J. Hyg., Camb. (1974), **73**, 401
Printed in Great Britain

An analysis of the susceptibilities of several populations of *Rattus norvegicus* to warfarin

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(*Received* 13 *May* 1974)

SUMMARY

An analysis was made of the dose-response of several populations of *Rattus norvegicus* fed upon baits containing 0·005 % warfarin for various numbers of days. Warfarin-susceptible populations fell within a narrow range, with LFP 50s and LFP 98s (lethal feeding periods in days to obtain 50 % and 98 % mortalities respectively) of up to 3·0 and 5·0 days respectively. The probability of an individual rat from these populations surviving a six-day feeding period was estimated at 0·003 or less. Populations with responses falling beyond these limits were regarded as warfarin-resistant.

Six of nine populations of *R. norvegicus*, from England, Germany and the United States, were determined to be warfarin-susceptible within the narrow limits given above. In all six cases, no animals survived the six-day WHO feeding test for anticoagulant susceptibility. In three populations from the United States, where rats survived six days feeding, their population responses clearly fell outside the measures given above. It is suggested, tentatively, that anticoagulant-resistant Norway rat populations be defined as those whose LFP 50 and LFP 98 exceeds 3·0 and 5·0 days respectively, and in which the probability of an individual animal surviving a six-day feeding upon 0·005 % warfarin is 0·01 or more.

INTRODUCTION

The purpose of the anticoagulant-resistance feeding test as originally proposed by Drummond (1966) and later modified and adopted by the World Health Organization (1970) is to measure the susceptibility of rodent populations to a given anticoagulant rodenticide. The proposed concentrations of anticoagulants and number of days of feeding were left open to the discretion of investigators until additional experience dictated the appropriate concentrations or time periods.

In a subsequent paper, Drummond & Wilson (1968) suggested that warfarin at 0·005 % concentration (by weight) in a suitable bait fed to *Rattus norvegicus* for six days provided a suitable screening test for detecting resistance in that species. Bentley (1969) subsequently defined resistant Norway rats in the United Kingdom as those that survived a standard feeding period of six days on 0·005 % warfarin in the laboratory. Other investigators, including ourselves (Brooks & Bowerman, 1973), have used this test as a basis for detection of anticoagulant-resistant rats in the United States. More recently, the six-day feeding test at

0·005 % warfarin has been used as a screening test to detect developing anticoagulant resistance in Norway rats in cities in the United States (Jackson, Brooks, Bowerman & Kaukeinen, 1973).

The original basis for using a six-day feeding at 0·005 % warfarin concentration rests upon the evidence provided by Drummond & Wilson (1968) of one population of susceptible Norway rats in England. Further confirmation was provided by ourselves (Brooks & Bowerman, 1973) from the testing and analysis of two warfarin-naïve Norway rat populations in New York State. In this present paper we re-examine the published data on the susceptibility of *Rattus norvegicus* to warfarin and also look at new evidence derived by ourselves on additional Norway rat populations. Our purpose will be to present an analysis of base-line warfarin susceptibility in *Rattus norvegicus* and to derive statistical criteria by which both normal warfarin-susceptible rat populations may be defined and those by which resistant populations may be characterized.

MATERIALS AND METHODS

Norway rats, both wild-caught and a domestic laboratory strain (Long-Evans), were individually caged after first being weighed and sexed. Sick, pregnant and immature animals were set aside. A basal diet of laboratory meal was provided for each animal and water was available *ad lib*. Animals were acclimated to cage conditions for a minimum of three weeks before testing.

The test procedure employed a pre-test baiting with either ground rolled oats or Purina laboratory chow. Rolled oats were used in our earlier testing and Purina chow has been used routinely as a standard test diet since April 1972. After feeding was stabilized, the amount eaten daily was measured for two days preceding the trial. Then, for periods ranging from one to twelve days, groups of rats were allowed unrestricted feeding upon a bait containing 0·005 % warfarin, by weight. Food consumption was measured daily, accounting also for any spillage caught on papers below each cage. The warfarin was supplied by the Wisconsin Alumni Research Foundation as a technical powder and was mixed into a master concentrate with corn starch or finely ground Purina lab chow. Animals were observed daily during the trial and for a ten-day period after the last warfarin feeding for symptoms of anticoagulant poisoning (bleeding, pilo-erection, sluggishness, bleached extremities) and for mortality. Dead animals were autopsied to verify anticoagulant effects. After the poisoning trial, animals were returned to the basal diet of laboratory meal. Animal weights were determined immediately before the warfarin baiting and a terminal weight was obtained. In some cases, animals weighed less than 150 g., but having been caged at least 60 days, were used in the trials.

