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The development and use of a test to identify resistance to the anticoagulant difenacoum in the Norway rat (*Rattus norvegicus*)*

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SUMMARY

Feeding tests were carried out in the laboratory to obtain basic data on the susceptibility of wild Norway rats to difenacoum. The results were used to derive a standard test procedure for the identification of difenacoum resistance in warfarin-susceptible and resistant rats. Details are given of tests on rats from suspected difenacoum-resistant infestations on farms.

INTRODUCTION

The discovery of resistance to warfarin in the Norway rat, *Rattus norvegicus*, and subsequently in the house mouse, *Mus musculus* and ship rat, *R. rattus* has been well documented (Boyle, 1960; Rowe & Redfern, 1965; Greaves, Rennison & Redfern, 1973). The problems that this phenomenon caused were partially overcome by the introduction of another anticoagulant, coumatetralyl. This was shown to kill both non-resistant and warfarin-resistant Norway rats, although there was a significant decrease in its toxicity to the latter (Greaves & Ayres, 1969). Coumatetralyl was found to kill a proportion of warfarin-resistant house mice (Rowe & Redfern, 1968).

A more significant breakthrough in rodent control occurred in 1974, when difenacoum was introduced by Sorex (London) Ltd. The compound was one of a new series of 4-hydroxycoumarin anticoagulants, and was shown to be especially effective against warfarin-resistant Norway rats (Hadler, Redfern & Rowe, 1975; Rennison & Hadler, 1975).

Although difenacoum has a much greater anticoagulant activity, it apparently acts as a vitamin K antagonist like other anticoagulants, and it is reasonable to suppose that resistance to it might occur in due course.

This paper describes laboratory tests to determine the susceptibility of *R. norvegicus* to difenacoum, the results of which were used to throw light on the degree of resistance of rats caught in five field infestations where control measures with difenacoum had been unsuccessful.

METHODS

Laboratory feeding tests were carried out on individually caged wild-caught rats that had been allowed to settle down in captivity for a minimum period of 3 weeks before being used. Rats susceptible to warfarin (and therefore presumably to difenacoum) were obtained either from a Midlands refuse destructor where anticoagulants had never been used, or from farms in central Wales. In the latter case susceptibility to warfarin was determined by injecting a small dose of warfarin and vitamin K-oxide and examining the clotting activity of the blood 24 h later (Martin, Steed, Redfern, Gill & Huson, in preparation). Warfarin-resistant rats were trapped on Welsh farms in the same area and identified as such either by the same procedure or by a 6-day feeding test on 0·005% warfarin in medium oatmeal (Drummond & Wilson, 1968). Suspected difenacoum-resistant rats were caught on farms in Hampshire and Berkshire. The degree of susceptibility to difenacoum in warfarin-resistant and suceptible rats was determined by establishing a base-line for each strain in accordance with the World Health Organization method (W.H.O., 1975), using 0·005% difenacoum in medium oatmeal for a number of different feeding periods. Preliminary sighting experiments had indicated that 0·001% was too low a concentration.

RESULTS AND DISCUSSION

Collection of base-line data and development of test for difenacoum resistance

The relation between length of feeding test on difenacoum and mortality (Table 1) was examined using a computer probit analysis program (Finney, 1971), and also the method of Litchfield & Wilcoxon (1949).

No significant heterogeneity was found between the sexes within each strain, or between the strains when the sexes were combined. When all the data were combined, the values obtained from probit analysis for the 'lethal feeding period' (LFP) 98 and 99 were 3·65 days (confidence limits 3·10–4·64) and 4·23 days (3·52–5·62) respectively. The slope of the probit line was 4·189 (S.E. ± 0·499).

Adopting the World Health Organization procedure (W.H.O., 1975) of taking the next whole number of days above the upper 95% confidence limit of the chosen lethal feeding period (in this case the LFP 98), the test for the identification of resistance to difenacoum in the Norway rat in the U.K. should be free feeding on 0·005% difenacoum in medium oatmeal for 5 days.

The somewhat less accurate Litchfield & Wilcoxon method gave the LFP 98 as 4·0 days (confidence limits 2·77–5·76), indicating a 6-day feeding period.

