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Genetical control of the anaphylactoid reaction in rats

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1. INTRODUCTION

A single injection of dextran into rats produces hyperaemia, pruritus and oedema of the face, ears and paws (Voorhees *et al.*, 1951; Morrison *et al.*, 1951*a*; 1951*b*). This response corresponds to the anaphylactoid reaction produced by egg-white as described by Selye (1937), but differs from anaphylaxis in that prior sensitization to the oedema-producing agent is not required.

In the course of investigating this reaction (Harris & West, 1961) animals were found which completely failed to show anaphylactoid oedema after intraperitoneal or intravenous injection either with dextran or with egg-white (see Plate I).

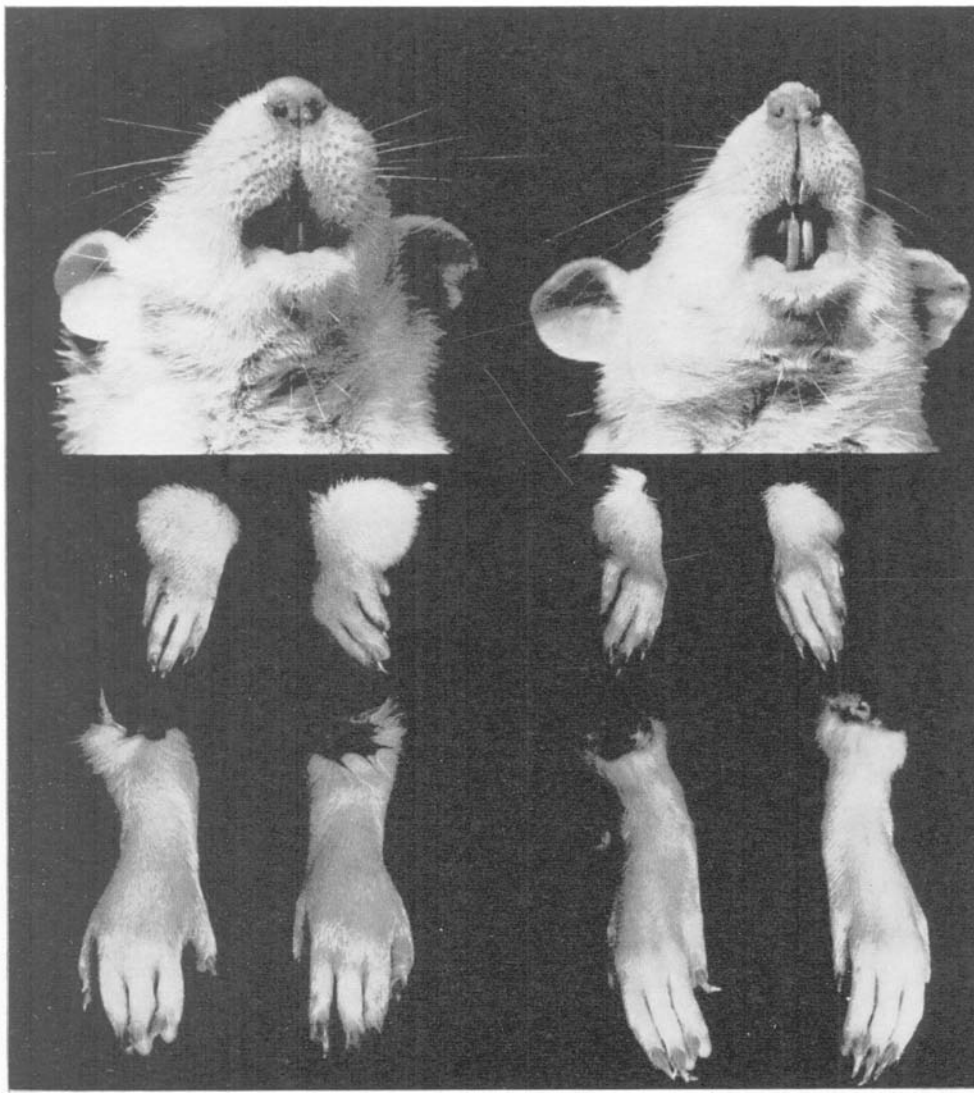
Occasional reports of non-reacting rats can be found in the literature. Thus Léger *et al.* (1947) found that 'the reaction to egg-white can readily be obtained in a high percentage of animals', and in a later paper Léger & Masson (1948) recorded that 25% of animals given egg-white did not show any symptoms of shock nor any oedema, but only hyperaemia. Kátó & Gözsy (1960*a*) using 8200 Sprague-Dawley rats found that one out of 182 animals was a 'non-reactor' or 'poor reactor', whilst in another paper (1960*b*) they state that 'a non-reacting rat is seldom found'. Levy & Vaillancourt (1960) using Wistar rats also noted that a few rats responded to the dextran with obvious reddening of the extremities but without evidence of oedema.

