Genet. Res., Camb. (1982), **40**, *pp.* 59–64
With 2 text-figures
Printed in Great Britain

Multiple allelism at the locus controlling warfarin resistance in the Norway rat

By J. H. GREAVES and P. B. AYRES

Tolworth Laboratory, Ministry of Agriculture, Fisheries and Food, Hook Rise South, Tolworth, Surrey KT6 7NF

(*Received 9 October 1981 and in revised form 6 January 1982*)

SUMMARY

Two warfarin-resistant strains of the Norway rat, *Rattus norvegicus*, derived independently from wild populations in Wales and Scotland and both homozygous for a major gene at the warfarin-resistance locus, *Rw*, were found to differ in their hypoprothrombinaemic response to simultaneous dosage with warfarin and vitamin K. The Welsh strain gave a small response and the Scottish strain a large response. These two response levels segregated in Mendelian fashion in various crosses involving the two resistant strains and a third, non-resistant strain. This indicates that the *Rw* locus has a series of three multiple alleles, denoted Rw^{w}, Rw^{s} and Rw^{+}. The results are discussed briefly in relation to biochemical, ecological and evolutionary aspects of warfarin resistance.

1. INTRODUCTION

It has been observed that strains of the Norway rat, *Rattus norvegicus*, derived from different wild populations polymorphic for resistance to the anticoagulant rodenticide, warfarin, show characteristic differences in the expression of the resistance, even though in each strain it appears to be controlled by a gene at the *Rw* locus. Thus, a strain of Welsh origin, when compared with a strain from Scotland shows greater resistance to warfarin, a different spectrum of resistance to other related anticoagulants and relatively high susceptibility to vitamin K deficiency (Greaves & Ayres, 1973, 1976; Martin, 1973). A third strain, from Denmark, has points of resemblance with both the first two strains (Greaves & Ayres, 1977) whilst a fourth strain, from Hampshire, shows a uniquely high resistance to the anticoagulant, difenacoum (Redfern & Gill, 1978).

These differences suggest either that different combinations of modifiers affect the expression of the same resistance gene to a varying extent in different strains, or that the *Rw* locus has multiple alleles, each allele having a distinctive effect upon the response to anticoagulants and vitamin K, and each strain carrying a different allele. To distinguish between these two possibilities it is necessary to show either that interbreeding between two strains erodes the difference between them, as

would be predicted from the modifier hypothesis or, alternatively, that the putatively different forms of resistance segregate from each other in Mendelian ratios as would be expected if they were due to different alleles. A difficulty in performing this type of experiment has been that, though mean interstrain differences are demonstrable, no established technique classifies individual animals with the precision required to demonstrate genetic segregation.

In this paper we report that a technique devised by O'Reilly (1971) to study the effect of vitamin K in preventing the hypoprothrombinaemic response to warfarin discriminates well between the Welsh and Scottish strains of warfarin-resistant rat. By applying the technique to the offspring of various crosses involving the two strains it is shown that the two forms of resistance segregate as required by the multiple allele hypothesis.

2. METHODS

Three parental strains of rats featured in the experiments: (1) a warfarin-resistant strain homozygous for the Welsh-derived resistance allele, Rw^{w}; (2) a resistant strain homozygous for the putative Scottish allele, Rw^{s}, and (3) a warfarin-susceptible Wistar stock, genotype Rw^{+} Rw^{+}. The alleles are denoted by letters rather than the previously employed numerical superscripts in order to conform to current genetical nomenclature rules for the rat (Festing, 1978). The three strains are closely related, the two resistant strains having been established after at least four generations of backcrossing to the Wistar stock.

The study was made in two stages. First, the parental strains and the offspring of the three crosses between them were tested to assess the ability of the technique employed to discriminate among animals of different resistance genotypes, and to establish reference levels of phenotypic response. Second, in order to study transmission of the two forms of resistance, four crosses were made with Welsh/Scottish heterozygotes (putative genotype Rw^{w} Rw^{s}) including the intercross, backcrosses to the two parental strains and an outcross to the warfarin-susceptible, Wistar strain. The rats were maintained in conventional animal house conditions with unlimited access to diet 41B and, except when under test, to tap water.

For testing, each animal was caged singly and received 250 ppm of warfarin sodium and 10 ppm of vitamin K_1 in the drinking water for 48 h. The average quantity of solution consumed during the 48 h was 17·0 g $\pm$ S.E. 0·2 g per 100 g of body weight, corresponding to mean dosages of 4·25 mg/100 g of warfarin sodium and 0·17 mg/100 g of vitamin K_1. At the end of the 48 h a blood sample was taken from the tail vein, under light ether anaesthesia, with a plastic syringe and 25 gauge (0·5 mm) needle and immediately mixed with 3·13 % sodium citrate solution. The one-stage blood clotting time was then measured with Thrombotest reagent and an automatic coagulation timer. The test is sensitive to deficiencies of the warfarin-sensitive, vitamin K – dependent clotting factors. The clotting times of untreated rats generally range up to about 30 s in this test.

