TOLERANCE SHOWN BY Rattus rattus TO AN ANTICOAGULANT RODENTICIDE

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ABSTRACT: Apart from using 0.005% concentration, the recommended field dose of 0.025% of the anticoagulant is used along with an alternate food for individual rats for a varying number of days. Those that had survived were taken as tolerant, provided they showed an mg/kg intake beyond the tolerance limit, survived a six days of feeding, exhibited bait-shyness and did not exhibit hemorrhage after death.

In determining the criteria for tolerance to an anticoagulant by a rat, one should take into account four composite factors. These are, six days of even 0.025% feeding, bait-shyness when alternate food is given, higher mg/kg intake than the tolerance level and a loss of intensive hemorrhage after death.

INTRODUCTION

Hayes and Gaines (1950) in their experiments with the rodenticide "Warfarin" concluded that it provided a completely new practical approach to rodent control in as much as, it did not cause bait-shyness, was self prebaiting and could be used as a residual rodenticide. Since then Warfarin has been the mainstay of inter epidemic anti-rat measures in plague control operations. However, later work of Boyle (1960), Cuthbert (1963) and Lund (1964) showed that the Norway rat, Rattus norvegicus had indicated resistance to this rodenticide.

Drummond (1966) anticipated that it will be more than just a few years before Warfarin will be virtually ineffective against most mouse populations.

In India Deoras (1965) showed that Warfarin was not as effective as zinc phosphide, during his field trials in the Vidarbha region of Maharashtra, when Rattus rattus from two places in Vidarbha, i.e. Nagpur and Chanda were tested in the Laboratory with field doses of Warfarin; they showed tolerance (Deoras 1966).

The most common rats in the town of Bombay are R. rattus; Bandicota bengalensis and Rattus norvegicus. Preliminary experiments carried out showed that the Bombay R. rattus and R. norvegicus were tolerant whereas and that B. bengalensis was very susceptible (Deoras 1967). The tolerance shown by the house rat posed a setback to the wide-spread use of Warfarin and this needed detailed studies for confirmation. Therefore, R. rattus were fetched in numbers from the town of Bombay and some other region of Maharashtra and were subjected to detailed trials to see their status to Warfarin tolerance. This paper gives an account of the studies done on the Bombay house rat R. rattus to confirm their susceptibility or otherwise to a locally manufactured anticoagulant.

MATERIALS AND METHODS

Rattus rattus were collected from different wards of Bombay and later from Sholapur, Osmanabad, Murud, Ambejogai, Pali, Gevrai, Daulatabad, and Pachod in wonder traps. They were made free of ectoparasites by brushing and held in the laboratory for 30 days. During this period, they were given water in bottles and weighed amount of food in hoppers. The food at this time consisted of crushed grains of wheat and Jowar (Andropogon sorgham). The rats were weighed at the beginning of the experiment. After acclimatization for 30 days, the rats were given the anticoagulant mixed in the above food, in predetermined doses. The anticoagulant tested was a locally manufactured Warfarin, developed and patented by the National Chemical Laboratory, Poona, marketed by UNICHEM and formulated by Pest Control (Pvt.) Ltd. The formula of this compound is as follows 3(alpha-phenyl-acctyl beta ethyl)4 hydroxycoumarin.

The concentrations used for experimentation were 0.005, 0.01, 0.25, 0.05, 0.1 and 0.5 per cent for Bombay rats and only 0.025 for rats from other places. The duration of feeding with Warfarin in the initial stages was up to 10 days. After trials it was reduced to 6 days, then the rats were kept on normal food for the remaining period even up to 20 days in a number of cases. To see further critical levels, these feedings with the anticoagulants were reduced to 2, 3, 4 days. In the third set of experiments, Warfarin was given along with the normal food as would be available in nature to estimate the daily intake and bait refusal if any. Each rat was dissected after death to see any internal hemorrhage due to the anticoagulant.

Table I - Showing the consumption of dry mixed cereal food by three common rats and the pellets given.