In our material it soon became apparent that this failure to respond (non-reactivity) occurred in some Wistar rats, but never in Lister, Sprague-Dawley or August rats. As we hope to show in this paper, the difference in reactivity is a simple Mendelian one, and is thus another example of a genetical polymorphism revealed by drugs (Kalow, 1962).

2. MATERIALS AND METHODS

Table 1 lists the original stocks of the rats used in the present investigation.

A 6% solution of dextran ('Intradex'—Glaxo) was injected intraperitoneally at a dose of 3 ml./kg. and the animals observed for 4½ hours. Rats failing to react within



Wistar albino rats, 'reactor' (*left*) and 'non-reactor' (*right*), 2 hours after intraperitoneal dextran (180 mg./kg.). Note the absence of swelling in the snout and paws of the non-reactor animal.

this period when tested on three consecutive occasions at weekly intervals were designated as 'non-reactors'. The details of the reaction are recorded in the paper by Harris & West (1963). All tests were carried out on rats at least 3 weeks old.

Table 1. *Original stocks of rats used in this work*

L.A.C. = Laboratory Animals Centre 'Catalogue of uniform strains'

Strain	Description	Colony	L.A.C. Number	L.A.C. Designation
Wistar	Albino	Agricultural Research Council (A.R.C.), Compton, Berkshire	189d	WAG/C
,,	,,	Glaxo Laboratories, Greenford, Middlesex	246a	WAG
,,	,,	Smith, Kline and French (S.K.F.), Welwyn Garden City	—	—
,,	,,	London School of Hygiene and Tropical Medicine	—	—
,,	,,	Wellcome Foundation, London	—	—
,,	,,	Messrs Tuck, Rayleigh, Essex	—	—
,,	,,	Institute of Psychiatry, London	163a	A
Sprague-Dawley	Albino	Animal Supplies (London) Ltd. (A.S.L.), London	—	—
Lister	Hooded (black)	A.R.C. Compton (see above)	189c	PVG/C
,,	,, ,,	Animal Supplies (London) Ltd., London	—	—
August	Hooded (yellow)	Chester Beatty Institute, Pollards Wood Research Station	568b	August

3. RESULTS

(i) *Non-reactors in various strains*

Tables 2*a* and 2*b* show the percentage of non-reactors in the various strains and colonies. Failure to react was observed in the A.R.C. Compton, School of Pharmacy, S.K.F. and School of Hygiene Wistar rats (W1, W2, W3, W5, W6 and W7) and not in any other Wistar colonies or in the August, Lister or Sprague-Dawley strains. Male and female non-reactors occurred at roughly the same proportion.

(ii) *Non-reactors in the A.R.C. Compton closed-population stock (W1)*

The original colony in which we detected non-reactors to dextran was the randomly-bred closed-population colony from the A.R.C. Compton stock. Table 3 shows more detailed results of screening tests performed on rats from this source over the last five years. The percentage of non-reactors has not varied very much from year to year.

