release any histamine yet polymyxin B was again effective, releasing an average of 9 μg of histamine.

Blood histamine. There was a marked rise (about ten-fold) in the blood histamine levels of reactor rats after intravenous dextran (control value 0.2 μg/ml.), and this rise lasted more than 2 hr. In contrast, intravenous dextran failed to change the blood histamine levels of non-reactors (control value 0.18 μg/ml.), even when such animals had been previously treated with insulin or tri-iodothyronine.

Tissue histamine and 5-hydroxytryptamine. The histamine and 5-hydroxytryptamine contents of three tissues (ears, skin of the feet and abdominal skin) of the two types of rat are shown in Table 6. The levels of these amines in non-reactors were always higher than in reactors, indicating that the failure to respond to dextran was in no way due to a deficiency of histamine or of 5-hydroxytryptamine in the skin.

Blood sugar. There was no difference between the blood sugar levels in reactor rats (106.5 ± 2.4 mg/100 ml.) and those in non-reactors (106.0 ± 3.0 mg/100 ml.). Thus non-reactivity is not the result of a diabetic state; this conclusion is supported by the absence of glycosuria.

Serum proteins. The total serum protein of non-reactor rats (range 5.9 to 7.5 g/100 ml.) did not differ from that of reactors (range 5.6 to 6.6 g/100 ml.). The

individual serum protein fractions, estimated as percentages of the totals, were also similar (Table 7).

Anaphylatoxin formation. The production of anaphylatoxin was possible with serum samples from both reactor and non-reactor rats. The dextran-serum incubates killed four out of six guinea-pigs in each instance.

TABLE 6

HISTAMINE AND 5-HYDROXYTRYPTAMINE CONTENTS OF TISSUES OF GROUPS OF SIX REACTOR AND NON-REACTOR RATS

	Histamine (μg/g)		5-Hydroxytryptamine (μg/g)	
Tissue	Reactors	Non-reactors	Reactors	Non-reactors
Ears	26·2	37·5	0·68	0·79
Skin of the feet	47·5	65·3	0·83	0·90
Abdominal skin	36·6	40·7	2·18	2·21

TABLE 7

SERUM PROTEINS OF REACTOR AND NON-REACTOR RATS, CALCULATED AS PERCENTAGES OF THE TOTAL PROTEINS

Values are the ranges for groups of five rats

Serum protein	Reactors	Non-reactors
Albumin	46–63	52–58
α_1-Globulin	8–13	9–11
α_2-Globulin	7–10	8–10
β-Globulin	11–18	13–22
γ-Globulin	8–15	5–15

TABLE 8

THE PERCENTAGE OF NON-REACTOR RATS IN GROUPS OF ANIMALS OBTAINED FROM VARIOUS COLONIES

Where colonies are listed in the Laboratory Animals Centre " Catalogue of Uniform Strains " the page reference is given in parentheses

Strain of rat	Colony	Mating regimen	Number tested	Non-reactors (%)
Wistar	Agricultural Research Council Field Station, Compton	Closed-population	1,585	23·4
	Agricultural Research Council Field Station, Compton (189d)	Brother–sister	134	100
	Glaxo Laboratories, Greenford (246a)	Brother–sister	55	0
	Smith, Kline & French, Welwyn Garden City	Brother–sister	40	17·5
	London School of Hygiene and Tropical Medicine	Brother–sister	132	100
	Institute of Psychiatry, London (163a, f, g)	Brother–sister	24	0
	Messrs Tuck, Rayleigh	Closed-population	130	0
	Boots Pure Drug Co., Nottingham	Closed-population	20	0
	Wellcome Foundation, London	Closed-population	40	0
	Liverpool School of Pharmacy	Brother–sister	12	0
August	Chester Beatty Institute, Pollards Wood Research Station (568b)	Brother–sister	107	0
	Alderwoods, London	—	24	0
Hooded Lister	Agricultural Research Council Field Station, Compton (189c)	Brother–sister	36	0
	A.S.L. Farms, London	Closed-population	100	0
Sprague Dawley	A.S.L. Farms, London	Closed-population	124	0
	Bengers, Holmes Chapel	Closed-population	10	0

Fig. 3. Comparison of the effects of an intraperitoneal injection of dextran (180 mg/kg) in a hooded Lister rat (left) and in an animal of similar fur colour from the pure non-reactor strain bred in this laboratory (right). Note that the animal on the right is resistant to the effects of dextran, showing no oedema of the paws and snout.

