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Laboratory evaluation of difenacoum as a rodenticide*

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SUMMARY

The efficacy of difenacoum as a new anticoagulant rodenticide was evaluated by blood coagulation studies and laboratory feeding tests using warfarin-resistant and non-resistant common rats (*Rattus norvegicus*), ship rats (*R. rattus*) and house mice (*Mus musculus*). Prothrombin assays indicated that the compound had as marked an activity with warfarin-resistant common rats as coumatetralyl had with non-resistant animals. Feeding tests confirmed that 0·005% would be a near-optimal concentration for field use, although there was some evidence of unpalatability.

Results with ship rats and house mice were less favourable. Trials with enclosed colonies of warfarin-resistant mice confirmed the laboratory finding that although difenacoum was more effective than all other currently used anticoagulants, it was unlikely to give complete control.

It is concluded that difenacoum is a valuable new rodenticide, especially for controlling warfarin-resistant common rats.

INTRODUCTION

The development of resistance to anticoagulant poisons by rodents in Britain has been well documented, and is now found in the three most important pest species – common rat, ship rat and house mouse. This fact, together with the paucity of satisfactory acute poisons led to the launching of various lines of research to develop new, more effective rodenticides. One of several approaches made by the Pest Infestation Control Laboratory (P.I.C.L.) of the Ministry of Agriculture, Fisheries and Food consisted of a routine procedure for screening a wide range of toxic compounds donated by the pharmaceutical industry. Another approach, made by Sorex (London) Ltd and consisting of the synthesis and examination of novel derivatives of known anticoagulant drugs, produced a series of 4-hydroxycoumarins, several of which were found to be considerably more active than currently available rodenticides.

The present paper describes joint work carried out on the compound 3-(3-*p*-diphenyl-1,2,3,4-tetrahydronaphth-1-yl)-4-hydroxycoumarin, a member of the series described by Hadler & Shadbolt (1975) for which the British Standards Institution proposed common name is 'difenacoum'. It has lately been released as a rodenticide under the trade name 'Neosorexa'.

Blood coagulation studies, to compare the activity of difenacoum with other anticoagulants, were carried out by Sorex (London) Ltd, and the feeding tests with wild rodents and the mouse colony trials were done by P.I.C.L.

METHODS

For most of the work wild rodents were used, including warfarin-resistant and non-resistant samples of common rat (*Rattus norvegicus*), ship rat (*R. rattus*) and house mouse (*Mus musculus*), as defined by Greaves, Redfern & King (1974). Two laboratory strains of rat (HW and HS), both homozygous for warfarin resistance, were used together with a non-resistant Wistar-derived strain. Some of the work with mice was performed on a wild strain obtained from Cambridge, showing an unusually high level of resistance to warfarin.

Prothrombin time determination

The activity of difenacoum in Wistar and HW rats was compared with that of other anticoagulant poisons using a modified one-stage method for prothrombin time determination (Quick, Stanley-Brown & Bankroft, 1935).

Each anticoagulant, dissolved in polyethylene glycol 300, was administered by intraperitoneal injection. Three daily doses were given at various dosage levels, with 3 rats per group. Blood samples were taken by cardiac puncture 24 hr. after the last injection, and prothrombin times determined. ED50 estimations were obtained by best line of fit.

Laboratory feeding tests

In accordance with the routine procedure for screening candidate rodenticides (Rowe, Greaves, Redfern & Martin, 1970), feeding tests were initially performed on singly caged laboratory rats and mice and subsequently on wild-caught animals. Equal numbers of males and females were used except in the case of the ship rat where the limited numbers of animals available precluded the completion of the full range of tests.

Feeding tests were of two types – 'no-choice' tests in which each animal was presented with the toxic bait only and 'choice' tests in which the same bait but without the poison was also given. Each day fresh bait was supplied and in the case of choice tests the positions of the two baits were interchanged. In the no-choice tests with laboratory rodents and in all choice tests animals were maintained on diet 41B until the start of the tests. In no-choice tests with wild rats and mice the bait-base without the poison was given for a few days before the start of the test, to condition the animals to eat freely. Feeding tests were mainly of 2 or 4 days duration, and used a mixture of pinhead oatmeal (90%), corn oil (5%) and wholemeal flour as a vehicle for the poison (5%). In the 'no-choice test to death' medium oatmeal bait was used.

Daily readings of bait consumption were made and the days to death of animals recorded. Typical haemorrhagic symptoms of anticoagulant poisoning were observed in autopsied animals.