(iii) *Breeding from Wistar rats*

Matings between non-reactors always produced only non-reacting offspring and thus a pure strain (W/N) was obtained. Matings between reactors sometimes segregated, suggesting that non-reactivity is recessive. Such segregation is shown

Table 2*a*. *Reactivity in various Wistar colonies*

Source of colony (as in Table 1)	Designation	Mating regimen	Generation tested	Number tested	Non-reactors No.	Non-reactors %
A.R.C. Compton	W1	Closed population (15 years) and additions from colony W2	Unknown	909 ♂ 700 ♀	213 165	23·4 23·6
	W2 (WAG/C)	Brother–sister	F76 to F80	71 ♂ 73 ♀	71 73	100 100
	W3	Brother–sister	F8 to F11	27 ♂ 23 ♀	27 23	100 100
Glaxo	W4 (WAG)	Brother–sister	F76 to F78	30 ♂ 25 ♀	0 0	0 0
	W4a	F1 substrain	*	1 ♂ 23 ♀	0 0	0 0
School of Pharmacy, London	W5	Brother–sister	F78 to F79	10 ♂ 11 ♀	10 11	100 100
S.K.F.	W6	Brother–sister (2 years)	Unknown	20 ♂ 20 ♀	4 3	20 15
London School of Hygiene	W7	Brother–sister	F79	48 ♂ 84 ♀	48 84	100 100
Wellcome	W8	Closed population (12 years)	Unknown	20 ♂ 20 ♀	0 0	0 0
Tuck	W9	Closed population (27 years)	Unknown	110 ♂ 20 ♀	0 0	0 0
Institute of Psychiatry	W10	Brother–sister	F81	3 ♂ 3 ♀	0 0	0 0

* Colony W4 brother-sister mated for breeding stock, sub-strain crossed at random for uniform F1 animals = W4a.

Table 2*b*. *Reactivity in strains other than Wistar*

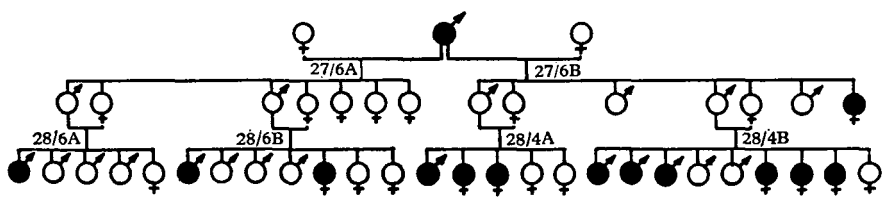
Strain	Source of colony	Designation	Mating regimen	Generation tested	Number tested	Non-reactors No.	Non-reactors %
Sprague-Dawley	A.S.L.	S1	Closed population (4 years)	Unknown	74 ♂ 50 ♀	0 0	0 0
Hooded Lister	A.R.C. Compton	H1 (PVG/C)	Brother–sister	F48 to F50	3 ♂ 3 ♀	0 0	0 0
	A.S.L.	H2	Closed population (2 to 3 years)	Unknown	50 ♂ 50 ♀	0 0	0 0
August	Chester Beatty	A1	Brother–sister	F21	6 ♂ 101 ♀	0 0	0 0

Table 3. *Percentages of non-reactors found among rats obtained from A.R.C. Compton closed-population colony (W1)*, 1959–63.

	Male rats			Female rats		
Year	Total	Non-reactors	% non-reactors	Total	Non-reactors	% non-reactors
1959	18	3	16·6	12	3	25·0
1960	767	178	23·2	167	27	16·2
1961	39	9	23·1	328	82	25·0
1962	73	19	26·0	181	49	27·1
1963	12	4	33·3	12	4	33·3
	909	213	23·4	700	165	23·6

in Text-fig. 1. It was however possible to produce a pure reactor strain (W/R) by selection (Table 4).

Three crosses between animals from the two pure strains produced 22 offspring, all of which were reactors. This agrees with the assumption that non-reactivity is recessive. The reactions of the heterozygotes were in no way different from those of the homozygotes in the W/R parental stock. The results of 10 intercrosses, that is matings between heterozygotes, was 48 reactors to 21 non-reactors; this does not



Text-fig. 1. Mating of a non-reactor male (♂) with a (presumably) homozygous reactor female (27/6A) and a heterozygous reactor female (27/6B). The F2 generations of brother–sister matings are also recorded. Non-reactors are shown as filled circles, reactors as open circles.