Anaphylaxis in guinea-pigs. The sera of reactor and non-reactor rats were equally effective as antigens in groups of four guinea-pigs, death on challenge always occurring within 3 min.

Anaphylaxis in rats. When the challenging dose of antigen was 1 ml., all rats died although the survival times of non-reactor rats (about 45 min) exceeded those of reactors (about 30 min). Using 0.5 ml. of antigen, only 50% of the rats of each type

died within 24 hr, and of these the non-reactors again survived longer than the reactors.

Testing for non-reactivity to dextran in other rat colonies. The percentage of non-reactors in groups of rats obtained from colonies other than the closed-population colony of the Agricultural Research Council's Field Station is shown in Table 8. Non-reactivity to dextran was present only in the Wistar strain and then only in a few of the sublines tested. The August, hooded Lister and Sprague Dawley colonies that were tested contained no non-reactors. However, by crossing Wistar non-reactors with hooded Lister reactors and selectively mating the offspring, a line of non-reactors which outwardly resemble hooded Lister rats has been established (Fig. 3). This line is genetically pure for non-reactivity, since all subsequent matings of animals from this line have yielded only non-reactors.

DISCUSSION

The present experiments show that in a colony of Wistar rats some animals, " reactors," responded to dextran while others, " non-reactors," showed no response. The classification into reactors and non-reactors was made by means of a simple test in which three injections of dextran were given at weekly intervals and the occurrence of oedema observed. The test was usually performed when the animals were at least 3 weeks old and animals then shown to be non-reactors always failed to respond in subsequent tests.

The percentage of non-reactors in male and female rats from the Compton closed-population colony varied from month to month, and this is possibly due to the fact that this colony is sometimes augmented (Moss, personal communication) by additions from the Compton brother-sister colony which is a pure line of non-reactor rats (Harris *et al.*, 1963). The closed-population colony therefore contains homozygote reactors (genotype Dx, Dx), heterozygote reactors (Dx, dx) and homozygote non-reactors (dx, dx), and by selective breeding from this colony pure non-reactor and pure reactor Wistar colonies have been established. Non-reactors have also been found in a few other colonies of Wistar rats but not in some other strains commercially available. However, a pure non-reactor strain of non-albino rats has now been established in this laboratory by outcrossing Wistar non-reactors with hooded Lister or August reactors and breeding selectively from the offspring of the second generation.

The optimal intraperitoneal dose of the standard dextran (Intradex) to produce oedema in the reactor Wistar rats was 180 mg/kg, but doses as high as 6 g/kg were ineffective in the non-reactors. There was no evidence of a " critical " dose with this sample of dextran, as suggested by Kátó & Gözsy (1960b). It complied with the requirements for Dextran Injection B.P. in having an intrinsic viscosity between 0.29 and 0.35 and an average molecular weight of 140,000, although small quantities of dextrans of higher and lower molecular weights were also present. Dextrans of average molecular weights other than 140,000 were effective in reactors but none produced oedema in non-reactors. The dextran samples of molecular weight between 97,000 and 1,780,000 were the most active, a result which agrees with that of Halpern (1956).

The results with fresh egg-white were similar to those with dextran, non-reactors again being resistant even to ovomucoid, the active fraction of the egg-white (Léger & Masson, 1948). Crude egg albumen produced oedema in reactor rats but a purified freeze-dried ovalbumin did not.

The procedures which potentiate the response of reactor rats to dextran failed to induce a response to dextran in non-reactors. For example, insulin, which potentiates the response to dextran in intact rats (Adamkiewicz & Langlois, 1957) and in spinal rats (Adamkiewicz, Langlois & Poirier, 1958), did not change the resistance of non-reactors to dextran. Similarly the oral hypoglycaemic drug, chlorpropamide, was ineffective in non-reactors but like tolbutamide it potentiated the response to dextran in reactors, probably by releasing insulin (Jasmin & Bois, 1959; Adamkiewicz, Fitko & Fortier, 1960). The procedures which inhibit the reaction to dextran include the production of alloxan-diabetes and previous treatment with glucose (Adamkiewicz & Adamkiewicz, 1959, 1960) or with 2-deoxyglucose (Goth, 1959). However, the non-reactors were not diabetic since their blood-sugar levels were not abnormally high and they had no glycosuria.

