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The effect of warfarin on plasma clotting time in wild house mice (*Mus musculus* L.)*

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INTRODUCTION

The results of studies of the toxicity of warfarin [3-(α-acetonyl)-4-hydroxycoumarin] to wild house mice (*Mus musculus* L.) have been reported earlier (Rowe & Redfern, 1964; 1965). The mice used were so-called 'normal' animals that had been hand-caught in corn-ricks not treated with warfarin, and suspected warfarin resistant animals that had been live-trapped in premises where prolonged but ineffective warfarin treatments had been carried out. In comparative feeding tests over 10 and 21 days with a cereal bait containing 0·025% warfarin it was found that mortality was lower and the time to death longer for the allegedly resistant mice.

The present experiments were designed to extend these findings by determining the clotting times of the plasma of normal and suspected resistant mice receiving warfarin and thus the changes in the combined level of three blood clotting factors—factor II (prothrombin), factor VII (proconvertin) and factor X (Stuart-Prower factor)—normally depressed by anticoagulants.

MATERIAL AND METHODS

Adult wild house mice only were used. Normal mice (N) were hand-caught in neighbouring corn-ricks. Suspected warfarin resistant mice were drawn from two sources: home-bred resistant animals (HBR) were 4-month-old animals whose parents had survived 21 days feeding on 0·025% warfarin in an oatmeal/mineral oil/sugar bait-base, and field resistant (FR) mice were taken in premises where extended treatments with 0·025% warfarin in medium oatmeal had been unsuccessful in eliminating infestations.

Warfarin was administered to the test animals either mixed in one of two cereal-type baits or by subcutaneous injection in a solvent, dimethyl sulphoxide. Mice given repeated warfarin doses by injection were dosed at intervals of 24 hr.

Blood samples, 0·15 ml., were withdrawn from the retro-orbital sinus of the mouse (Riley, 1960) and collected into 0·015 ml. of 3·8% sodium citrate. The blood was centrifuged, and the combined level of factors II, VII and X determined by a modification of the Quick one-stage prothrombin test, using 'Two Seven Ten' reagent (supplied by Diagnostic Reagents Ltd., Thame, Oxfordshire) and normal saline (0·85%) as diluent. Plasma that failed to clot after 15 min. was recorded as uncoagulable.

RESULTS

The clotting times of diluted plasma from normal mice

The relationship between clotting time and plasma dilution was first established for normal mice. For this purpose blood samples were taken from twelve males and twelve females. After centrifuging, the plasma from mice of the same sex was pooled and diluted by adding varying amounts of 0·85 % saline. The clotting time of the diluted plasma was then determined as in Table 1, where '100 % plasma' refers to plasma and saline in the ratio of 60:40, '50 % plasma' to plasma and saline in the ratio of 60:140, etc. It can be seen from Table 1 that increases in clotting time and hence falls in the combined level of factors II, VII and X became more marked with increasing plasma dilution.

Table 1. *Mean clotting times of normal male and female mouse plasma at various dilutions*

Percentage of 60:40 plasma/saline	Clotting time (sec.) and number of determinations			
	Male		Female	
	Mean	Range	Mean	Range
100	17·4 (4)	17·1–17·6	16·9 (3)	16·7–17·3
50	22·4 (4)	20·5–23·4	22·1 (4)	21·8–22·3
25	30·2 (4)	29·1–31·2	28·9 (4)	27·5–30·2
12·5	49·7 (4)	46·8–51·7	43·5 (4)	41·2–46·0
6·25	91·0 (4)	86·8–92·9	65·9 (4)	64·2–68·3
3·13	144·3 (4)	115·8–178·8	123·2 (4)	115·0–176·1

Table 2. *Mean plasma clotting times of untreated mice*

Source of mice (see text)	Sex	Number	Clotting time (sec.)	
			Mean	Range
N	M	75	16·8	13·7–29·5
	F	81	16·6	14·0–22·3
HBR	M	63	16·7	14·6–25·7
	F	65	16·3	14·3–18·9
FR	M	19	16·3	13·5–21·6
	F	19	16·2	14·2–19·3

Variation in the clotting times of untreated mice

Table 2 shows the mean clotting times determined for mice from the three sources before administration of warfarin. Considerable individual variation in resting clotting times was observed but the differences between the mean values for mice of the same sex from the different sources and between those of males and females from the same source were not significant, the probability level being greater than 0·1 in each comparison.

Blood samples were also taken from twelve N mice daily for 1 week. The clotting times were found to vary daily in all individuals and tended to decline slightly over the period (six males averaged 15·3 and 14·1 sec.; and six females averaged 16·2 and 14·8 sec. on days 1 and 7 respectively).