3. RESULTS

The clotting times recorded for rats of the three parental strains and the offspring of crosses between them are summarized in Figure 1. Of the parental strains, the Welsh (Fig. 1 A) gave the smallest response with times all 35 s or less, as compared with the Wistar susceptibles (Fig. 1 C) whose times all exceeded 100 s. The Scottish strain (Fig. 1 B) was intermediate and more variable in response,

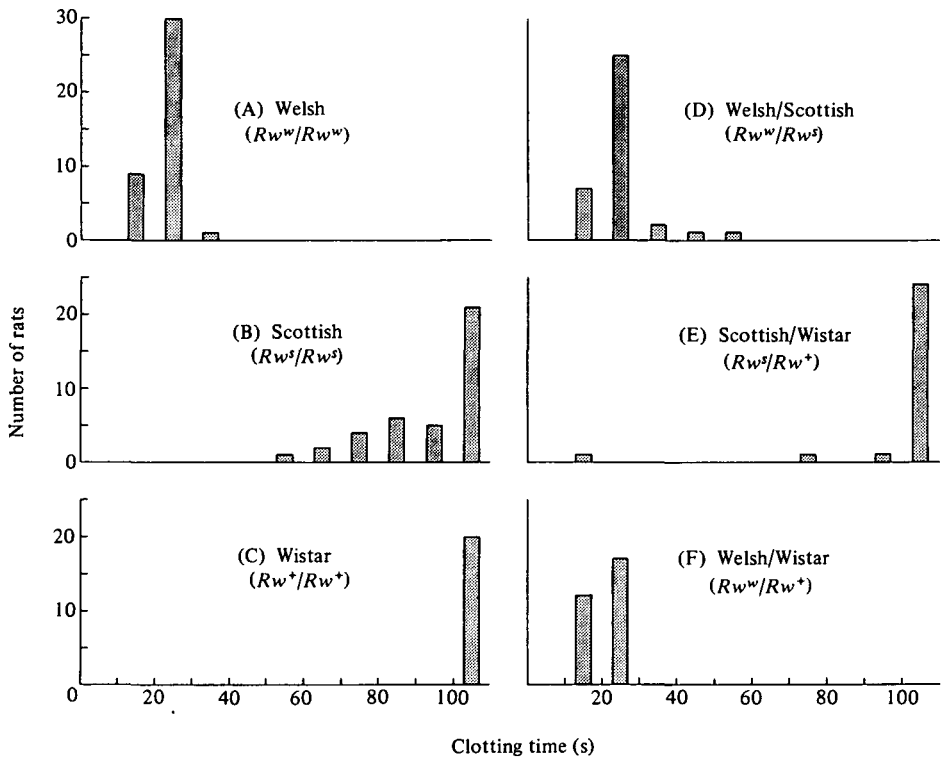

Fig. 1. Distribution of clotting times of rats after receiving warfarin sodium (250 ppm) and vitamin K_1 (10 ppm) in their drinking water for 48 h.

overlapping substantially with the Wistar, but not with the Welsh strain. A slightly more variable pattern of response is apparent in the offspring of the crosses between the strains. It can be seen, however, that the Welsh × Scottish (Fig. 1 D) and Welsh × Wistar (Fig. 1 F) offspring had generally much shorter clotting times than the offspring of the Scottish × Wistar (Fig. 1 E) cross. A cut-off time of 50 s correctly identifies 104/105 carriers of the Welsh allele and 85/86 non-carriers. These results indicate that, under the conditions of the experiment, Welsh-type resistance is dominant to the Scottish-type resistance and to susceptibility, and that the Scottish type is almost recessive to susceptibility. The recessivity of the