Kind of Rat	Month	Average weight of rats in grm.	Average weight of food consumed by the rats in 24 hours in grm.	Average amount of water consumed by the rats in 24 hours in cc.	Average No. of pellets given by the rats in 24 hours.	Average weight of each pellet in grm.
Rr.	January	103	9.3	24.	36	0.0196
	February	103	10.1	23.7	39	0.0197
	March	103	8.1	25.	39.6	0.019
Rn.	January	122	14.5	25.2	41	0.054
	February	122	12.9	25.1	40.3	0.054
	March	122	12.2	34.9	31.2	0.05
Bb.	January	232	14.2	26	49	0.037
	February	232	15.1	25.2	43	0.041
	March	232	12.7	34.9	43.2	0.04

Rr. Rattus rattus, Linn; Rn. Rattus norvegicus, Berkenhout; Bb. Bandicota bengalensis, Grey and Hardwicke.

Table II - Summary of trial of Warfarin in various concentrations for ten days with 10 R. rattus each, from Bombay.

Name of the product	Concentration used Percentage	Average weight of rats	Average bait food consumed till death in gms.	Mg/Kg active ingredient consumed till death.	Mean day of death	Percentage mortality
Warfarin	0.0025	105.3	31.9	7.6	5.8	70
	0.005	101.	34.9	15.	6.	70
	0.01	105.8	34.3	32.4	7.7	90
	0.025	104.4	29.9	71.	5.9	100
	0.05	108.6	29.7	135.	5.2	100
	0.1	95.	20.	209.	6.	40
	0.5	104.	21.25	100.	4.75	80
Control	0.025	469.2		12.97	5.8	100

Table III - Experimentation of Individual R. rattus with 0.025% Warfarin given for varying No. of days.

Sr. No.	Sex	Weight of Rats At start	Weight of Rats At death	Difference Wt. in gms. up to death	Lethal dose mg/kg till death	Day of death	Days of survival on control food	Warfarin consumed by surviving rats on mg/kg basis	Wt. of surviving rats in gms.
				TWO DAYS OF WARFARIN FEEDING.					
1.	M	152	-			S	12	26.31	162
2.	M	164	118	-46	12.19	7			
3.	M	164				S	12	22.52	170
4.	F	87				S	12	36.78	80
5.	F	82	70	-12	30.48	5			
6.	M	160				S	24	31.25	164
7.	M	134	119	-15	44.7	7			
8.	F	103				S	24	77.6	139
9.	F	114	102	-12	63.6	4			
10.	F	102	112	+10	31.8	3			
				THREE DAYS OF WARFARIN FEEDING.					
1.	M	127	113	-14	37.4	4			
2.	M	114				S	23	143.6	160
3.	M	172				S	23	149.5	179
4.	F	109	107	-2	68.8	5			
5.	F	87	90	+3	86.2	3			
				FOUR DAYS OF WARFARIN FEEDING.					
1.	M	142				S	10	8.8	152
2.	M	115				S	10	80.0	120
3.	M	104	70	-34	67.3	4			
4.	F	100	95	-5	95.0	3			
5.	F	82	75	-7	115.8	4			
				SIX DAYS OF WARFARIN FEEDING.					
1.	M	123	118	-5	18.2	2			
2.	M	127	140	+13	49.2	4			
3.	F	108	118	+10	94.4	5			
4.	F	119	127	+8	168.1	7			
5.	F	108	105	-3	46.29	3			
6.	M	152				S	8	70.39	142
7.	M	137	95	-42	74.4	8			
8.	M	89	72	-17	75.2	5			
9.	F	127	115	-12	23.62	4			
10.	F	109	95	-14	36.69	4			
				EIGHT DAYS OF WARFARIN FEEDING.					
1.	M	82	100	+18	173.1	5			
2.	M	92	90	-2	51.6	4			
3.	M	169				S	18	118.34	165
4.	F	105	103	-2	109.52	5			
5.	F	144	120	-24	22.5	3			

S. Surviving; M. Male, F. Female.

The rats used in the experiments were R. rattus rufuscens and Bandicota bengalensis, Grey and Hardwicke. The latter were used as controls for comparison.