Tests on suspected difenacoum-resistant rats

Since the summer of 1975, various reports have been received of farm rat infestations proving difficult to control with difenacoum: all of these were in or near an area of warfarin resistance on the Hampshire–Berkshire border. Rats were trapped on five of these, and brought into the laboratory for further investigation. Because collection of the base-line data given above was incomplete at the

Table 1. *Mortality in* R. norvegicus *after unrestricted feeding on* 0·005% *difenacoum in medium oatmeal for varying numbers of days*

	Feeding period (days)	Sex	Mean body wt (g)	Mortality	Lethal dose of active ingredient (mg/kg)		Survived dose of active ingredient (mg/kg)		Days to death	
					Mean	Range	Mean	Range	Mean	Range
Warfarin-resistant	1	M	249	6/20	3·1	1·6– 3·7	2·9	2·0– 4·5	7·7	4–14
		F	229	6/20	3·5	2·5– 4·6	3·8	2·5– 4·6	6·7	4–13
	2	M	314	44/45	6·5	3·0–10·8	7·4	—	6·6	3–14
		F	252	41/49	6·6	1·7–10·7	6·2	1·9–11·3	6·7	2–12
	3	M	305	33/35	8·4	4·2–12·7	4·4	3·5– 5·2	5·9	4– 8
		F	207	43/49	10·2	2·2–15·7	10·7	5·6–16·7	6·3	4–13
	4	M	297	20/20	7·3	3·1–10·2	—	—	5·4	2– 8
		F	242	29/30	10·2	5·5–17·0	14·5	—	6·7	5–10
Warfarin-susceptible	1	M	306	5/10	3·1	2·8– 3·4	2·6	2·3– 3·5	6·8	5–10
		F	263	1/5	2·8	—	4·0	2·7– 4·7	5·0	—
	2	M	276	8/10	7·4	5·7–10·5	2·7	1·3– 4·1	5·8	5– 7
		F	197	9/10	7·8	4·5–10·5	4·2	—	7·7	5–12
	3	M	303	10/10	10·0	6·0–14·6	—	—	6·4	6– 8
		F	164	10/10	11·5	7·4–15·6	—	—	6·9	6– 9
	4	M	276	5/5	14·1	10·7–16·8	—	—	6·0	4– 7
		F	219	5/5	14·3	9·9–19·7	—	—	7·1	6–10
Combined data	1	M	268	11/30	3·1	1·6– 3·7	2·8	2·0– 4·5	7·3	4–14
		F	236	7/25	3·4	2·5– 4·6	3·8	2·5– 4·7	6·5	4–13
	2	M	307	52/55	6·6	3·0–10·8	4·3	1·3– 4·1	6·5	3–14
		F	243	50/59	6·8	1·7–10·7	5·9	1·9–11·3	6·9	2–12
	3	M	305	43/45	8·7	4·2–14·6	4·4	3·5– 5·2	5·5	4– 8
		F	200	53/59	10·5	2·2–15·7	10·7	5·6–16·7	6·4	4–13
	4	M	293	25/25	8·7	3·1–16·8	—	—	5·5	2– 8
		F	239	34/35	10·8	5·5–19·7	14·5	—	6·7	5–10

Table 2. *Mortality in* R. norvegicus *from suspected difenacoum-resistant infestations after unrestricted feeding on* 0·005% *difenacoum in medium oatmeal for varying numbers of days*

Source	Feeding period (days)	Sex	Mortality	Total	Lethal dose of active ingredient (mg/kg) Mean	Lethal dose of active ingredient (mg/kg) Range	Survived dose of active ingredient (mg/kg) Mean	Survived dose of active ingredient (mg/kg) Range	Days to death Mean	Days to death Range
Farm A (Berks.)	3	M	4/6	8/13	11·1	4·3–19·7	13·8	13·8	3·8	3– 5
		F	4/7		9·3	8·9– 9·6	13·6	11·3–16·9	7·0	3–13
	6*	M	0/2	2/5	—	—	14·8	14·2–15·3	—	—
		F	2/3		10·2	8·0–12·3	20·0	—	7·0	6– 8
	3	M	2/2	2/6	8·8	8·4– 9·2	—	—	5·0	4– 6
		F	0/4		—	—	9·5	7·5–11·7	—	—
	6	M	3/3	6/7	11·7	10·3–13·7	—	—	7·0	6– 9
		F	3/4		12·4	10·6–14·2	18·3	—	8·0	5–13
	8	M	2/2	5/6	15·5	9·0–21·9	—	—	7·5	5–10
		F	3/4		12·0	7·4–15·1	35·4	—	7·7	6–10
Farm B (Berks.)	3	M	3/3	3/5	12·7	9·4–16·7	—	—	6·0	6
		F	0/2		—	—	16·2	15·8–16·5	—	—
	6*	F	2/2	2/2	6·5	2·0–11·0	—	—	6·5	4– 9
Farm C (Hants.)	3	M	10/10	12/12	10·6	6·9–18·8	—	—	6·2	5– 9
		F	2/2		12·1	9·5–14·7	—	—	6·5	6– 7
Farm D (Hants.)	9	M	3/3	8/8	8·3	6·5– 9·5	—	—	7·0	6– 8
		F	5/5		14·1	8·5–23·0	—	—	8·2	7– 9
Farm E (Hants.)	9†	M	3/3	7/8	7·6	7·4– 8·0	—	—	5·3	4– 6
		F	4/5		19·1	13·1–28·3	37·3	—	8·0	7–10