Table 4. *Production of a pure reactor line (W/R) and a pure non-reactor line (W/N)*

Parents							
Reactors				Non-reactors			
Generation	No. of brother–sister matings	Reactors	Non-reactors	Generation	No. of brother–sister matings	Reactors	Non-reactors
F1	1	6	0	F1	1	0	6
F2	2	12	0	F2	2	0	14
F3	3	22	0	F3	2	0	14
F4	4	17	0	F4	5	0	37
F5	3	20	0	F5	3	0	29
F6	6	51	0	F6	5	0	36
F7	3	27	0	F7	3	0	20

differ significantly from 3:1. Eleven backcrosses to the non-reactor type produced 36 reactors and 39 non-reactors, whilst 2 backcrosses to the reactor type produced 11 reactors only.

(iv) *Crosses between Wistar non-reactors and reactors from other strains*

Non-reactivity has been bred from Wistar (albino) into Lister (black-hooded, dark-eyed) and August (yellow-hooded, pink-eyed) rats (Table 5) and a pure stock of non-albino, non-reactor rats has been obtained. These results confirm that non-reactivity to dextran is an autosomal recessive character and we propose the symbol *dx* for the gene responsible for the non-reactivity and the symbol *Dx* for the dominant allele.

Table 5. *Outbreeding of non-reactivity into two reactor strains of rat*

	F1			F2		
Cross	Matings	Reactors	Non-reactors	Matings	Reactors	Non-reactors
Wistar (non-reactors) × Lister (reactors)	5	41	0	13	98	31
Wistar (non-reactors) × August (reactors)	3	22	0	8	71	23
Totals	8	63	0	21	169	54

In the intercrosses (F2) summarized in Table 5 and in some backcrosses, certain colour characters as well as the dextran reactivity were found to segregate. Table 6 shows independent 3:1 segregation of non-reactivity and albinism in the reciprocal crosses. Table 7 shows a similar independent segregation of non-reactivity and black

Table 6. *Independent segregation of the albino gene* (c) *and the non-reactor gene* (dx) *in intercrosses (F2) from non-reactor Wistar rats crossed with reactor Lister (black-hooded), or reactor August (yellow-hooded) rats*

Grandparents		Coat colour in F2			
		Albino		Non-albino	
♀	♂	Reactors	Non-reactors	Reactors	Non-reactors
Wistar non-reactors ×	Lister reactors	13	4	44	14
Lister reactors ×	Wistar non-reactors	11	6	30	7
August reactors ×	Wistar non-reactors	6	0	12	5
Wistar non-reactors ×	August reactors	15	4	38	14
	Totals	45	14	124	40
		59		164	

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hood-colour in the Lister crosses, and of non-reactivity and yellow hood-colour in the August crosses.

The entire F1 offspring from crosses between albino Wistar rats and the hooded Lister or August rats were hooded, indicating that all three strains were homozygous for this gene (*hh*), and that its presence in the Wistar stock is masked by the simultaneously present epistatic albino gene in the homozygous form (*cc*). This was borne out by the observation that no non-hooded rats ever occurred among the coloured (non-albino) offspring of later generations. In the absence of segregation for hooded, no linkage tests were possible.

Table 7. *Segregation of the coat colour genes* (Aa *and* Pp *respectively*) *and the reactor genes,* (Dx, dx) *in intercrosses (F2). Note that yellow coat colour is associated with pink eyes. This table is a further analysis of the non-albino animals recorded in Table 6*

Grandparents		Coat colour in F2			
		Reactors		Non-reactors	
		Black	Agouti*	Black	Agouti*
♀	♂				
Wistar non-reactors ×	Lister reactors	11	33	2	12
Lister reactors ×	Wistar non-reactors	7	23	0	7
Totals		18	56	2	19
		Yellow	Agouti*	Yellow	Agouti*
August reactors ×	Wistar non-reactors	5	7	1	4
Wistar non-reactors ×	August reactors	13	25	5	9
Totals		18	32	6	13

* Agouti is the 'wild-type' colour.