Prior treatment with tri-iodothyronine produces a severe reaction to dextran (Spencer & West, 1962), yet this treatment was ineffective in non-reactors. Both cortisone and corticotrophin inhibit the anaphylactoid reaction (Selye, 1949), and therefore hyperactivity of the adrenal glands is a possible cause of non-reactivity. However, the adrenal glands in reactors and non-reactors were similar in size, and adrenalectomy in the non-reactors did not alter their resistance to dextran. Thus hypersecretion of the adrenal glands is unlikely to be the chief mechanism involved.

Both histamine and 5-hydroxytryptamine have been suggested as possible mediators of the anaphylactoid reaction in rats (Rowley & Benditt, 1956), though 5-hydroxytryptamine perhaps plays the more important role (Parratt & West, 1957b). Antagonists of 5-hydroxytryptamine inhibit this reaction, whilst antihistamines are only weak inhibitors. In addition, exogenous 5-hydroxytryptamine is many times more active than histamine in producing oedema in rats (Sparrow & Wilhelm, 1957; Parratt & West, 1958). The present work shows that the skin of non-reactor rats was not deficient either in histamine or in 5-hydroxytryptamine, and these rats were no less sensitive to injected histamine than were the reactors. Although dextran released no histamine from the perfused hind-quarters of the non-reactors, more powerful histamine liberators such as Polymyxin B were effective. Dextran raised the blood histamine levels of reactors but did not alter the levels of non-reactors even when they had been previously treated with insulin or tri-iodothyronine.

An investigation of the sera of reactor and non-reactor rats was carried out, since Archer (1959) suggested the presence of an antibody to dextran and the formation of a potent histamine-releasing substance when dextran was added *in vitro* to a β- and γ-globulin fraction of serum. However, the electrophoretic patterns of the sera and the production of anaphylatoxin in rats of the two types were similar. It was also possible to produce both anaphylaxis in guinea-pigs using non-reactor serum as antigen and anaphylaxis in non-reactor rats using horse serum as antigen.

Tests involving the inhibition of dextran-induced oedema in rats have been described for the quantitative evaluation of antihistamines (Stucki & Thompson,

1958; Kátó & Gözsy, 1960c, 1961) and anti-inflammatory compounds (Setnikar, Salvaterra & Temelcou, 1959). The present results show that, when a strain of rat which contains some non-reactors is used, then by chance the resistant animals may be unequally distributed between the test and control groups. It is important therefore in this work to test all animals for reactivity to dextran before use.

Although histamine and 5-hydroxytryptamine are released when dextran is injected into reactor rats, these two amines are present in the skin of non-reactors but are not released by dextran. It is possible therefore that the combination of dextran with a blood or tissue component necessary to effect the release of amines does not occur in non-reactors. This component may be an enzyme, a metabolic product or an antibody. Since dextran reactivity is closely linked with carbohydrate transport as exemplified by the actions of glucose and insulin and by the recent work of Beraldo, Dias da Silva & Lemos Fernandes (1962) with sugars other than glucose, an abnormal metabolic intermediate may be formed. If an antigen-antibody reaction is involved in the production of dextran oedema—and dextran may be antigenic in man (Kabat & Berg, 1953)—then it is likely that this antibody to dextran is lacking in non-reactor rats. The difference between the reactions to dextran in rats of the two types used in the present study is in any case genetically controlled.

We are grateful to Dr H. Kalmus, of the Galton Laboratory, for much assistance in the genetical work, and to the many people who supplied rats and gave information about their colonies. Some of the dextran samples were kindly supplied by Bengers; ovomucoid by Dr J. Williams, the University of Cambridge; and sodium chlorpropamide by Pfizers. The electrophoretic patterns of the sera were determined in the Department of Clinical Chemistry, Royal Infirmary, Edinburgh, through the kindness of Dr R. Kapeller-Adler. One of us (J. M. H.) wishes to thank the Pharmaceutical Society of Great Britain for an Educational Award.