OBSERVATION

Table No. I gives the normal intake of food and water by R. rattus, during the year under experimentation for comparison with the data of intake in anticoagulant experiments.

Table No. II gives the intake and mortality of R. rattus with varying concentrations of the anticoagulant, starting from 0.0025% to 0.5%. It is noted here that there is 100% mortality in 10 days for 0.025 and 0.05% concentration only. In higher concentrations the intake of the bait material is reduced. There are survivors even if the mg/kg concentration is higher. The mean day of death for the 0.025 and 0.05% is 5.9 and 5.2 respectively. B. bengalensis taken as control had died off within 10 days with 0.05% and 0.025% concentration.

These two concentrations i.e. 0.025 and 0.05% that have left no survivors were taken for observing the minimum days of intake of poison to start mortality. Table III, therefore, shows the mortality in R. rattus when fed for 2, 3, 4, 6 and 8 days. In each case after feeding on the anticoagulant for the requisite number of days, they were switched on to the normal food. B. bengalensis were kept as control (Table IV) and the mortality timings in them indicate that even with 2 days of feeding there is mortality, while in R. rattus there are survivors at 8 days also.

Table IV. Experimentation on individual Bandicota bengalensis with 0.025% Warfarin given for varying No. of days.

Sr. No.	Sex	Weight of rats in gms.		Difference wt. in gms. up to death	Lethal dose mg/kg till death	Day of death	Average day of death
		At start	At death				
				TWO DAYS OF WARFARIN FEEDING.			
1.	M	430	390	-40	21.39	5th	
2.	M	425	402	-23	14.58	6th	
3.	F	437	382	-55	11.44	7th	5.8
4.	F	542	515	-27	7.38	4th	
5.	F	515	480	+65	11.06	7th	
				FOUR DAYS OF WARFARIN FEEDING.			
6.	M	384	372	-12	36.9	5th	
7.	M	552	448	+6	40.72	6th	
8.	F	442	407	-35	14.705	5th	5.4
9.	F	480	428	-52	22.29	5th	
10.	F	482	443	-39	11.92	6th	
				SIX DAYS OF WARFARIN FEEDING.			
11.	M	392	400	+8	60.48	5th	
12.	M	292	300	+8	46.89	4th	
13.	F	435	357	-73	5.10	6th	5.6
14.	F	469	453	-16	21.9	5th	
15.	F	487	400	-87	7.19	8th	
				EIGHT DAYS OF WARFARIN FEEDING.			
16.	M	564	528	-36	32.26	6th	
17.	M	427	443	+16	48.00	6th	
18.	F	327	312	-15	45.87	5th	5.6
19.	F	409	490	+81	48.89	7th	
20.	F	452	438	-14	22.12	4th	

M = Male; F = Female.

Table V - Warfarin trials with Rattus rattus. (Both Warfarin & Control food offered) Warfarin Concentration 0.025%

Sr. No.	Sex	Wt. in gms.				Quantity of Warfarin as food consumed each day up to 20th day along with control food in gms.																				Total consumption gms.	Day of death or surviving.	Mg/kg of Warfarin for 20 days or death
		Starting	At death or after 20 days	Diff. in wt.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20			
1.	M	91	108	+17	R	2	3	3	3	2	-	-	-	3	-	-	-	1	1	-	-	3	-	3	2	26	S	71.40
					C	8	7	6	6	9	2	8	7	-	11	14	-	-	4	5	6	5	4	6	5	133		
2.	M	110	116	+6	R	2	2	2	1	2	-	1	-	1	-	-	5									16	12	36.3
					C	5	6	8	13	10	3	9	11	7	-	-	-									72		
3.	M	85	86	+1	R	3	2	1	2	4	-	-	-	3	-	1	1	3	-							20	14	58.8
					C	5	2	-	13	3	12	-	-	3	4	1	2	5	10							60		
4.	M	128	125	-3	R	4	2	2	4	3	-	-	-	-	-	4	-	-	3	1	-	-	-	-	1	24	S	46.9
					C	8	8	12	3	8	15	5	14	6	6	5	7	8	3	4	2	2	2	4	4	126		
5.	M	141	151	+10	R	6	2	7	4	3	2	1	2	2	1	5	1	3	-	1	1	1	1	-	5	52	S	92.4
					C	3	3	6	5	3	4	9	6	8	-	1	5	6	7	4	5	5	2	-	-	82		
6.	F	78	77	-1	R	3	2	3	2	3	-	-	2	-	1	6	9	5	4	-	-	-				40	17	12.8
					C	4	3	6	4	6	6	4	2	8	6	1	0	17	9	-	-	-				76		
7.	F	112	86	-26	R	2	4	2	9	4	-	-	-	-	-	4	8	3	-							36	14	80.6
					C	-	2	4	2	4	8	7	8	8	6	1	0	3	1							54		
8.	F	84	85	+1	R	2	2	5	3	2	-															14	6	41.6
					C	2	4	7	9	4	3															29		
9.	F	76	65	-11	R	2	2	2	3	1	1	-	-	8	-											19	10	62.4
					C	3	2	4	5	4	5	3	8	1	-											35		
10.	F	99	72	-27	R	3	4	2	3	-	-	-	-	1	1	-	1									15	12	37.8
					C	3	3	4	1	2	3	3	3	4	3	3	1									33		