Feeding trials with enclosed mouse colonies

Tests were carried out on family groups of mice. These were bred from the descendants of wild stock (different from those used in the laboratory tests) the individuals of which had been found mostly to be highly warfarin-resistant, 94·3 % being able to survive a 21-day period of feeding on bait containing 0·025 % warfarin.

Difenacoum and warfarin treatments were conducted in large rectangular metal pens (9·5 × 2·5 m) as part of a wider study designed to compare the efficacy of various acute and chronic poisons against mice (Rowe & Bradfield, 1975). Four treatments were conducted employing difenacoum at 0·005 % and 0·01 %. For comparative purposes, two treatments were also undertaken using warfarin at 0·025 %. Alternative plain food (whole wheat and powdered diet 41B) was made available to the mice during each 21-day poison treatment. Both difenacoum and warfarin were included in the pinhead oatmeal/wholemeal flour/corn oil bait-base used in the laboratory tests.

After a 7-day recovery period the survivors of each poison treatment were transferred to a smaller circular pen (1·7 m diameter). They were then subjected to a further 21-day poison treatment, but in the absence of alternative food.

Additional 21-day no-choice poison treatments were conducted in the small pens. Apart from difenacoum (at 0·005 % in bait) and warfarin (0·025 %), three other anticoagulant rodenticides that are in current use against mice were examined. These compounds were coumatetralyl (0·05 %), chlorophacinone (0·025 %) and diphacinone (0·0125 %).

RESULTS AND DISCUSSION

Prothrombin time determination

A summary of the prothrombin assays is given in Table 1, where prothrombin ED 50 has been calculated as the daily dose predicted to raise the prothrombin time to a mean value of 112 sec., the midway point between 12 sec., the mean normal prothrombin time, and 212 sec., the mean prothrombin time obtained when the level of clotting factors is at the minimum recordable value. The results obtained by best line of fit indicate that while difenacoum is more active than the other compounds against Wistar rats, its level of superiority is much greater against resistant rats. The 'resistance index' (see footnote of Table 1) of 1·9 for difenacoum is much lower than that for the other anticoagulants, indicating that difenacoum is very active against resistant as well as non-resistant rats.

Of the standard materials, coumatetralyl has a resistance index of about 16, while the other compounds appear to be resisted at least 100 times. In view of the

Table 1. *Prothrombin ED 50 values for male Wistar and HW rats after three daily injections of various anticoagulants*

Anticoagulant	Prothrombin ED 50 (mg/kg/day × 3) Wistar	HW	Resistance index*
Warfarin S (−) isomer	0·30	> 50·0	> 166
Warfarin R (+) isomer	3·3	> 50·0	> 15
Coumatetralyl	0·31	*ca.* 4·4	*ca.* 16
Diphacinone	0·22	*ca.* 50·0	*ca.* 227
Chlorophacinone	0·22	> 20·0	> 90
Difenacoum	0·17	0·32	1·9

* Resistance index = ED 50 HW (resistant)/ED 50 Wistar (susceptible).

low activity of the most active isomer of warfarin, it may be concluded that the commercial product is resisted by a factor considerably in excess of 100.

Laboratory feeding tests

It is useful to evaluate the potential of a new anticoagulant poison by comparing three important aspects of its performance with those of two currently used rodenticides, namely warfarin which continues to be an extremely good poison for the common rat in areas in which resistance has not developed, and calciferol, a compound especially useful against warfarin-resistant common rats and house mice (Greaves *et al.* 1974; Rennison, 1974; Rowe, Smith & Swinney, 1974), and incorporated with warfarin in the commercially available formulation 'Sorexa CR'.

Toxicity of difenacoum bait

Most feeding tests were carried out at 0·005 %, the concentration shown to be optimal by Sorex (London) Ltd.

Two-day no-choice tests (Table 2) gave kills of common rats of 10/10 and 9/10 non-resistant and resistant animals respectively. With calciferol at 0·1 % (in medium oatmeal), the concentration at which it is used in Sorexa CR bait, Greaves *et al.* (1974) obtained complete kills with both types of animal: with 0·025 % warfarin a kill of 21/23 was obtained with non-resistant rats (Bentley & Larthe, 1959), and with resistant rats no mortality would be expected.

With mice the kills of 7/10 and 9/10 with non-resistant and resistant animals respectively were less good than those of 0·1 % calciferol (10/10 and 10/10), but much better than those of warfarin. With the last poison, for example, Rowe & Redfern (1964) only obtained a kill of 6/30 after 4 days feeding with non-resistant mice and no kill would of course be expected with resistant mice.