All mortality data were evaluated using the dose-response analysis as proposed by Litchfield & Wilcoxon (1949). Data were first evaluated for males and females separately, and if no significant differences were found in slope function ratios and potency ratios, their combined data were used. Significant differences occurred only between males and females from Cambridge, New York and results for them are presented separately.

Table 1. *Mortality to Norway rats from several populations after unrestricted feeding on baits containing* 0·005 % *warfarin for various numbers of days*

Population	Days feeding	Mortality					
		Males		Females		Both sexes	
		No.	Percent	No.	Percent	No.	Percent
Refuse destructor, English Midlands (Drummond & Wilson, 1968)	1	0/2	0·0	5/10	50·0	5/12	41·7
	2	3/7	42·8	16/22	72·7	19/29	65·5
	3	16/16	100·0	14/14	100·0	30/30	100·0
	4	7/7	100·0	4/4	100·0	11/11	100·0
	6	3/3	100·0	4/4	100·0	7/7	100·0
Refuse disposal site, Merrick, N.Y. (Brooks & Bowerman, 1973)	1	3/12	33·3	1/11	9·1	5/23	21·7
	2	19/22	86·3	5/10	50·0	24/32	75·0
	3	10/10	100·0	10/10	100·0	20/20	100·0
Chicken farm, Sharon Springs, N.Y.	1	1/8	12·5	2/8	25·0	3/16	18·7
	2	9/10	90·0	5/10	50·0	14/20	70·0
	4	8/8	100·0	9/10	90·0	17/18	94·4
	6	11/11	100·0	11/11	100·0	22/22	100·0
Refuse tip, Borkum, Germany (Telle, 1971)	2	12/29	41·4	20/32	62·5	32/61	52·4
	3	10/11	90·9	8/8	100·0	18/19	94·7
	4	15/15	100·0	16/16	100·0	31/31	100·0
	5	40/40	100·0	18/18	100·0	58/58	100·0
	6	4/4	100·0	6/6	100·0	10/10	100·0
Refuse disposal site, Berlin, N.Y. (Brooks & Bowerman, 1973)	2	1/10	10·0	1/7	14·3	2/17	11·8
	3	9/10	90·0	7/8	87·5	16/18	88·8
	4	10/10	100·0	10/14	71·4	20/24	83·3
	5	5/5	100·0	9/9	100·0	14/14	100·0
Long-Evans laboratory rats	1	—	—	0/10	0·0	0/10	0·0
	2	1/10	10·0	4/10	40·0	5/20	25·0
	3	8/10	80·0	8/10	80·0	16/20	80·0
	4	10/12	83·3	10/10	100·0	20/22	90·9
	5	9/10	90·0	—	—	—	—
	6	12/12	100·0	2/2	100·0	14/14	100·0
Refuse disposal site, Pittstown, N.Y. (Brooks & Bowerman, 1973)	2	3/10	30·0	3/12	25·0	6/22	27·2
	3	9/15	60·0	5/12	41·7	14/27	51·8
	4	9/10	90·0	9/11	81·8	18/21	85·7
	5	11/11	100·0	9/10	90·0	20/21	95·2
	6	9/9	100·0	10/11	90·9	19/20	95·0
	7	—	—	14/14	100·0	14/14	100·0
Feed mill, Albany, N.Y.	2	0/6	0·0	0/8	0·0	0/14	0·0
	4	4/8	50·0	2/8	25·0	6/16	37·5
	5	7/8	87·5	4/8	50·0	11/16	68·7
	6	15/15	100·0	24/25	96·0	39/40	97·5
Turkey farm, Cambridge, N.Y. (Brooks & Bowerman, 1973)	4	1/8	12·5	1/8	12·5	2/16	12·5
	6	6/8	75·0	5/8	62·5	11/16	68·7
	8	8/8	100·0	6/8	75·0	14/16	87·5
	10	12/12	100·0	10/12	83·3	22/24	91·6
	12	—	—	9/10	90·0	—	—

Table 2. *Base-line susceptibilities of several populations of* Rattus norvegicus *to* 0·005 % *warfarin* (95 % *confidence limits in days given for each lethal feeding period*)

The probability of a rat from each population surviving a six-day feeding upon 0·005 % warfarin is estimated using a log-normal distribution fit, where the mean is estimated as the log LFP 50 and the standard deviation is estimated as the log-slope function(S).