REFERENCES

ADAMKIEWICZ, V. W. & ADAMKIEWICZ, L. M. (1959). Alloxan diabetes and dextran "anaphylactoid" inflammation. *Amer. J. Physiol.*, **197**, 377–379.

ADAMKIEWICZ, V. W. & ADAMKIEWICZ, L. M. (1960). Glucose and the dextran "anaphylactoid" inflammation. *Amer. J. Physiol.*, **198**, 51–53.

ADAMKIEWICZ, V. W., FITKO, R. J. & FORTIER, A. A. (1960). Hypoglycemic drugs and the dextran "anaphylactoid" inflammation. *Canad. J. Biochem.*, **38**, 823–827.

ADAMKIEWICZ, V. W. & LANGLOIS, Y. L. (1957). Sensitization by insulin to the dextran "anaphylactoid" reaction. *Canad. J. Biochem.*, **35**, 251–256.

ADAMKIEWICZ, V. W., LANGLOIS, Y. L. & POIRIER, L. J. (1958). Sensitization by insulin to dextran anaphylactoid inflammation in spinal rats. *Amer. J. Physiol.*, **195**, 635–638.

ARCHER, G. T. (1959). Release of histamine from rat mast cells by blood treated with dextran. *Nature (Lond.)*, **184**, 1151–1152.

BERALDO, W. T., DIAS DA SILVA, W. & LEMOS FERNANDES, A. D. (1962). Inhibitory effects of carbohydrates on histamine release and mast cell disruption by dextran. *Brit. J. Pharmacol.*, **19**, 405–413.

BUTTLE, G. A. H., D'ARCY, P. F., HOWARD, E. M. & KELLETT, D. N. (1957). Plethysmometric measurement of swelling in the feet of small laboratory animals. *Nature (Lond.)*, **179**, 629.

EDLUND, T., LOFGREN, B. & VALI, L. (1952). Toxicity of dextran in rats. *Nature (Lond.)*, **175**, 125.

FELDBERG, W. & MONGAR, J. L. (1954). Comparison of histamine release by compound 48/80 and octylamine in perfused tissues. *Brit. J. Pharmacol.*, **9**, 197–201.

GIERTZ, H., HAHN, F., OPFERKUCH, W. & SCHMUTZLER, W. (1961). Vergleichende Untersuchungen über den anaphylaktischen Schock und den Anaphylatoxinschock an der isolierten Meerschweinchenlunge. *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.*, **242**, 42–64.

GOTH, A. (1959). Inhibition of anaphylactoid edema in the rat by 2-deoxyglucose. *Amer. J. Physiol.*, **197**, 1056–1058.

GOTH, A., NASH, W. L., NAGLER, M. & HOLMAN, J. (1957). Inhibition of histamine release in experimental diabetes. *Amer. J. Physiol.*, **191**, 25–28.

HAGEDORN, H. C. & JENSEN, B. N. (1923). The microdetermination of blood sugar by means of ferricyanide. *Biochem. Z.*, **135**, 46–58.

HALPERN, B. N. (1956). Histamine release by long chain molecules. In *Ciba Foundation Symposium on Histamine*, ed. WOLSTENHOLME, G. E. W. and O'CONNOR, M., pp. 97–98. London: Churchill.

HARRIS, J. M., KALMUS, H. & WEST, G. B. (1963). Genetical control of the anaphylactoid reaction in rats. *Genet. Res.*, in the press.

HARRIS, J. M. & SPENCER, P. S. J. (1962). A modified plethysmographic apparatus for recording volume changes in the rat paw. *J. Pharm. (Lond.)*, **14**, 464–466.

HARRIS, J. M. & WEST, G. B. (1961). Anaphylactoid reaction in rats. *Nature (Lond.)*, **191**, 399–400.

JASMIN, G. & BOIS, P. (1959). Effect of insulin, fasting and tolbutamide on dextran edema in rats. *Proc. Soc. exp. Biol. (N.Y.)*, **101**, 656–658.

KABAT, E. A. & BERG, D. (1953). Dextran—an antigen in man. *J. Immunol.*, **70**, 514–532.