The colour of the hood of the F1 animals was agouti, irrespective of the colour of the parents (black Lister or yellow August). In the F2 generations, agouti coat colour segregated against black or yellow respectively. This suggests that the constitution of the parent stocks, when based on comparative studies on coat colour genes by Little (1958) and Deol (1963) is as follows:

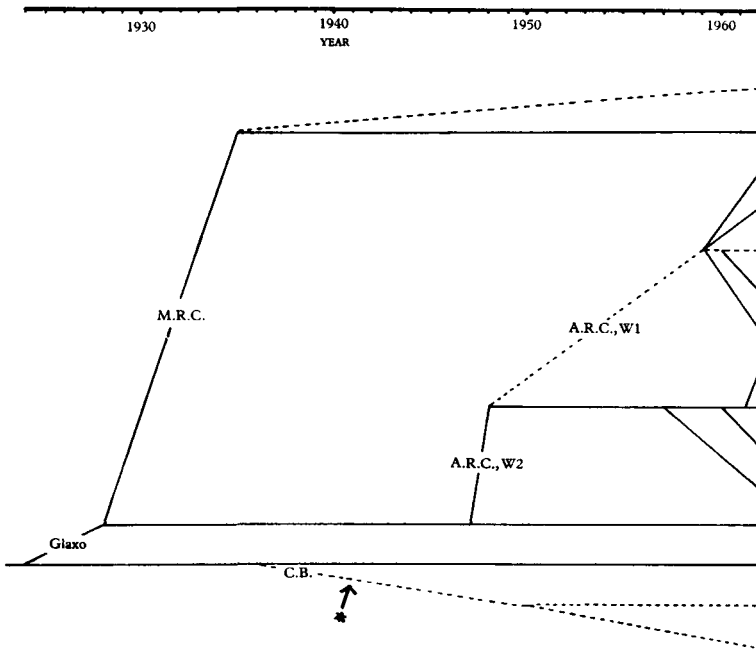
	C-locus (albino)	A-locus (agouti)	P-locus* (pink-eyed yellow)	H-locus (hooded)	Dx-locus (reactivity)
Wistar	*cc*	*AA*	*PP*	*hh*	*dxdx*
Lister	*CC*	*aa*	*PP*	*hh*	*DxDx*
August	*CC*	*AA*	*pp*	*hh*	*DxDx*

* A mimic called red-eyed yellow (r) would produce similar effects.

If this interpretation is accepted then the figures in Table 7 are indicative of independent segregation of *Dx* and *A* in the Lister crosses, and of independent segregation of *Dx* and *P* in the August crosses, and are not compatible with closer linkage.

(v) *History of the 'Wistar' colonies used*

As mentioned above, non-reactors to dextran were found in some of the albino Wistar sub-strains, and not in any other strain of rat tested. In attempting to explain the origin of non-reactivity, it is necessary to reconstruct the history, relationships and breeding regimen of the various Wistar sub-strains that we have used. The breeding procedure tabulated in Table 2 and also shown in Text-fig. 2 was partly derived from the records and partly from conversations with the people responsible for keeping the various stocks. Historical data of this kind are always somewhat uncertain.



COLONY		REACTION TO DEXTRAN
Tuck	W9	R
M.R.C.	—	—
School of Pharmacy	W/R	R
School of Pharmacy	W/N	N
A.R.C.	W1	R+N
S.K.F.	W6	R+N
A.R.C.	W3	N
School of Pharmacy	W5	N
A.R.C.	W2	N
School of Hygiene	W7	N
Institute of Psychiatry	W10	R
Glaxo	W4	R
Wistar Institute	—	—
Chester Beatty	—	—
Wellcome	W8	R

Text-fig. 2. The history, breeding regimen and present-day reactivity to dextran of some 'Wistar' rat colonies used. —— = brother-sister mating, --- = closed population mating, *——→ indicates probable admixture here. R = reactors, N = non-reactors, R + N = both reactors and non-reactors.

In 1924 eight rats obtained from the Wistar Institute in the U.S.A. were used to found a colony at Glaxo Laboratories, Greenford—the WAG stock (our designation W4)—and this has now been inbred to 78 generations by brother–sister mating. Many of the colonies tested by us have been derived from the WAG line via the WAG/C brother–sister mated line started in 1947 at the A.R.C. Field Station at Compton. This line (W2) was at F42 when founded at Compton and is now at F80. Two other Wistar stocks (W1 and W3) are also kept at Compton. W1 is a closed-population colony originally founded in 1948 with rats from the W2 line, and usually kept at a strength of several hundred individuals but occasionally some additions

are made to it, again from the brother–sister line W2. Another brother–sister line (W3) has recently been started with rats from this closed-population stock and is now at F11. Our pure reactor (W/R) and pure non-reactor (W/N) lines were produced by selective breeding from the W1 stock.