Table 3. *The clotting time response of mice given a single dose of warfarin*

	Dose (mg./kg.)	Sex	Clotting time (sec.) after warfarin administration									
			12 hr.		24 hr.		36 hr.		48 hr.		72 hr.	
			Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
N	1	M	32·1 (5)	26·3–36·8	41·6 (5)	24·3–64·5	20·1 (5)	15·2–31·6	17·5 (5)	14·8–25·2	15·1 (5)	13·0–16·2
		F	35·9 (5)	22·7–43·8	35·9 (6)	18·3–55·4	20·7 (6)	14·9–36·5	16·4 (5)	14·9–18·2	15·0 (5)	14·0–16·0
	5	M	—	—	97·5 (5)	49·5–140·3	—	—	17·7 (5)	14·8–24·5	—	—
		F	—	—	167·8 (5)	30·1–462·0	—	—	17·9 (5)	14·9–25·1	—	—
	50	M	—	—	124·7 (4)	72·6–177·0	—	—	24·8 (4)	18·3–34·1	—	—
		F	—	—	174·9 (5)	134·7–225·4	—	—	18·5 (5)	15·6–23·4	—	—
HBR	1	M	31·0 (5)	25·6–42·6	45·5 (5)	25·2–83·4	72·4 (5)	15·3–271·0	31·3 (5)	16·2–77·1	16·1 (5)	14·3–20·8
		F	22·9 (5)	17·7–34·6	17·2 (4)	16·3–17·7	20·0 (4)	14·3–34·3	14·5 (4)	13·9–15·1	13·6 (4)	13·1–14·4
	5	M	—	—	57·1 (5)	21·3–107·7	—	—	16·8 (5)	14·6–19·7	—	—
		F	—	—	26·4 (4)	14·3–44·8	—	—	14·9 (4)	13·5–15·4	—	—
	50	M	—	—	87·8 (4)	33·3–142·1	—	—	18·8 (4)	15·3–24·8	—	—
		F	—	—	41·3 (4)	26·8–68·3	—	—	15·9 (4)	14·2–16·9	—	—
FR	1	M	33·0 (6)	25·0–42·2	32·2 (7)	18·0–59·4	20·8 (7)	16·8–26·2	18·9 (6)	17·0–20·8	16·4 (7)	15·2–20·0
		F	17·0 (6)	16·4–18·2	25·0 (6)	16·2–33·5	18·8 (6)	16·5–27·2	19·9 (6)	16·2–28·5	14·8 (6)	14·0–15·2
	5	M	—	—	76·6 (6)	29·7–137·6	—	—	19·8 (6)	16·3–27·4	—	—
		F	—	—	23·2 (6)	17·8–33·2	—	—	19·9 (6)	15·4–35·5	—	—
	50	M	—	—	137·3 (7)	48·1–175·8	—	—	26·1 (7)	17·4–39·4	—	—
		F	—	—	24·3 (5)	17·8–40·2	—	—	18·5 (5)	17·5–19·9	—	—
	100	M	—	—	117·6 (7)	88·4–166·6	—	—	—	—	—	—
		F	—	—	24·0 (5)	16·9–36·1	—	—	—	—	—	—

Figures in paretheses indicate number of animals observed.

The effect of a single dose of warfarin on the clotting time of mouse plasma

Normal

A single dose of 1 mg. of warfarin/kg. body weight was administered by injection to each of eleven N mice and clotting time determinations made 12, 24, 36, 48 and 72 hr. later. After 2–3 week recovery periods the survivors were given further doses of 5 and 50 mg./kg. and clotting time determinations made after 24 and 48 hr. in each case.

Mean clotting times for the three doses of warfarin are given in Table 3. A marked increase in clotting time was found 12 hr. after the administration of the lowest dose of warfarin and clotting times were still prolonged after 24 hr. They then decreased and in six of the eleven animals investigated they had returned to normal levels after 36 hr. One mouse that still had an extended clotting time after 36 hr. died later. After 72 hr., the individual clotting times in eight of the ten survivors were slightly shorter than those determined before the administration of warfarin.

Longer clotting times were also apparent 24 hr. after the administration of the two higher warfarin dosages but as with the lowest dosage given the clotting times, with one exception, were nearly back to normal after 48 hr.

Home-bred resistant

In a similar study, ten HBR mice were given, at intervals, injected doses of 1, 5 and 50 mg. warfarin/kg. body weight. Clotting times were determined between 12 and 72 hr. after each dose. As with N mice, individual variation in response was observed and clotting times similarly increased with increasing warfarin dosages. The overall response was less marked however particularly in the case of females (Table 3).

Field resistant

The response of FR males given single injected doses of 1, 5 and 50 mg. of warfarin/kg. body weight was observed to be as marked as that found to occur in similarly treated N males (Table 3). FR females however, like HBR females, were markedly less responsive to the same three warfarin doses. After 24 hr. the mean clotting times of FR females (25·0, 23·2 and 24·3 sec. respectively) were considerably lower than the comparable values obtained with N females (32·2, 76·8 and 137·2 sec. respectively). Furthermore, increasing the dose of warfarin administered to female FR mice from 50 to 100 mg./kg. body weight did not result in any further increase in clotting times.