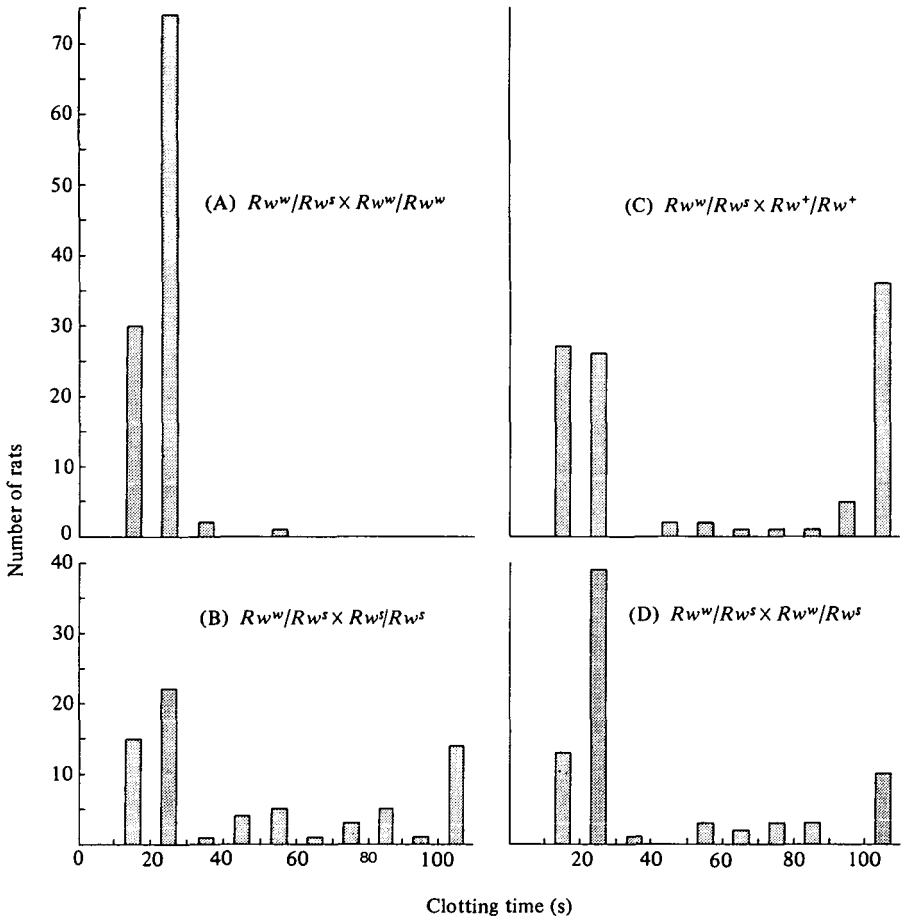

Fig. 2. Distribution of clotting times of rats after receiving warfarin sodium (250 ppm) and vitamin K_1 (10 ppm) in their drinking water for 48 h. The rats are the offspring of Welsh/Scottish heterozygotes in the following crosses: A, backcross to the Welsh strain; B, backcross to the Scottish strain; C, outcross to the Wistar strain; D, intercross.

Table 1. *Single factor ratios in the offspring of Welsh/Scottish heterozygotes*

	Offspring			
Cross	Numbers with clotting times $\geqslant 50$ s/< 50 s	Expected ratio	Chi squared	*P*
Intercross $Rw^w/Rw^s \times Rw^w/Rw^s$	21/53	1:3	0·29	0·5–0·7
Outcross $Rw^w/Rw^s \times Rw^+/Rw^+$	46/55	1:1	0·64	0·3–0·5
Backcross $Rw^w/Rw^s \times Rw^s/Rw^s$	29/42	1:1	2·02	0·1–0·2

https://doi.org/10.1017/S0016672300018905 Published online by Cambridge University Press

Scottish type, as against its apparent dominance in a previous study (Greaves & Ayres, 1976) is due to the use here of a larger dose of warfarin; this neatly illustrates how the environment may determine whether a gene is dominant or recessive in expression. The small response of carriers of the Welsh allele confirms the observation of O'Reilly (1971) that Welsh-type resistance produces an increased sensitivity to the antidotal effect of vitamin K on warfarin-induced hypoprothrombinaemia.

The results for progenies in which evidence of segregation of the Welsh and Scottish alleles was sought are shown in Fig. 2. As expected from the dominance of the Welsh over the Scottish type of resistance, the distribution of clotting times is unimodal where all offspring were carriers of the Welsh allele (Fig. 2A) and bimodal in the three crosses where both carriers and non-carriers were expected. Table 1 shows that the cut-off time of 50 s divides each of the three segregating progenies into two classes which are a good fit to the expected Mendelian ratios.

4. DISCUSSION

The bimodal distribution of clotting factor responses, and their corresponding twofold classification into Mendelian proportions in progenies where both carriers and non-carriers of the Welsh allele were expected to occur, indicate unequivocally that the Welsh and Scottish types of resistance are due to different alleles. Since both forms of resistance appear to be controlled by the *Rw* locus this may be taken as strong evidence of multiple allelism at the *Rw* locus. Since the presence of n different alleles at a locus permits $0{\cdot}5\ n\ (n+1)$ different genotypes to be formed, then with the three alleles Rw^{w}, Rw^{s} and Rw^{+} six genotypes are possible. At the ecological level this gives natural populations the potential to become highly polymorphic. As each allele, and therefore each genotype, produces a different response to various anticoagulants and to vitamin K it is evident that such populations would tend to be more responsive to selection with anticoagulant rodenticides and able to maintain a higher frequency of resistance in the absence of such selection by minimizing adverse effects of resistance, such as the tendency to spontaneous haemorrhage (Partridge, 1980).