Our brother–sister colony W5 was started with rats from F77 of the A.R.C. W2 stock, and the Institute of Psychiatry colony (W10) was founded in January 1957 with rats from F68 of this same W2 stock (W10 has been brother–sister mated ever since and is now at F81). The London School of Hygiene rats (W7) were obtained from A.R.C. Compton at F75 and are still brother–sister mated (now at F79). The S.K.F. colony (W6) was derived from the closed-population A.R.C. Compton stock but has been brother–sister mated since its foundation in 1960.

The Tuck colony (W9) is a closed-population colony derived from Glaxo via the Medical Research Council, Hampstead, stock. It was obtained by M.R.C. in 1927/28 and the Tuck sub-line started in about 1935.

The Wistar colony W8 was from the Wellcome Laboratories. This was derived from rats brought over from the Wistar Institute to the Chester Beatty Institute in the late 1930's. The line was maintained as a closed-population colony for some years but in 1942 had to be evacuated for the duration of the war. When re-established at the Chester Beatty Institute after 1945 some black-coloured animals appeared in the offspring. This colour was bred out and the colony is now albino but is called the Chester Beatty (CB) strain because it is not pure Wistar. The Wellcome colony was founded with rats obtained from Chester Beatty (CB) in March 1950 and is now bred as a closed-population.

(vi) *Origin and maintenance of non-reactivity*

Non-reactor rats only occurred in stocks derived from the A.R.C. Compton colonies, and five of these lines were pure non-reactors. Two each are kept at A.R.C. Compton and the School of Pharmacy and the fifth is at the London School of Hygiene. All of these are propagated by brother–sister matings. However, 6 animals derived from a sub-strain of W2 kept at the Institute of Psychiatry (W10) were all reactors.

The A.R.C. Compton closed-population line (W1) contains reactors and non-reactors. A similar mixture is maintained in rats in a sub-line (W6) kept at S.K.F. but now brother–sister mated.

As non-reactors only occurred in lines derived from A.R.C. Compton, the spread of non-reactivity must have started in one of these colonies, either from a new or old mutation, or from the chance introduction by contamination. That in fact mutations occur spontaneously in laboratory-kept rodents has been shown by the work of Deol *et al.* (1960) and Grewal (1962), who found that mouse sub-lines from pure stocks separated some time ago tended to differ in some hereditary metrical characters.

We know that W1 was from time to time reinforced by animals from W2 (presumably non-reactors) and thus the situation can be explained by assuming that the mutation arose in W2 at some time, perhaps since W10 was separated (i.e.

within the last 6 years). On the other hand, it is possible that it had arisen much earlier in W2. Whichever assumption is accepted, it can be seen that the reactivity has been completely lost in W2, W5 and W7, whilst the majority type of rats in W10 were reactors.

The existence of both non-reactor (W3) and mixed (W6) lines in the colonies derived from the mixed A.R.C. Compton W1 stock which itself consists of several hundred rats bred in a closed-population might also be explained by chance fixation. On the other hand the persistence of both alleles after about 6 generations of brother–sister mating in W6 may possibly be indicative of selection against the homozygotes.

4. SUMMARY

1. A recessive autosomal gene, *dx*, preventing the anaphylactoid reaction has been discovered in a stock of Wistar albino rats from the Agricultural Research Council, Compton.

2. Segregation occurred in the F2 generation derived from crossing pure-bred reactor rats and pure-bred non-reactor rats.

3. Non-reactivity was outbred into non-albino stocks and was shown to segregate independently of the albino gene *c* and not to be closely linked to the colour genes, *P* and *A*.

4. The mutation from *Dx* to *dx* may have arisen a few years ago in one of the Wistar colonies before it was split into sub-lines.

5. The *dx* gene is fixed in several brother–sister mated sub-lines and appears to be in equilibrium with *Dx* in some less inbred closed-populations.

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