The effect of two standard doses of warfarin at 3 weeks interval on the clotting time of normal mouse plasma

Three groups of six N mice (three males and three females) were given by injection two identical doses of either 1, 5 or 50 mg. of warfarin/kg. body weight with an interval of 3 weeks between doses. Clotting times were determined before and after the administration of each dose.

Individual mice differed in their response to the same first dose of warfarin. The greater prolongation in clotting times that occurred with increasing warfarin dosage was evident 24 hr. after dosing. Most individuals responded similarly to the two identical warfarin doses (Table 4).

Table 4. *The clotting time (sec.) of normal mice given two doses of warfarin with an interval of 3 weeks*

Dose		Sex	Time after dose: 12 hr.	Mean	24 hr.	Mean	48 hr.	Mean
1 mg./kg.	1st	M	24·7, 26·8, 39·1	30·2	52·0, 18·3, 32·5	34·3	14·2, 16·3, 15·1	15·2
	2nd	M	36·4, 29·4, 39·2	35·0	20·3, 28·7, 27·3	25·4	16·9, 16·4, 16·2	16·5
	1st	F	28·4, 29·3, 29·9	29·2	20·0, 19·6, 21·6	20·4	17·2, 14·7, 15·7	15·9
	2nd	F	30·7, 32·3, 37·0	33·3	23·4, 18·0, 30·3	23·9	15·7, 14·7, 17·1	15·8
5 mg./kg.	1st	M	29·8, 31·6, 36·7	32·7	31·6, 33·7, 29·5	31·6	15·5, 15·3, 15·9	15·6
	2nd	M	27·8, 36·2, 35·6	33·2	71·5, 21·6, 78·8	57·7	20·4, 15·6, 15·5	17·2
	1st	F	27·3, 30·4, 40·6	32·8	54·4, 135·7, 145·5	111·9	14·3, 550·0, 18·1	194·1
	2nd	F	31·4, 29·0, 34·4	31·6	51·2, 62·9, 125·3	79·8	16·0, 15·4, 19·1	16·8
50 mg./kg.	1st	M	30·3, 37·2, 39·3	35·6	93·7, 78·0, 196·5	122·7	15·8, 13·9, 18·3	16·0
	2nd	M	27·3, 30·6, 25·6	27·8	88·8, 80·2, 68·5	79·2	15·8, 27·6, 24·7	22·7
	1st	F	30·4, 31·2, 33·2	31·6	93·6, 103·3, 98·7	98·5	15·7, 16·5, 16·3	16·2
	2nd	F	30·0, 31·6, 33·4	31·7	110·0, 104·7, 115·4	110·0	15·9, 16·2, 16·9	16·3

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The effect of daily standard doses of warfarin on the clotting time of mouse plasma

Normal

Three groups of ten N mice were given daily for 10 days single doses of either 1, 5 or 50 mg. of warfarin/kg. body weight (Table 5). Clotting times were determined before the administration of the first dose (not shown in Table 5) and at 24 hr. intervals thereafter.

Table 5. *Mean clotting times (sec.) of normal and home-bred resistant mice given daily doses of warfarin*

		Dose of warfarin					
		1 mg./kg.		5 mg./kg.		50 mg./kg.	
	Days after first dose	M	F	M	F	M	F
Normal	1	34·5	37·4	84·3	154·1	125·0	89·9
	2	29·8	34·3	108·8 (1)*	164·2 (2)	141·0 (2)	458·4 (2)
	3	17·7	23·0	87·5 (2)	107·1 (2)	138·6 (1)	205·9 (2)
	4	21·7	25·9	105·0	— (3)	135·6 (1)	175·5 (3)
	5	19·6	28·6	39·2 (1)	— (2)	128·0 (1)	284·2 (1)
	6	22·1	29·9	34·9 (1)	— (1)	44·3 (2)	— (3)
	7	18·5	19·5	40·0 (1)	—	105·7 (1)	— (2)
	8	20·6	23·4	63·2	—	79·0 (1)	— (2)
	9	16·4	20·2	54·9	—	315·5	— (1)
	10	17·4	22·6	124·4	—	— (2)	— (1)
	Mortality	0/3	0/7	3/5	5/5	3/5	4/5
	Days to death	—	—	4,6,8	3,4,5,6,7	4,7,8	5,5,7,9
Home-bred resistant	1	28·0	17·4	48·2	27·1	119·7	46·5
	2	43·7	18·0	194·4	46·9	214·9	46·7
	3	53·8	16·9	175·0 (1)	43·6	58·0	31·6
	4	45·4	17·3	80·8	33·2	41·9	22·2
	7	74·0	15·6	97·5	26·5	57·9	18·0
	8	31·3	16·5	36·6	19·8	57·8	18·5
	9	24·2	16·4	35·5	23·9	48·9	17·3
	10	37·7	16·7	84·9	19·1	83·5	22·5
	Mortality	0/3	0/3	0/2	0/3	2/3	0/3
	Days to death	—	—	—	—	3,7	—

* Figures in parentheses indicate no. of animals with uncoagulable plasma.