* Survivors of 3-day test above.
† Survivors of 9-day test on 0·001% difenacoum.

time, the rats were subjected to feeding tests of varying duration, details of which are given in Table 2.

It is clear that rats from Farm C were not resistant to difenacoum and no useful conclusions may be drawn from Farm D. Assuming that the 3-day test on rats from Farm B did not influence the result of the later 6-day test, it is probable that this infestation was not resistant; one rat died on day 4, while the other had reduced its intake of poisoned bait by day 5.

Eighteen rats from Farm A were given tests of 6 or 8 days, and the five that survived continued to feed well throughout the test period and can be classified as resistant to difenacoum.

Another case of resistance is shown by the one female from Farm E. This rat survived a 9-day test on 0·005 % difenacoum, having eaten well throughout: it ate a total of 123·9 g of poisoned bait, representing an intake of 37·3 mg/kg of active ingredient.

It is concluded that the six rats that survived periods of feeding longer than the prescribed 5 days showed a considerable degree of resistance to difenacoum. By analogy with the relationship between field and laboratory evidence for warfarin resistance, it seems likely that rats shown to possess this degree of resistance to difenacoum in the laboratory would be extremely difficult if not impossible to kill with this material in the field, but further evidence is needed to confirm this.

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REFERENCES

Boyle, C. M. (1960). Case of apparent resistance of *Rattus norvegicus* Berkenhout to anticoagulant poisons. *Nature, London* **188**, 517.

Drummond, D. C. & Wilson, E. J. (1968). Laboratory investigations of resistance to warfarin of *Rattus norvegicus* Berk. in Montgomeryshire and Shropshire. *Annals of Applied Biology* **61**, 303–12.

Finney, D. J. (1971). *Probit Analysis*. 3rd ed. Cambridge University Press.

Greaves, J. H. & Ayres, P. B. (1969). Some rodenticidal properties of coumatetralyl. *Journal of Hygiene* **67**, 311–15.

Greaves, J. H., Rennison, B. D. & Redfern, R. (1973). Warfarin resistance in the ship rat in Liverpool. *International Pest Control* **15** (2), 17.

Hadler, M. R., Redfern, R. & Rowe, F. P. (1975). Laboratory evaluation of difenacoum as a rodenticide. *Journal of Hygiene* **74**, 441–8.

Litchfield, J. T. & Wilcoxon, F. (1949). A simplified method of evaluating dose-effect experiments. *Journal of Pharmacology and Experimental Therapeutics* **96**, 99–113.

Rennison, B. D. & Hadler, M. R. (1975). Field trials of difenacoum against warfarin-resistant infestations of *Rattus norvegicus*. *Journal of Hygiene* **74**, 449–55.

Rowe, F. P. & Redfern, R. (1965). Toxicity tests on suspected warfarin-resistant house mice (*Mus musculus* L.). *Journal of Hygiene* **63**, 417–25.

Rowe, F. P. & Redfern, R. (1968). Comparative toxicity of the two anticoagulants, coumatetralyl and warfarin to wild house mice (*Mus musculus* L.). *Annals of Applied Biology* **62**, 355-61.

World Health Organization (1975). Instructions for determining the susceptibility or resistance of rodents to anticoagulant rodenticides. *W.H.O./V.B.C./75.595*.