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The effect of sex and age on the response to warfarin in a non-inbred strain of mice*

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INTRODUCTION

The results of a study of the toxicity of warfarin [3-(α-acetonylbenzyl)-4-hydroxycoumarin] to wild house-mice (*Mus musculus* L.) indicated that there was a difference in susceptibility attributable to sex (Rowe & Redfern, 1964). In free-feeding tests lasting from 4 to 28 days, proportionally more male than female mice were killed after feeding on bait containing 0·025 % warfarin and the average time to death of females was longer than that of males. Furthermore, although the mice tested were of unknown age, the data also suggested that the heaviest, and therefore probably the oldest females were the most difficult animals to kill. These findings prompted the following further investigation of the possible influence of sex and age on the response to warfarin in mice.

METHODS

The mice employed in this study were a non-inbred strain (L. A. C. Grey) developed at the Laboratory Animals Centre, Medical Research Council Laboratories, Carshalton. Preliminary sighting tests showed that this laboratory strain of mice was more susceptible to warfarin poisoning than the wild species. Twelve out of twenty L.A.C. Greys were killed after feeding on bait containing 0·005 % warfarin for 3 days compared with only six out of thirty wild mice fed on bait containing 0·025 % warfarin over the same period.

One hundred (fifty male and fifty female) L. A. C. Grey mice were paired at random when they were 6 weeks old; each pair was placed in a container measuring 12 × 5 × 4 in. and supplied with a composite diet (diet 41*b*) and water *ad lib.* The date of birth of litters was recorded and the litters taken from their parents when they were 3 weeks old. Littermates were housed together until they were 5 weeks of age and then isolated in metal test-cages measuring 14 × 11 × 6 in.

The adult pairs were allowed to breed a second time and each male was removed from its breeding female as soon as the latter was again clearly pregnant. The dates of birth of second litters were recorded and the young removed from their mothers when they were 3–4 weeks old. Littermates of the same sex were kept together until they had reached the age of 22 weeks at which time they were isolated in test cages. Parent mice were similarly caged 2 weeks prior to poisoning.

Apart from some of the females of second litters, all mice were tested in the same manner. Each animal was weighed and on the following day diet 41*b* was removed from its cage; it was then presented with a plain bait-base consisting of pinhead oatmeal, caster sugar, and a technical grade 'white oil' in the proportions by weight of 17:1:1 respectively for 4 days. At the end of this period an excess amount of bait-base containing 0·005% warfarin was offered to the mouse daily for 3 days. The poison was dispersed thoroughly in fine oatmeal to give a master-mix containing 0·1% warfarin and one part of the master-mix was then added to 19 parts of the bait-base. The total amounts of bait-base and poison bait eaten were recorded, paper placed in the tray underneath each cage facilitating the collection of spilled food. After the end of the 3-day test period, the warfarin bait was replaced by diet 41*b*. The times of death and the weights of mice that died were recorded and the dead animals autopsied. Animals tested in this manner included the established breeding pairs when they were 6 months old, first litter males and females at the age of 6 weeks, and all the males and a proportion of the females of second litters at the age of 6 months.

The remaining females from second litters were used in two toxicity tests undertaken to determine the effect on the mortality level of incorporating the substituted male hormone, methyl testosterone, in their feed. In both these tests, animals were divided into two groups so that as far as possible each group contained the same number from a given litter. In the first test, nine females were offered bait containing 0·005% warfarin, and ten others bait containing 0·05% methyl testosterone and 0·005% warfarin, each over a period of 3 days. In a later test, thirty females were fed on crushed diet 41*b* containing 0·05% methyl testosterone for a 2-week period before they were presented with bait containing 0·005% warfarin and 0·05% methyl testosterone for 3 days; simultaneously a control group of twenty-nine females were offered bait containing only 0·005% warfarin for 3 days. Details of other tests are given below.

RESULTS

The results of the 3-day free-feeding toxicity tests with bait containing 0·005% warfarin are summarized in Table 1.

The data in Table 1 show that there was a clear-cut sex difference in response to warfarin poisoning in mice 6 months old, significantly more male than female parent and second litter mice succumbing to the toxic effects of warfarin. Although the mean body weight of male animals was greater than that of females the higher comparable mortality of 6 month old males cannot be attributed to any greater food intake on their part: for adult female mice survived better than adult males despite consuming on average a higher dose of poison (Table 2). No sex difference in the action of warfarin was evident in mice 6 weeks old and there was no marked difference in the amount of poison consumed by surviving males and females of this age class.

The results of the two experiments in which adult female mice were administered methyl testosterone in their diet are given in Table 3.

No marked differential mortality occurred between adult female mice treated with either warfarin bait alone or with bait containing warfarin and methyl testosterone over a 3-day period. The average dose of warfarin received by mice in each of the two groups during this period was 23·2 and 20·0 mg./kg. respectively.

Table 1. *Effect of sex and age on toxicity of warfarin in L.A.C. Grey mice.*

Group	Age	Mortality	Mortality (%)	Significance of difference
Parent males	6 months	35/46	76·1	$P = < 0{\cdot}001$
Parent females	6 months	7/47	14·9	
First litter males	6 weeks	44/141	31·2	$P = 0{\cdot}1–0{\cdot}5$
First litter females	6 weeks	37/138	26·8	
Second litter males	6 months	80/120	66·7	$P = < 0{\cdot}001$
Second litter females	6 months	14/44	31·8	

Table 2. *The average dose of warfarin consumed by male and female L.A.C. Grey mice of two age classes*