KÁTÓ, L. & GÖZSY, B. (1960a). Effect of phenothiazine derivatives on dextran-induced edema. *J. Pharmacol. exp. Ther.*, **129**, 231–236.

KÁTÓ, L. & GÖZSY, B. (1960b). Kinetics of edema formation in rats as influenced by critical doses of dextran. *Amer. J. Physiol.*, **199**, 657–660.

KÁTÓ, L. & GÖZSY, B. (1960c). Quantitative evaluation of antihistaminic effects. *Toxicol. appl. Pharmacol.*, **2**, 144–150.

KÁTÓ, L. & GÖZSY, B. (1961). Improved method for quantitative evaluation of drug effects on dextran edema in the rat. *Toxicol. appl. Pharmacol.*, **3**, 145–152.

LÉGER, J. & MASSON, G. M. C. (1948). Studies on eggwhite sensitivity in the rat. *Ann. Allergy*, **6**, 131–143.

LÉGER, J., MASSON, G. & PRADO, J. L. (1947). Hypersensitivity to egg-white in the rat. *Proc. Soc. exp. Biol. (N.Y.)*, **64**, 366–370.

LEVY, S. W. & VAILLANCOURT, DE G. (1960). Effects of inflammation on proteins and enzymes in rat plasma. *Canad. J. Biochem.*, **38**, 575–584.

MORRISON, J. L., BLOOM, W. L. & RICHARDSON, A. P. (1951). Effect of dextran on the rat. *J. Pharmacol. exp. Ther.*, **101**, 27–28.

MORRISON, J. L., RICHARDSON, A. P. & BLOOM, W. L. (1951). Effect of antihistaminic agents on the reaction of the rat to dextran. *Arch. int. Pharmacodyn.*, **88**, 98–105.

PARRATT, J. R. (1957). Thyroxine and histamine release in the rat. *J. Physiol. (Lond.)*, **138**, 51–52*P*.

PARRATT, J. R. & WEST, G. B. (1957a). 5-Hydroxytryptamine and tissue mast cells. *J. Physiol. (Lond.)*, **137**, 169–178.

PARRATT, J. R. & WEST, G. B. (1957b). 5-Hydroxytryptamine and the anaphylactoid reaction in the rat. *J. Physiol. (Lond.)*, **139**, 27–41.

PARRATT, J. R. & WEST, G. B. (1958). Inhibition by various substances of oedema formation in the hind-paw of the rat induced by 5-hydroxytryptamine, histamine, dextran, eggwhite, and compound 48/80. *Brit. J. Pharmacol.*, **13**, 65–70.

PARRATT, J. R. & WEST, G. B. (1960). Hypersensitivity and the thyroid gland. *Int. Arch. Allergy*, **16**, 288–302.

ROWLEY, D. A. & BENDITT, E. P. (1956). 5-Hydroxytryptamine and histamine as mediators of the vascular injury produced by agents which damage mast cells in rats. *J. exp. Med.*, **103**, 399–412.

SELYE, H. (1937). Studies on adaptation. *Endocrinology*, **21**, 169–188.

SELYE, H. (1949). Effect of ACTH and cortisone upon an "anaphylactoid" reaction. *Canad. med. Ass. J.*, **61**, 553–556.

SETNIKAR, I., SALVATERRA, M. & TEMELCOU, O. (1959). Antiphlogistic activity of iproniazid. *Brit. J. Pharmacol.*, **14**, 484–487.

SPARROW, E. M. & WILHELM, D. L. (1957). Species differences in susceptibility to capillary permeability factors: histamine, 5-hydroxytryptamine and compound 48/80. *J. Physiol. (Lond.)*, **137**, 51–65.

SPENCER, P. S. J. & WEST, G. B. (1962). Further observations on the relationship between the thyroid gland and the anaphylactoid reaction in rats. *Int. Arch. Allergy*, **20**, 321–343.

STUCKI, J. C. & THOMPSON, C. R. (1958). A screening procedure for substances which inhibit dextran edema in the rat. *Amer. J. Physiol.*, **193**, 275–282.

VOORHEES, A. B., BAKER, H. J. & PULASKI, E. J. (1951). Reactions of albino rats to injections of dextran. *Proc. Soc. exp. Biol. (N.Y.)*, **76**, 254–256.