Population	Number of rats	Slope function (S)	LFP 50 (days)	LFP 98 (days)	Probability of survival
Merrick, New York	75	1·42	1·44 (1·26–1·64)	3·00 (2·29–3·93)	0·0005
Borkum, Germany	179	1·29	1·97 (1·79–2·17)	3·36 (2·77–4·06)	0·0005
English Midlands	89	1·72	1·35 (1·05–1·73)	3·80 (2·14–6·72)	0·003
Sharon Springs, New York	76	1·64	1·54 (1·22–1·94)	4·25 (2·74–6·59)	0·003
Long Evans rats	96	1·37	2·46 (2·14–2·83)	4·75 (3·89–5·79)	0·002
Berlin, New York	73	1·34	2·58 (2·28–2·92)	4·82 (3·79–6·13)	0·002
Pittstown, New York	125	1·48	2·77 (2·43–3·16)	6·20 (3·95–9·73)	0·024
Albany, New York	86	1·26	4·35 (3·91–4·91)	7·00 (5·69–8·61)	0·082
Cambridge, New York (males)	36	1·24	5·15 (4·15–6·38)	8·05 (5·88–11·03)	0·238
Cambridge, New York (females)	46	1·80	5·40 (4·91–5·94)	17·7 (—)	0·428

In all cases, lines were fitted to the observed mortalities on log-probability paper until the best fit was obtained. Then the LFP 50s and LFP 98s (Lethal Feeding Periods, in days, to obtain 50 % and 98 % mortalities, respectively) were obtained and the 95 % confidence limits at these dosage levels were estimated (Litchfield & Wilcoxon, 1949). We are following the terminology as suggested by the British workers (Rowe & Redfern, 1964; Drummond, personal communication) of Lethal Feeding Period, rather than Effective Dose, as being a better description of the nature of the measurement. The LFP 50 is also known as the mean Lethal Feeding Period. The slope functions, which are 'the fold change in the line required to produce a unit standard deviation change in response along the line' (Litchfield & Wilcoxon, 1949) are given also as part of the descriptive parameters.

RESULTS AND DISCUSSION

Data are presented on nine Norway rat populations (Table 1). Six populations were examined from published literature (Drummond & Wilson, 1968; Telle, 1971 and Brooks & Bowerman, 1973) and three are populations recently tested by ourselves.

Populations are ranked by increasing order according to their LFP 98s in Table 2. The first six populations could all be described as warfarin-susceptible, since in all cases 100 % mortality was achieved in six days' feeding or less. These six warfarin-susceptible populations are drawn from widely separated geographic areas and represent rats both with and without past exposure to anticoagulants. Telle (1971) describes the rats from Borkum as coming from an indigenous

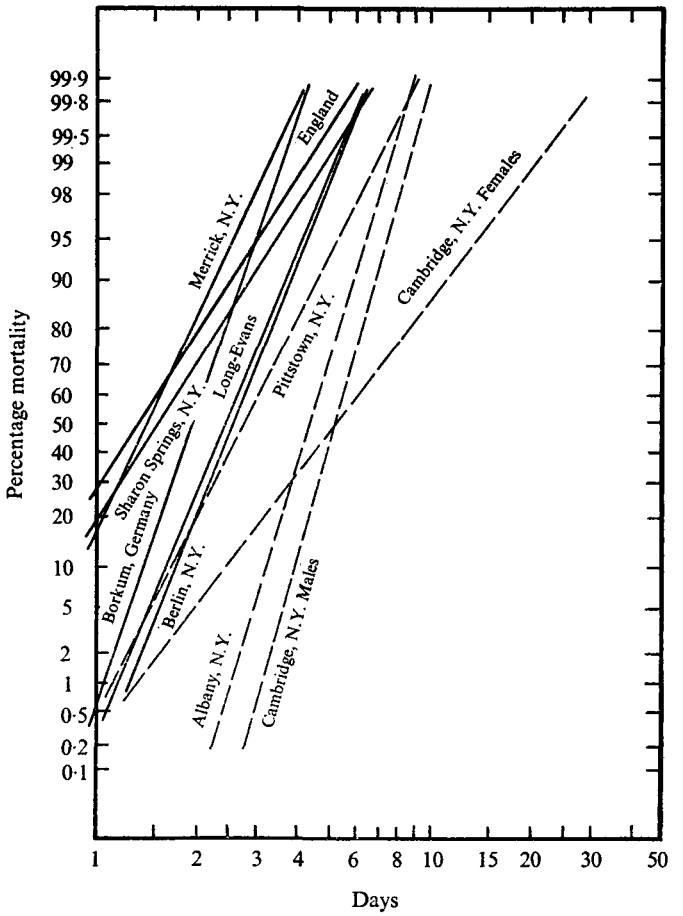

Fig. 1. Dose–response lines of several populations of *Rattus norvegicus* to 0·005 % warfarin. Solid lines are considered warfarin-susceptible; dashed lines are defined as warfarin-resistant.