At the lowest dosage only one mouse (a female) of the ten examined failed to respond. Maximum elevation of clotting times occurred from 24 to 48 hr. after the first dose. Clotting times then fell and the final values were similar to or less than those obtained before the administration of warfarin in all but two of the mice. At the two higher dosages, clotting times after 24 hr. were more elevated. The duration of the response was also greater, and after the second dose of warfarin the plasma of some individuals was found to be uncoagulable. Furthermore, fifteen animals died and the clotting times of the survivors (four males and one female) were still elevated after the tenth and final dose of warfarin.

Home-bred resistant

In a similar study with HBR mice, marked daily fluctuations in clotting times occurred in two of the three males given daily doses of 1 mg. of warfarin/kg. body weight (Table 5). The mean response of all three however was greater than that of the similarly treated N males. In contrast the response shown by the three HBR females to 1 mg./kg. was slight over the whole test period. Males were also clearly more affected than females when they were given either 5 or 50 mg./kg. doses of warfarin daily and two of the three males receiving the highest dose died. An initial prolongation in clotting time was observed when HBR females were given the two higher doses of warfarin, but after 10 days the clotting times obtained approached the pre-treatment values. No HBR females died.

The effect of 0·025 % *warfarin in medium oatmeal on the clotting time of mouse plasma*

Table 6 shows the results of feeding mice from the three different sources on 0·025 % warfarin in oatmeal and the proportion of animals having uncoagulable plasma at varying intervals during the test periods.

Table 6. *Comparative mortality and plasma coagulability of wild mice from three different sources when fed baits containing 0·025 % warfarin*

		Bait					
		Medium oatmeal			POFOSO		
Source		N	HBR	FR	N	HBR	FR
No. of mice		27	62	44	32	67	8
Proportion of mice with uncoagulable plasma on day	3	27/27	11/19	—	27/27	20/20	—
	4	—	13/15	8/12	9/9	—	—
	5	—	8/16	—	—	44/45	—
	7	4/4	9/12	3/29	5/5	—	—
	10	1/1	11/16	0/4	—	6/6	—
	14	—	3/13	1/25	—	—	—
	21	—	3/22	1/34	1/1	5/5	0/1
Mortality		27/27	40/62	10/44	31/32	62/67	7/8
% mortality		100	64·5	22·7	96·9	92·5	87·5
Range of days to death		4–12	6–17	5–11	1–10	4–19	7–20

N = Normal. HBR = Home-bred, warfarin-resistant. FR = Field-caught, suspected warfarin-resistant.

Normal

Twenty-seven N mice were treated. The clotting times of a group of three males and four females were found to be elevated on day 1 (range 44·7–70·7 sec.), had risen further by day 2 and the plasma of all seven individuals was uncoagulable by day 3. By day 7 three mice had died and the plasma of the other four was still uncoagulable. The plasma of the remaining twenty mice (ten males and ten females) was similarly found to be uncoagulable on day 3. All twenty-seven animals were dead after 12 days.

Home-bred resistant

Sixty-two HBR mice were similarly fed for 21 days. Clotting times were found to be elevated in all the thirty-one mice examined on day 2. In contrast with N mice, however, the plasma of eight mice examined on day 3 was coagulable (Table 6). Furthermore on days 10, 14 and 21 some individuals still had plasma that was coagulable. Again, in comparison with the complete kill obtained with N animals, mortality in HBR mice was low (64·5 %).

Examination of the clotting time values determined in the surviving animals (three males and twelve females) from which blood samples were taken repeatedly showed that they declined considerably during the test period and in some individuals were approaching near normal values by day 21 (range 15·0–146·0 sec.). The clotting times of the seven survivors (four males and three females) from which blood was taken on day 21 only, were comparatively higher, ranging between 51·4 and 308·8 sec. (four mice) and uncoagulable (three mice).

Field resistant

Forty-four FR mice drawn from four different localities were used in further comparative 21-day feeding tests. Eight of the twelve mice (seven males and five females) which were drawn from two localities and examined on day 4 had uncoagulable plasma on that day and died later. On the same day the clotting time values of the four female survivors were 52·6, 21·6, 30·0 and 31·5 sec. Lower values were determined on days 7, 10 and 14 and by day 21 the values had declined to 15·2, 17·3, 15·4 and 17·1 sec. respectively. Only three of twenty-four mice (thirteen males and eleven females) from the other two localities that were examined on day 7 had plasma that was uncoagulable and two, both males, were the only animals that died. Clotting times were found to be prolonged in all but one of the remaining twenty-one mice, but they then declined and on day 21 they were either below or approaching (range 14·4–36·7 sec.) the pre-treatment values.

A further eight individuals (six males and two females) from one of the latter localities all survived the feeding test and on day 21 the plasma of each mouse was found to be coagulable (range of clotting times 16·4–26·8 sec.). The overall mortality of FR mice was very low (10/44, 22·7 %).