M = Male rats; F = Female rats; R = Warfarin bait; C = Control bait food only; S = Survivors

Table VI - Warfarin trials with Rattus rattus. (Both Warfarin & Control food offered) Warfarin Concentration 0.05%

Sr. No.	Sex	Wt. in gms. Starting	At death or after 20 days.	Diff. in wt.		Quantity of Warfarin as food consumed each day up to 20th day along with control food in gms. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Total consumption gms.	Day of death or surviving.	Mg/kg of Warfarin consumed for 20 days or death.
1.	M	133	120	-13	R	2	2	2	3		1	-	-	-	1	-	1	2	1	2	2	4	1	-	-	24	18	90.2
					C	10	4	4	9	13	5	4	6	5	7	4	2	7	9	4	2	2	1			98		
2.	M	69	73	+4	R	3	2	3	3	2	-	-	-	-	-	-	-	2	-	2	1	-	-	-	-	18	S	130.4
					C	3	5	8	7	5	2	1	7	7	7	6	5	10	4	8	2	2	6	7	7	109		
3.	M	126	138	+12	R	3	2	3	-	-	1	12	-	-	-	1	-	-								22	13	87.2
					C	6	8	4	17	3	15	2	14	11	5	3	-	-								85		
4.	M	113	130	+12	R	3	3	3	4	-	-	-	-	2	-	2	-	-	-	1	-	-	-	-	-	19	S	84.7
					C	7	5	8	10	11	9	5	7	7	5	4	8	6	8	6	9	4	5	5	7	137		
5.	M	106	104	-2	R	-	4	5	5	2	-	-														16	7	75.4
					C	6	5	10	17	-	-	-																
6.	F	62	56	-6	R	3	4	1	2	-																10	5	80.6
					C	7	4	4	2	3																20		
7.	F	112	122	+10	R	6	2	2	3	1	-	2	-	-												16	9	71.4
					C	7	4	10	9	6	4	7	6	-												53		
8.	F	126	113	-13	R	3	2	2	-	2	9	1	-	-	-	-	-									19	12	75.3
					C	6	6	6	14	8	8	6	7	4	7	1	-									73		
9.	F	83	90	+7	R	3	1	4	4	1	-															13	6	78.2
					C	8	6	10	9	7	-															40		
10.	F	80	79	-1	R	5	3	2	4	2	-	-	-	-	-	-	-	-	-	-	3	2	-	1	-	22	S	137.5
					C	4	1	8	10	6	3	1	4	6	3	7	6	5	7	8	2	2	6	4	5	98		

M = Male rats; F = Female rats; R = Warfarin bait; C = Control bait food only; S = Survivors

Rats were then observed with 0.025% and 0.05% anticoagulant and an alternate food at the same time. This is the condition as will be available in nature. Tables V and VI give in detail the performance of 0.025% and 0.05% concentrations. Individual rats were observed for 20 days with 0.025% and 0.05% poison along with food. Table V and VI shows that rats on an average take the food in more quantity when the choice is available. Those that have survived in Table V, have taken the anticoagulant continuously for 20 days except a break of 2 days. Secondly the mean day of death has gone from 5.9 and 5.2 to 12 and 11, respectively, for 0.025 and 0.05% concentrations.