Current theory suggests that warfarin acts by inhibiting a membrane-bound reductase (or reductase system) that converts vitamin K_1 epoxide to the biologically active form of the vitamin (Bell, 1978; Fasco & Principe, 1980). Welsh-type resistance has been attributed to structural change in the reductase that makes it less sensitive to inhibition by warfarin (Zimmerman & Matschiner, 1972, 1974; Bell & Caldwell, 1973). While the possibility cannot yet be excluded that *Rw* represents a tightly linked series of genes controlling, perhaps, a functionally related group of enzymes involved in vitamin K metabolism, the locus is not known to be divisible by recombination. Assuming, therefore, that *Rw* is a single structural gene for the reductase, it can be surmised that the Scottish allele, Rw^{s}, represents a further variant of the reductase, whose binding affinity for warfarin and other molecules involved in vitamin K metabolism is intermediate between the two forms represented by the Welsh allele, Rw^{w} and the wild-type allele, Rw^{+}.

Membrane-bound enzymes, such as the reductase, are thought to be relatively intolerant of structural variation owing to their need to maintain accurate steric relations with other molecules associated with the membrane. Consequently, and in contrast to many soluble enzymes, they tend to be conserved by natural selection in a monomorphic state. This picture is consistent with the general observation that warfarin-resistance alleles seem to be very rare until selection with warfarin-type poisons has been practised for several years and that, in populations that have become polymorphic for resistance, there are large differences in relative fitness between different genotypes (Greaves, Redfern, Ayres & Gill, 1977; Partridge, 1979). It is thus of some interest not only that different resistant mutations have occurred in the Scottish and Welsh rat populations but also that the same membrane-bound reductase may have been modified in different ways to produce two different forms of resistance.

REFERENCES

BELL, R. G. (1978). Metabolism of vitamin K and prothrombin synthesis: anticoagulants and the vitamin K-epoxide cycle. *Federation Proceedings: American Societies for Experimental Biology* **37**, 2599–2604.

BELL, R. G. & CALDWELL, P. T. (1973). Mechanism of warfarin resistance. Warfarin and the metabolism of vitamin K_1. *Biochemistry* **12**, 1759–1762.

FESTING, M. F. W. (1978). Nomenclature. *Rat News Letter* **4**, 1–3, 10–12.

FASCO, M. J. & PRINCIPE, L. M. (1980). Vitamin K_1 hydroquinone formation catalyzed by a microsomal reductase system. *Biochemical and Biophysical Research Communications* **97**, 1487–1492.

GREAVES, J. H. & AYRES, P. (1973). Warfarin resistance and vitamin K requirement in the rat. *Laboratory Animals* **7**, 141–148.

GREAVES, J. H. & AYRES, P. B. (1976). Inheritance of Scottish-type resistance to warfarin in the Norway rat. *Genetical Research* **28**, 231–239.

GREAVES, J. H. & AYRES, P. B. (1977). Unifactorial inheritance of warfarin resistance in *Rattus norvegicus* from Denmark. *Genetical Research* **29**, 215–222.

GREAVES, J. G., REDFERN, R., AYRES, P. B. & GILL, J. E. (1977). Warfarin resistance: a balanced polymorphism in the Norway rat. *Genetical Research* **30**, 257–263.

MARTIN, A. D. (1973). Vitamin K requirement and anticoagulant response in the warfarin resistant rat. *Biochemical Society Transactions* **1**, 1206–1208.

O'REILLY, R. A. (1971). Vitamin K in hereditary resistance to oral anticoagulant drugs. *American Journal of Physiology* **221**, 1327–1330.

PARTRIDGE, G. G. (1979). Relative fitness of genotypes in a population of *Rattus norvegicus* polymorphic for warfarin resistance. *Heredity* **43**, 239–246.

PARTRIDGE, G. G. (1980). Vitamin K deficiency syndromes in warfarin-resistant brown rats (*Rattus norvegicus*). *Laboratory Animals* **14**, 193–195.

REDFERN, R. & GILL, J. E. (1978). The development and use of a test to identify resistance to the anticoagulant difenacoum in the Norway rat (*Rattus norvegicus*). *Journal of Hygiene* **81**, 427–431.

ZIMMERMAN, A. & MATSCHINER, J. T. (1972). The biochemical basis of hereditary resistance to warfarin in the rat. *Federation Proceedings: American Societies for Experimental Biology* **31**, 405.

ZIMMERMAN, A. & MATSCHINER, J. T. (1974). Biochemical basis of hereditary resistance to warfarin in the rat. *Biochemical Pharmacology* **23**, 1033–1040.