The effect of 0·025 % warfarin in an oatmeal/mineral oil/sugar bait-base on the clotting time of mouse plasma

Normal

Thirty-two N mice (sixteen males and sixteen females) were fed 0·025 % warfarin in a bait-base comprising 85 % pinhead oatmeal, 5 % fine oatmeal, 5 % mineral oil and 5 % sugar (POFOSO) for up to 21 days and blood samples were taken daily. As with N mice fed 0·025 % warfarin in medium oatmeal, plasma withdrawn on day 3 failed to coagulate (Table 6). All but one of the thirty-two mice investigated died within 10 days. The survivor, a female, had uncoagulable plasma at the end of the test period.

Home-bred resistant

Two groups of forty-nine and eighteen HBR mice were fed 0·025 % warfarin in POFOSO bait for 21 and 26 days respectively. All twenty mice examined on day 3 had uncoagulable plasma and the overall mortality was high (62/67, 92·5 %). The plasma of the survivors (one male and four females) was found to be still uncoagulable at the end of the test periods.

Field resistant

In a single test, seven out of eight mice died after feeding on 0·025 % warfarin in POFOSO for 21 days. The plasma of the survivor (a female) was still coagulable at the end of the test period (clotting time 40·5 sec.).

Table 7. *Clotting times of seven mice following prolonged exposure to various warfarin baits*

		Sex of mice						
	Day	M	F	F	F	F	F	F
Before exposure	−1	16·5	18·1	17·7	16·4	15·5	17·8	16·5
0·025% warfarin in	2	115·4	121·3	281·0	890·0	181·0	345·0	183·0
oatmeal	21	41·6	25·2	27·9	34·6	70·0	87·1	39·8
	84	28·7	22·7	17·7	24·6	17·6	33·3	25·5
0·025 % warfarin in	86	214·8	244·7	104·4	271·0	46·3	270·6	—
POFOSO	89	UC*	UC	UC	UC	420·0	UC	UC
0·025 % warfarin in	92	780·0	188·5	26·1	D(90)†	18·6	72·4	30·8
oatmeal	96	32·2	18·5	17·9	—	18·4	23·8	18·1
0·1% warfarin in	99	279·1	129·7	38·3	—	156·5	331·9	148·6
oatmeal	124	UC	44·3	38·2	—	84·8	153·5	51·3
Diet 41 b	131	14·9	14·0	15·1	—	12·5	14·2	13·7
0·025 % warfarin in	133	125·4	26·4	33·8	—	36·6	36·9	33·9
oatmeal	138	34·7	25·0	17·6	—	15·4	21·8	27·8
0·025 % warfarin +	141	69·8	40·4	23·1	—	16·6	31·7	540·0
0·025 % sulphaquin-	152	107·6	28·5	20·8	—	27·2	76·5	65·4
oxaline in oatmeal								
0·025% warfarin in	155	281·0	26·8	22·1	—	50·4	90·1	226·2
oatmeal + 5 % corn	159	94·7	18·8	20·5	—	16·2	22·2	39·7
oil + 5 % sugar								
0·2 % warfarin in	163	UC	145·5	47·8	—	UC	UC	UC
oatmeal	209	D(197)	D(209)	UC	—	D(186)	UC	200·7

* UC = Uncoagulable. † D(90) = died on day 90.

The effect of prolonged exposure to various warfarin baits on the clotting time of mouse plasma

Seven of the twenty-two HBR mice (one male and six females) that had survived 21 days feeding on 0·025 % warfarin in plain oatmeal (column 2 of Table 6) were maintained on the same diet for an additional 9-week period. Clotting time determinations made on a further fifteen occasions showed that individual clotting

times varied irregularly during this extended feeding period. However, in all seven mice, clotting times were observed to be lower on day 84 than on day 21, and no mice died (Table 7).

The same animals were then subjected to other feeding tests. After 2 days feeding on 0·025 % warfarin in POFOSO, clotting times were found to be markedly elevated. Three days later (day 89) the plasma of six of the mice was found to be uncoagulable and the clotting time was prolonged in the case of the seventh animal. All seven mice were then once more fed on 0·025 % warfarin in plain medium oatmeal. One mouse died on day 90 but blood samples taken from the other six on two occasions during the next 7 days showed that clotting times were again declining or had returned to normal values. When the concentration of warfarin in the medium oatmeal bait was increased from 0·025 to 0·1 % (day 96) an increase in clotting time occurred. Determinations made on five occasions during the next 28 days showed that individual clotting times fluctuated irregularly. The plasma of one animal failed to coagulate at each of three successive samplings taken over a period of 14 days but on day 124 the clotting times of the other five individuals had again declined from peak values.