There were three survivors after 20 days, while in B. bengalensis (Table IV) all had died by the 6th day.

Having seen the performance in R. rattus from Bombay, rats from 8 places in Marathwada were given a 0.025% dose. Table No. VII, therefore, shows the performance by 0.025% of the anticoagulant. There are survivors from Murud, Gevrai, Daulatabad and Pachod even after 20 days.

Out of this lot, Sholapur, Osmanabad and Ambejogai rats that had shown mortality were given normal food along with the 0.025% and 0.05% concentrations there were survivors from the 1st two places even after 20 days, when alternate food is given.

The survivors in R. rattus in Table III have gained weight in 3 cases and lost only 4 to 10 gm. in the other two cases; but in spite of loss in weight the rat has survived. The big rat in Table IV has lost weight from 28-42 gm. and died.

In Table V is shown the individual gain and loss in weight. The first surviving rat in Table V has gained 17 gm. It took the anticoagulant for 5 days, then stopped it for 3 days and so on. The survivors have taken 71.40, 46.9 and 92.4 mg/kg of the anticoagulant which is far more than the one taken by the control at IV.

In the case of B. bengalensis, one always saw a bleeding through nose, mouth and anus. The viscera showed extensive hemorrhage. In the case of R. rattus, there was no case seen of bleeding through nose, mouth or anus. The internal organs were just pale and there was no indication of bleeding inside the R. rattus.

The minimum lethal dose per mg/kg to kill the control (Table IV) rat is 7.19 and the maximum is 60.48. In the case of R. rattus the minimum for the susceptible rat is 12.19 and the maximum is 173.1. The surviving rats have taken 149.6 mg/kg which is far more than the 60.48 mg/kg of the controls (Table IV).

DISCUSSION

Rattus rattus, refuscens, is the common house rat in Bombay. Recent work at Haffkine Institute had indicated that in Vidarbha and Marathwada regions of Maharashtra this was the most predominant rat in the fields also (Deoras 1966). The common raticides in use in India were barium carbonate, strychnine and zinc phosphide. Firstly, nearly all of them develop bait-shyness, require prebaiting and the last two unless used carefully are dangerous to poultry and cattle. In view of these, the development of a new type of raticide in India was a handy addition to rat control operations in plague preventive measures.

In field trials with zinc phosphide and anticoagulant (Warfarin), Deoras (1965) showed that Warfarin was not giving as good a kill as the first poison. The predominant rats were fetched from Chanda and Nagpur and were tested along with similar rats from Bombay. The doses tried were 0.005% which had shown resistance in R. norvegicus by Lund (1964). It was shown by Deoras (1966) that the Chanda rats took a much longer time and further, even with the field dose of 0.025% the rat continued to live under field conditions. But B. bengalensis, which is a much bigger rat than R. rattus, died within 6 days even with the smaller dose of 0.005% of the poison (Deoras 1967).

Hayes and Gaines (1950) have used specific concentrations based on the weight of the individual rat and giving it the calculated weight of poison as a definite intake. This we think is too artificial. We have given specific concentrations over or below 0.025%, the recommended field dose and let the rat eat it as a choice. The mg/kg intake is worked out as we know the weight of each rat. Bentley and Rowe (1956) have established that both Pival and Warfarin at 0.025% are equally toxic and equally acceptable to R. rattus. A W.H.0. notification (1966) mentions the mortality as a criteria for recognizing resistance

to Warfarin in _R. norvegicus by the use of 0.005% concentration for this rat, fed for 6 days, while the dose of Warfarin tolerated by the survivors was between 10.9 to 34.6 mg/kg. Lund (1964) used R. norvegicus along with R. rattus and declared resistance in R. norvegicus for 0.005% concentration of Warfarin of 6 days of feeding.