population on a refuse tip; survivors were caught after routine control campaigns. The rats from Sharon Springs, New York, were trapped on a chicken farm and had a history of 10 to 15 years' past exposure to anticoagulants on an irregular basis.

These six populations indicate that the base-line susceptibility of *Rattus norvegicus* to 0·005 % warfarin is rather narrowly defined. Their LFP50s are characterized as between 1·35 to 2·58 days (with an upper 95 % confidence limit of 2·92 days). Their LFP98s, which are of most concern to us, fall between 3·0 and 4·82 days (with an upper 95 % confidence limit of 6·72 days). In Fig. 1, these fitted dose-response lines are shown graphically.

An examination of the three populations where one or more rats survived a six-day feeding period on 0·005 % warfarin indicates that they fall outside the normal narrowly circumscribed limits of the susceptible groups. Pittstown, a refuse-disposal-site rat population with a history of moderate anticoagulant pressure in the recent past, exhibits a dose-response line falling within the 95 %

https://doi.org/10.1017/S0022172400042753 Published online by Cambridge University Press

confidence limits of susceptible rats, but the LFP98 is 6·20 days, a value significantly increased from the susceptible groups. Pittstown, however, is considered as an example of incipient resistance; a site to be monitored again in a year or so.

The Albany population was trapped from a feed mill in the port of Albany, New York. The population here had been periodically poisoned with anticoagulants and acute rodenticides by a commercial pest-control operator for a number of years. Here the population response to warfarin has clearly shifted toward resistance. The LFP50 has increased to 4·38 days and the LFP98 to 7·0 days. The dose-response line falls outside the 95% confidence limits for susceptible rats at these dosage levels.

Finally, the resistance site at Cambridge, New York, a turkey farm, is an example of a warfarin-resistant population. Approximately 30% of the rats here survived a six-day feeding period. The response of the sexes was such that they required separate analysis. Males had an LFP50 of 5·15 days, a value reasonably close to that of the females, 5·40 days. However, their slope functions differed significantly. Their respective LFP98s were 8·05 days for males and 17·7 days for females. No confidence limits were estimated for Cambridge females because they were meaningless.

In Table 2 is also given the probability of a rat from each of the several populations surviving a six-day feeding period using the log-normal distribution fit, where the means are estimated as the log-LFP50 and the standard deviations are estimated as log-slope function. In this example, the probabilities for the first six populations do not exceed 0·003. Thus the chances of 'normal' rats surviving a six-day feeding test are extremely remote. In contrast, the probabilities of survival increase from 0·02 in Pittstown up to 0·43 in Cambridge females.

Based upon these evaluations of the responses of nine Norway rat populations to 0·005% warfarin, we would suggest that warfarin-susceptible Norway rat populations tentatively be defined as those whose dose-responses do not exceed LFP50s of 3·0 days and LFP98s of 5·0 days and whose individual member's probability of survival of a six-day feeding period is no more than 0·01. It is suggested that population responses falling beyond these measures should be regarded as warfarin-resistant. Furthermore, the validity of the six-day feeding test of 0·005% warfarin in oatmeal or Purina lab chow is clearly established and the survival of even one animal, as Drummond states (1966), should be regarded as an alert calling for further investigation. It should be pointed out that the data reviewed and presented here are applicable only to populations of *Rattus norvegicus*. Comparable dose-response data need to be developed for other species of pest rodents which are frequently and repeatedly poisoned with anticoagulants.

We wish to acknowledge the advice and assistance of Mr Philip Quickenton, Biostatistician, New York State Department of Health, in computing the probabilities of survival of the various rat populations. We wish to thank D. C. Drummond, W. M. Jackson and D. E. Kaukeinen for kindly reviewing the manuscript and providing us with their critical remarks. We especially and gratefully acknowledge the technical assistance of our staff members in the laboratory

and field aspects of this study: Richard Butterfield, Henry J. McFerran, Jr., Meredith Thompson, Jr., John Burns, Constance Padula, Florence Bartlett, Joan Lingle and Delarue Conway.

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