In 10-day no-choice tests, a 5/5 kill of female HW rats with difenacoum was obtained at as low a concentration as 0·002 %; at 0·001 % the mortality was 7/10. In comparison coumatetralyl at 0·02 % killed 1/5 HW rats.

In 2-day no-choice tests with non-resistant ship rats, both difenacoum at 0·005 % (Table 2) and calciferol at 0·1 % (Greaves *et al.* 1974) gave kills of 10/10,

Table 2. *Mortality and bait consumption of wild rodents given a sole diet of* 0·005 % *difenacoum in pinhead oatmeal/corn oil bait for* 2 *days*

Species	Type*	Sex	Mean body weight	Mortality	Mean daily bait intake (g.)		Lethal dose of active ingredient (mg./kg.)		Survived dose of active ingredient (mg./kg.)		Days to death	
					Prebait†	Poison	Mean	Range	Mean	Range	Mean	Range
Rattus	NR	M	179	5/5	18·2	14·2	8	1–17	—	—	4·4	4–6
norvegicus	NR	F	149	5/5	16·1	11·9	8	5–10	—	—	6·0	4–10
	R	M	211	5/5	17·6	13·3	7	5–10	—	—	4·8	3–6
	R	F	208	4/5	16·3	12·7	7	4–12	6	—	7·3	6–8
Mus	NR	M	13	4/5	2·4	2·4	18	13–22	20	—	6·0	5–7
musculus	NR	F	13	3/5	2·1	2·6	20	17–22	21	18–24	7·3	6–9
	R	M	15	5/5	3·1	2·6	18	9–23	—	—	5·4	2–7
	R	F	15	4/5	2·3	2·6	19	12–22	—	—	5·4	2–7
	R	F	15	4/5	2·3	2·6	19	12–22	14	—	7·5	6–10
Rattus	NR	M	124	5/5	8·2	8·6	7	6–8	—	—	6·6	4–8
rattus	NR	F	99	5/5	8·2	8·0	8	5–11	—	—	5·6	4–7
	R	M	158	3/5	12·1	10·6	7	4–10	8	7–8	9·3	9–10
	R	F	122	2/5	9·5	9·1	7	6–7	8	8–9	9·5	6–13

* NR = non-resistant, R = resistant to warfarin.
† Last day only.

Table 3. *Bait consumption and mortality in wild* R. norvegicus, R. rattus *and* M. musculus *given a choice between plain and poisoned baits*

Species	Type	Mean body weight (g.)	Duration of test (days)	Concentration (%)	Mean daily bait intake (g.) Poison	Mean daily bait intake (g.) Plain	Significance (P) of Student's 't'	Mortality
Rattus norvegicus	NR	190	2	0·3	1·5	15·3	$<0·001$	14/20
	NR	178	2	0·03	6·0	10·1	$<0·01$	18/20
	NR	176	4	0·01	5·6	10·0	$<0·01$	29/30
	NR	185	4	0·005	6·7	9·0	$<0·02$	29/30
	R	224	2	0·3	4·6	12·1	$<0·01$	15/20
	R	289	2	0·03	7·2	10·6	0·1–0·05	13/20
	R	212	4	0·01	5·9	10·6	$<0·001$	27/30
	R	246	4	0·005	6·3	9·8	$<0·001$	28/29
Mus musculus	NR	13	2	0·3	0·4	2·2	$<0·001$	7/10
	NR	13	2	0·03	1·0	1·7	$<0·01$	8/10
	NR	12	4	0·01	1·4	1·6	0·2–0·1	19/20
	NR	14	4	0·005	1·5	1·3	0·5–0·4	18/20
	R	14	2	0·3	0·7	2·2	$<0·001$	7/10
	R	14	2	0·03	1·1	1·6	$<0·001$	7/10
	R	17	4	0·01	1·6	1·8	$<0·02$	8/10
	R	17	4	0·005	1·6	1·4	0·6–0·5	19/20
Rattus rattus	NR	127	4	0·005	3·3	7·9	$<0·01$	10/10
	R	152	4	0·01	4·3	9·5	0·3–0·2	3/5
	R	156	4	0·005	2·3	6·6	$<0·01$	4/10

with very similar means and ranges for days to death. Less good results were obtained with resistant animals: difenacoum (5/10) was slightly less effective than calciferol (7/10). These results with both groups of animals are considerably better than would be expected with 0·025 % warfarin (Greaves & Redfern, in preparation).