The mice were then transferred to a laboratory diet, diet 41*b*, (Bruce & Parkes, 1949) for 1 week, at the end of which (day 131) individual clotting times were below the pre-treatment values. The response of the mice to a further feeding period on 0·025 % warfarin in oatmeal was next investigated. After 2 days (day 133), prolonged clotting times in all six individuals were observed but the response was less marked than at the corresponding time in the first 21-day test period, when the same warfarin bait was used. Clotting times also tended to return to near normal values earlier than in the initial test period. The addition of 0·025 % sulphaquinoxaline to medium oatmeal bait containing 0·025 % warfarin in a further 14-day feeding period (beginning on Day 138) resulted in irregular but elevated clotting times. When the mice were fed warfarin in an oatmeal/corn oil/sugar bait-base, a further initial increase in clotting times occurred but on the seventh and last day (day 159), the clotting times had again declined considerably. In a final feeding period lasting 50 days, the mice were fed on 0·2 % warfarin in plain medium oatmeal. A marked rise in clotting times occurred and at some time during the test period the plasma of all individuals was rendered uncoagulable. Three mice died on days 186, 197 and 209 respectively.

The effect of a single dose of warfarin by injection and 0·025 % warfarin in medium oatmeal on the clotting time of mouse plasma

Normal

Clotting times of twelve N mice (six males and six females) were determined 3 days before and 24 and 48 hr. after each animal was injected with 5 mg. warfarin/kg. body weight. After a recovery period of 15 days the mice were fed on 0·025 % warfarin in medium oatmeal for 21 days. One animal, a female, survived the feeding period. In the females but not in the males (mean clotting time 59·3 sec.) there was some evidence of a negative correlation between the clotting time following

the injection of warfarin and the survival time during the subsequent feeding test. Female clotting times determined after 24 hr. were 103·8, 46·3, 50·5, 33·9, 25·8 and 26·5 sec. (mean 47·8 sec.) corresponding to deaths on days 6, 8, 10, 17, 19 and survival respectively.

In a similar test employing a dose of 10 mg. warfarin/kg. body weight, the clotting times of six males (mean 90·1 sec.) and four females (mean 71·5 sec.) were more elevated and all but one animal, a male, died during a subsequent feeding test. There was no obvious relationship between clotting and survival times in either males or females.

Home-bred resistant

In a single test with HBR mice, seven males and eleven females were injected with 10 mg. warfarin/kg. body weight before they were fed on 0·025 % warfarin in oatmeal for 21 days. Clotting times were determined 24 hr. before and after warfarin was injected. One male and four females survived the feeding test. Again with male animals, no obvious relationship was evident between clotting time (range 20·5–82·1 sec.) after the injection of warfarin and survival time during the feeding period. The response of the four female survivors, however, to the injected dose of warfarin (range of clotting times 15·2–21·4 sec.) was less than that of those that died (range of clotting times 30·9–168·1 sec.).

Table 8. *The clotting and survival times of field resistant mice receiving successive doses of warfarin and fed on* 0·025 % *warfarin bait*

	Clotting time after 24 hr. (sec.) Dose (mg./kg.)				
Sex	1	5	50	100	Day to death during 21-day feeding period
M	18·0	60·9	175·8	166·6	8
M	22·1	121·2	175·0	163·8	7
M	29·0	40·5	110·1	88·4	5
M	40·6	—	172·8	104·3	10
M	34·0	70·5	145·3	105·4	7
M	22·6	29·7	48·1	101·1	11
M	59·4	137·6	133·7	93·8	8
F	33·5	18·7	17·8	25·1	Survived
F	16·2	17·8	17·8	16·9	Survived
F	25·0	19·4	21·5	23·2	Survived
F	31·0	30·0	24·2	18·8	Survived
F	16·2	33·2	40·2	36·1	11

Field resistant

A shortage of test animals prevented a straightforward comparative test using FR animals. However, the FR mice referred to in Table 8 were also fed 0·025 % warfarin in medium oatmeal for 21 days after recovering from injected doses of warfarin. The individual clotting times 24 hr. after each of these mice had been given an injected dose of 1, 5, 50 and 100 mg. of warfarin/kg. body weight and the survival times in the feeding test given later are shown in Table 8. All seven

males died during the feeding test but, as with N and HBR males, no relationship was found between clotting time after dosing and the survival period. With females, however, the general lack of response even to the higher warfarin doses was in keeping with the low mortality (1/5) obtained during the feeding period.

DISCUSSION

Investigations into the changes in prothrombin times following the administration of coumarin and indanedione anticoagulants have been made in man and various laboratory and domesticated animals. It is clear from these studies that there is considerable inter-specific and intra-specific variation in response to these drugs. Using dicoumarol (3,3′-methylene-bis-4-hydroxycoumarin), Millar, Jaques & Henriet (1964) and Chandrasekhar, Hickie & Millar (1965) were able to classify rabbits and rats respectively into broad groups—non-reactors, poor reactors, reactors and hyper-reactors—on the basis of the change of prothrombin time. The wide range of days to death and percentage mortality that occurred when wild house mice were fed on bait containing warfarin (Rowe & Redfern, 1964) and other anticoagulants (Rowe & Redfern, unpublished) for fixed periods ranging from 4 to 28 days showed that this species also varies considerably in its susceptibility to anticoagulants. A further study (Rowe & Redfern, 1965) suggested that resistance to warfarin in mice was under genetical control.