Sex	Result	Number	Average weight (g.)	Average total warfarin dose (mg./kg.)
Parents (6 months)				
M	Died	35	37·5	13·4
	Survived	11	39·3	16·3
F	Died	7	32·1	15·2
	Survived	40	33·0	21·6
First Litters (6 weeks)				
M	Died	44	21·1	22·5
	Survived	97	20·0	27·4
F	Died	37	18·2	26·2
	Survived	101	18·2	25·8
Second Litters (6 months)				
M	Died	80	38·1	15·7
	Survived	40	38·8	20·3
F	Died	14	28·5	13·7
	Survived	30	31·0	26·6

Table 3. *Effect of methyl testosterone on mortality in 6-month-old female mice fed on bait containing 0·005 % warfarin*

Treatment	Days	Mortality	Mortality (%)
0·005 % warfarin	3	1/9	11·1
0·005 % warfarin plus 0·05 % methyl testosterone	3	0/10	0·0
0·005 % warfarin	3	9/29	31·1
0·005 % warfarin plus 0·05 % methyl testosterone*	3	16/30	53·3

* Having first been fed for 2 weeks on bait containing 0·05 % methyl testosterone

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The response to warfarin in the second test was greatest in those females presented with methyl testosterone in their feed for 2 weeks before they were poisoned with warfarin, but the difference in mortality between treated and untreated animals was not significant ($\chi^2 = 2{\cdot}2$, $P = 0{\cdot}1$–$0{\cdot}5$). It is possible, however, that the mortality in females pre-treated with methyl testosterone would have been greater had they eaten as much as the untreated females. The pre-treated mice ate 15·2 mg./kg. on the average and the untreated females ate 19·5 mg./kg.

In a similar test undertaken later, female littermates were divided into three groups soon after they were weaned. When they were 6 months old mice in each of the three groups were offered 0·005% warfarin bait over a 3-day period. Females of the first group were given methyl testosterone in their feed for 2 weeks before poisoning; the second and third groups of females were subjected to warfarin poisoning only, animals in the last group, however, being first allowed to breed. The mortality obtained in each of the three groups of females was 10/25, 7/26 and 8/26 respectively.

DISCUSSION

The response of laboratory rats and mice to a number of chemical substances has been observed to vary with sex. For example, Quinn, Axelrod & Brodie (1958) showed that the duration of action of hexobarbitone was about four times longer in female rats than in males, while DuBois, Doull, Salerno & Coon (1949) found that parathion was more toxic to female than to male rats.

Venho (1959) studied the effect of sex on the toxicity of the anticoagulants dicoumarol and phenylindanedione to mice and found that females were more resistant than males to these drugs. A similar sex variation in response to warfarin was found in adult laboratory mice in the present study and supports the earlier observation made on wild mice (Rowe & Redfern, 1964).

Warfarin is antagonistic to vitamin K and the symptoms of warfarin poisoning and vitamin K deficiency—hypoprothrombinemia and haemorrhage—are identical. Metta & Johnson (1960) who maintained rats on a diet deficient in vitamin K found that females were less susceptible than males to vitamin K deficiency. It is considered probable therefore that female mice survived warfarin poisoning better than males in the present study because, like female rats, they are less susceptible to vitamin K deficiency.

Quinn *et al.* (1958) further showed that the metabolism of hexobarbitone could be influenced by sex hormones, oestradiol administration increasing the response of male rats to the drug and testosterone reducing the response of females. From the results of their study Metta & Johnson (1960) concluded that the greater susceptibility of male rats to an artificially induced vitamin K deficiency was not associated with coprophagy or due to any higher food intake on their part compared with females but was a true sex difference under hormonal control. Some evidence that sex hormones also play a part in determining the response of male mice to anticoagulants was provided by Venho (1959) who found that the administration of oestradiol to castrated male mice decreased the mortality due to dicoumarol. Further indirect evidence to support the viewpoint that sex hormones influence

the response of male mice to anticoagulants is given by the mortality data (Tables 1 and 2) of immature and mature male animals given warfarin. A marked lower mortality occurred in the younger animals although on average they ate relatively more poison than the older animals—showing that male mice become more susceptible to warfarin poisoning with maturity. This result is in accordance with the work of Mellette & Leone (1960) who kept male rats of differing ages on a diet deficient in vitamin K and found that susceptibility to hypoprothrombinemia increased with age.

These authors also found the reverse situation in female rats, the latter becoming less susceptible to a lack of dietary vitamin K with age. Furthermore, they reported that the decline in the prothrombin level of female rats maintained on a vitamin K free diet and injected with testosterone propionate was greater than that of females maintained on a vitamin K free diet only. There was no evidence in the present study, however, that female mice become more resistant to warfarin with age. The mortality in female mice 6 weeks old was not significantly different from that of either 6-month-old parent or second litter females ($\chi^2 = 2{\cdot}1$, $P = 0{\cdot}1$–$0{\cdot}5$; $\chi^2 = 0{\cdot}2$, $P = 0{\cdot}5$–$0{\cdot}9$ respectively), despite the fact that again the younger animals consumed relatively more poison (Tables 1 and 2). Although the mortality data of Table 1 suggested the possibility that 6-month-old females that had bred were more susceptible to warfarin than virgin animals of the same age, this was not substantiated by further experiments. The present experiments also showed that the prior administration of methyl testosterone did not significantly enhance the toxicity of warfarin to adult female mice and this is similar to the finding of Venho (1959) that the administration of testosterone to castrated female mice did not change the mortality due to dicoumarol.

SUMMARY

1. A marked sex variation in susceptibility to warfarin was found in 6-month old L.A.C. Grey mice, males being more susceptible than females. No sex difference in response was evident in young mice 6 weeks old.

2. Susceptibility to warfarin poisoning increased markedly with age in males but not in females.

3. No significant increase in mortality following warfarin poisoning occurred in adult female mice given methyl testosterone.

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