Speed of rodenticidal action

A series of no-choice feeding tests to death were carried out using difenacoum at 0·005 % to investigate the speed of rodenticidal action. With non-resistant and resistant common rats bait consumption remained normal for 2 days. On the third day 5/20 rats ate less than half the quantity they had eaten on day 1: this ratio increased to 13/20, 19/20 and 20/20 on days 4, 5 and 6 respectively. The time to death ranged from 4 to 10 days, the last animal to die being from the non-resistant group. This feeding pattern is reminiscent of that found with non-resistant common rats on warfarin.

In tests to death with five resistant house mice, one animal ate well for 3 days and died on day 4. The others continued to eat well for a further 3 days, but on day 7 virtually stopped feeding. Two of these mice died on days 10 and 12 respectively. The remaining two mice resumed feeding again on day 9 and continued a reasonably normal daily intake until their deaths on days 22 and 30.

This phenomenon of a marked reduction in appetite, presumably due to the effects of hypoprothrombinaemia, followed by a period of normal food consumption

Table 4. *Results of 'no-choice' feeding tests on enclosed colonies of wild mice with five anticoagulants*

Poison and concentration	Mortality	Mortality (%)	Days to death
0·005 % difenacoum	14/15	93·3	4–22
0·025 % warfarin	2/13	15·4	15–23
0·05 % coumatetralyl	3/13	23·1	7–9
0·025 % chlorophacinone	6/13	46·2	5–19
0·0125 % diphacinone	0/9	0·0	—

closely resembles that observed with resistant mice and warfarin (Rowe & Redfern, 1965). It is reasonable to infer, therefore, that this particular mechanism that may play a role in causing resistance to warfarin may also protect mice from difenacoum poisoning, which would seem to rule out the possibility of difenacoum becoming a very successful mouse poison. With non-resistant mice feeding had virtually stopped by day 5, and all animals died on days 7 or 8. Similar tests with resistant and non-resistant ship rats were inconclusive; several rats ate very small quantities of poison despite adequate pre-baiting.

Palatability of difenacoum bait

The results of choice tests between poisoned and plain foods are summarized in Table 3. In 4-day tests only the first 2 days' readings of bait consumption were used as a measure of palatability because symptoms of poisoning would be expected to affect the feeding behaviour from the third day. With the common rat and house mouse the generally significant unpalatability of difenacoum at 0·3 % and 0·03 % and the mortalities obtained would preclude the use of the compound as an acute rodenticide. Although at 0·005 % there was still some discrimination by common rats against the poison, the kills of warfarin-resistant and non-resistant groups were very high. In a further 2-day choice test using nine non-resistant rats, and comparing the consumption of 0·005 % difenacoum bait with that of 0·025 % warfarin, the mean daily intakes of the two poisons in an oatmeal/corn oil bait were 10·5 g and 8·9 g respectively, showing an insignificant preference for difenacoum ($P = < 0{\cdot}4$). With non-resistant house mice there was no discrimination against difenacoum at 0·01 % or 0·005 % and kills were high (19/20 and 18/20 respectively). At 0·005 % warfarin-resistant mice actually ate more poisoned food than plain. The anomalous results obtained with resistant common rats at 0·03 % and resistant ship rats at 0·01 % are difficult to explain.

Feeding trials with enclosed mouse colonies

In the trials in which groups of mice were given both poisoned food and the diet to which they were accustomed mortality was high in those offered difenacoum at either 0·005 % or 0·01 % bait in the large pens (72/81 or 88·9 % and 65/67 or 97·0 % respectively). In contrast none of the 60 mice treated with 0·025 % warfarin bait died.

In the follow-up no-choice tests in the small pens, three of the eight mice that

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had survived treatment with 0·005 % difenacoum bait in the large pens also survived 21 days feeding on the same poison bait alone. Both of the 0·01 % difenacoum treatment survivors died in the small pens (days to death 14 and 16) when given the same poison bait. The two groups of 0·025 % warfarin survivors were fed either 0·005 % or 0·01 % difenacoum treated bait in their small pen. The 23 and 37 animals comprising each group all died (days to death 4–13 and 3–24 respectively).

In the further no-choice feeding tests in the small pens, difenacoum gave the most effective kill of mice (Table 4). The single survivor of the 0·005 % difenacoum treatment also survived however a successive 21-day feeding period on 0·01 % difenacoum bait. This individual, a female, died 2 days after the concentration of difenacoum was increased still further to 0·02 %. The results of the pen feeding tests thus support the inference drawn from the earlier laboratory work that difenacoum is the most effective anticoagulant investigated so far against mice but that, all the same, it would be unlikely to eradicate all mouse populations resistant to other anticoagulant rodenticides.

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