As shown above, while untreated wild mice in the present study differed somewhat in their resting blood clotting factor levels, the mean clotting time values of untreated male and female mice from three different sources, or of males and females from the same source did not differ significantly. Nor was there any evidence of a clear-cut relationship between pre-treatment clotting time and the prolongation in clotting time that occurred when warfarin was administered.

The fluctuations and overall fall in clotting times that occurred when blood samples were taken daily from untreated mice is in keeping with the daily changes observed in rats following successive samplings (Pyorälä, 1965). Pyorälä comments that the stress imposed on experimental animals through repeated blood samplings may increase the activity of the sympathetic nervous system and lead to an increased production of blood clotting factors. In the present experiments therefore it is possible that the clotting factor levels of the individual mice from which blood was withdrawn regularly may have been abnormally increased and the effect due to warfarin reduced. Even so, such an effect cannot account for the marked differences in response to warfarin described in this paper both between mice derived from the same source and from the different sources.

All N mice responded to a single dose of warfarin at 1 mg./kg., and at higher doses (5 and 50 mg./kg.) clotting times became even more prolonged (Table 3). The overall response of HBR and FR mice given a single dose of 1 mg. of warfarin per kg. body weight (Table 3) was less marked than that found to occur in N individuals and was mainly due, in both cases, to a high degree of tolerance displayed by females. At the higher doses the increase in clotting time observed in HBR and FR male mice was not appreciably different from that seen in N males.

Chandrasekhar *et al.* (1965) point out that there are conflicting opinions concerning the development of tolerance to dicoumarol by rats. Mogenson, Fisher & Jaques (1958) and Boyd & Warner (1948) concluded that rats developed a tolerance after 1 and 2 weeks dicoumarol treatment respectively, whereas Slätis (1958) found no evidence that rats became tolerant to this anticoagulant following its administration for 5 weeks. Chandrasekhar *et al.* (1965) observed no development of tolerance following short-term administration of dicoumarol but found that tolerance developed during longer term administration. A similar conclusion with regard to warfarin is drawn from the present studies. The clotting times of N mice given two identical doses at an interval of 3 weeks were found to be similar. Most N individuals given 1 mg./kg. doses of warfarin daily for 10 days, however, appeared to develop a tolerance to the drug (Table 5). Not unexpectedly, the development of tolerance appeared to be dependent on the dose level of warfarin administered, the proportion of mice showing tolerance decreasing with increasing dosage. Home-bred resistant mice were able to develop tolerance to daily doses of warfarin at higher dosage levels than N mice. No male or female HBR mice died after receiving daily doses of either 1 or 5 mg. of warfarin/kg. body weight for 10 days and clotting times tended to decline from peak values during the test periods. At 50 mg./kg. some HBR males died but no females.

Differences in the response to warfarin of N, HBR and FR mice as judged by the relative amounts of poison bait consumed, clotting time determinations and overall mortality were clearly evident in the comparative feeding experiments (Table 6). Of twenty-seven N mice fed 0·025 % warfarin in oatmeal only eight were well enough to feed during the second week. Examination of blood samples showed that each animal had uncoagulable plasma by the third day and all the mice were dead after 12 days. In the similar study with sixty-two HBR mice, however, forty-seven animals continued to feed in the second week and twenty-eight in the third week. Of the twenty-two survivors, seventeen fed fairly evenly throughout the 3-week period; the bait consumption of the other five declined markedly during the second week but increased in the third week. Although some individuals had uncoagulable plasma after 3 days feeding, the plasma of all but three of the twenty-two surviving mice (mortality 64·5 %) was still coagulable on day 21, the last of the test period. In the study of FR mice, forty of the forty-four animals fed during the second week and thirty-four continued to feed during the third week and survived the test period. All but five of the thirty-four survivors (mortality 22·7 %) fed adequately throughout the test periods. On day 21 the clotting time of all but one of the survivors was near the pre-treatment value. In the above tests with HBR and FR mice relatively more males died (35/59) than females (15/47) the difference being highly significant [$\chi^2 = 6{\cdot}8$; $P = 0{\cdot}01$–$0{\cdot}001$]. This result is in agreement with our earlier findings that a sex difference in susceptibility to warfarin exists in mice (Rowe & Redfern, 1964, 1965, 1967). A similar conclusion was reached by Roll (1966).

The coagulability of the plasma of N, HBR and FR mice was impaired comparatively earlier when they fed on 0·025 % warfarin in the oily bait-base POFOSO instead of in plain oatmeal and mortality was also higher (Table 6). Markedly

prolonged clotting times were also observed within 2 days when seven HBR mice were fed on warfarin in POFOSO after having fed on warfarin in plain oatmeal for 12 weeks (Table 7). Neither an increase in the concentration of warfarin to 0·1 % nor the inclusion of sulphaquinoxaline or corn oil in the latter bait was as effective as 0·025 % warfarin in POFOSO in decreasing the coagulability of the plasma of these individuals. Although no marked increases in plasma clotting times were observed in other tests when mice were fed plain POFOSO, it is possible that as with rats (Drummond, 1967), mineral oil produces a situation favourable to the action of warfarin by interfering with the absorption of vitamin K from the gastro-intestinal tract.

At the present time mice are screened for resistance to warfarin in this laboratory on the basis of their ability to withstand a standard 21-day feeding test on 0·025 % warfarin in plain medium oatmeal. Clearly it would be advantageous to assess levels of resistance by a less laborious and time consuming method such as the change in clotting time following the injection of a single dose of warfarin. The results of injection and feeding tests on mice in the present study are sufficiently encouraging in the case of female animals to warrant further investigation of this aspect.

The data presented here support our previous conclusion that wild mice differ considerably in their susceptibility to warfarin. The abnormal level of resistance to warfarin shown by HBR mice also supports the earlier conclusion that warfarin resistance in wild mice is inherited. A similar conclusion may be drawn from the differences in response to warfarin shown by different laboratory strains of mice (Rowe & Redfern, unpublished) and from the increased level of resistance found by Roll (1966) when two strains of laboratory mice were exposed to warfarin in breeding and selection experiments conducted over several generations. Selection for resistant individuals is also likely to occur when free-living wild house mouse populations are continually exposed to warfarin during poison treatments and, as a result, populations having an order of resistance shown by the FR mice examined in this study may arise. The physiological mechanisms involved in warfarin resistance in mice are unknown, but the present experiments clearly show that some individuals that survived prolonged feeding on 0·025 % warfarin in oatmeal developed a tolerance to the drug. The combined level of factors II, VII and X in their blood diminished initially but then tended to recover. It is suggested that the development of tolerance is most likely an outcome of an increased ability to metabolize warfarin and that detoxification results from increased enzyme activity (Brodie, Maickel & Jondorf, 1958).

Although the response of mice to warfarin was found to increase in the presence of mineral oil it was also found that a few individuals could survive 21 days feeding on 0·025 % warfarin in bait containing mineral oil. These animals showed no haemorrhagic tendencies although their blood was probably uncoagulable for most of that time. In these very resistant individuals it may be further necessary therefore to interfere with other haemostatic mechanisms such as the adhesiveness of the blood platelets or vascular integrity in order to bring about haemorrhage and death (Jaques, 1959).

SUMMARY

The coagulability of the plasma of blood taken from the retro-orbital sinus of untreated and warfarin-treated wild house mice (*Mus musculus* L.) was determined. Individual differences were observed in the resting clotting times of animals drawn from three different sources, so-called 'normal' (N) mice, home-bred warfarin resistant (HBR) mice and field-caught suspected warfarin resistant (FR) mice. Mice from the three sources also showed wide individual variation in response to injected doses of 1, 5 and 50 mg. of warfarin/kg. body weight. The overall response shown by HBR and FR mice, particularly females, was less than that shown by N animals. Some FR females failed to respond to a dose of 100 mg. of warfarin/kg. body weight.

Normal mice showed a similar response to two identical doses of warfarin (1, 5 or 50 mg./kg. body weight) given at an interval of 3 weeks. They developed a tolerance to repeated daily injected doses of 1 mg. of warfarin/kg. body weight but not to the higher doses (5 and 50 mg./kg.) to which some HBR animals became tolerant.

In comparative 21-day feeding tests with 0·025% warfarin in medium oatmeal, the mortality in N, HBR and FR mice was 100% (27), 64·5% (40/62) and 22·7% (10/44) respectively. Whereas the plasma of all N mice was rendered uncoagulable after 3 days and the animals died within 12 days, the clotting times of the surviving HBR and FR mice (24/59 males and 32/47 females) either were not at any stage appreciably increased or had declined to near normal values by the end of the test period.

In similar tests with 0·025% warfarin in oatmeal bait containing 5% mineral oil, it was found that the clotting ability of the plasma of N, HBR and FR mice was impaired earlier and that mortality in HBR and FR animals was significantly higher (62/67, 92·5% and 7/8, 87·5% respectively).

Three of seven HBR mice died after prolonged feeding on various warfarin baits but only after 186, 197 and 209 days respectively. The inclusion of mineral oil in bait containing 0·025% warfarin was more effective in decreasing the coagulability of the blood of these mice than either increasing the concentration of warfarin (to 0·1 and 0·2%) or including sulphaquinoxaline or corn oil.

Studies on mice given warfarin by injection and in feed indicated that in females the level of resistance to warfarin may be assessed on the basis of their response to a single injected dose of warfarin.

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