Drummond (1966) mentions that most resistant rats are able to survive not only much longer feeding periods than the five days required to kill nearly all susceptibles, but also higher concentrations of Warfarin.

R. norvegicus available in Bombay is a much heavier rat than R. rattus. As there were no such figures of concentration tolerance available for R. rattus, this concentration of 0.005% and higher were undertaken initially to test the tolerance in a simple way. These lower concentrations have been tested up to 10 days and continued up to 20 days for a rat which is lighter in weight than R. norvegicus. If the percentage concentrations of 0.005, 0.01, 0.025, 0.05, 0.1 and 0.5 of the "Warfarin" compound used and mentioned at Table II are taken and these compared with their mean days of death in a graph, it will be noticed that the curve rises from .005% to 0.01% and then it falls as the concentrations are increased. This probably means that if a dose beyond 0.01% is given, the rat may die slightly quicker, simply because the anticoagulant concentration is high, though intake is low. There are survivals of 20%, 30% and 10% even at these higher concentrations, the least being for 0.01%. It will also be seen that the mean day of death for 0.005, 0.025 and 0.05 percentages for Bombay rats come between 5.2 and 5.9 days (Table II). In fact this day in 0.005% is only 6 while at 10 times the concentration i.e. 0.05% it is 5.2. It consumed more bait with lower concentration.

Table No. I shows the food intake of the three kinds of rats in 24 hours. This is to show the comparison of ingestion of food by the rats in the raticide trials. This table as compared to the amount ingested seen in Table V indicates that the rat took 10 gm. on the 1st day, and second day but gradually it fell off by the 6th day. In all rats the anti-coagulant consumption was either left or reduced, but the control food was taken all along. In all cases the rats had consumed more of control food. It selected normal food, showing bait-shyness, as would be seen in nature. After 10 days, the food intake had gone down in all cases. The surviving rats tolerated up 92.4 mg/kg. The rats have alternate food in nature. They, therefore, would take it for a few days then stop it and then take and there would be no dead rats. This table again is an indication of bait-shyness as a form of tolerance.

The concentration between 0.01 and 0.05% leaves no survivors and beyond this they start varying. The midpoint between these two is 0.025% and this is taken as base for testing tolerance. This is also the figure taken for field trials. R. rattus showing tolerance for this figure may then be deemed to show resistance. Statistical analysis of these results, particularly the day of death, have shown significance and indicated tolerance in R. rattus.

Table III very significantly shows that of 10 R. rattus (after taking only 2 days of poison bait), only 5 have died on 3-4 and 7th day. They were not given Warfarin on 3rd, 4th, 5th and 6th day. There was no continuity of feeding with the poison beyond 2 days. The lethal dose taken by dead rats was from 12-19 to 30-48 of mg/kg. In the case of those that survived, the consumption of Warfarin equivalent to this lethal dose was between 22.52 to 77.6 mg/kg, as compared to even 23-62 of the one that died. In the case of 8 days the survivors have taken 118.34 mg/kg. The huge quantity of 173.1 mg/kg taken by one of the dead rats in this series shows the increasing tendency to tolerate a bigger dose. This is not the case with B. bengalensis. Even those that ate for 2 days have died within 7 days with an mg/kg level from 7.38 to 21.39. Even for a longer period of feeding for 8 days, the rats have died within 7 days and nothing remained on the 8th day. The mg/kg was between 22.12 and 48.89 which is much lower than what is tolerated by R. rattus.

This individual rat experiment at Table III demonstrates that once taken, the Warfarin will act, even if it is taken for just two days, provided the rat does not show tolerance. The mg/kg lethal dose can indicate for the minimum but maximum will again show a kind of tolerance.

Bentley (1967) has mentioned "for practical purposes to earn the label 'resistant' we require rats to survive a standard feeding period of 6 days on 0.005% Warfarin in the laboratory." In Table III, 16% and 20% of rats have survived this period for a much higher dose.

Table VII and VIII shows the performance of R. rattus from a number of towns in the Marathwada region of Maharashtra. The places are at least 300 miles from Bombay and never was Warfarin used there. In these trials, except for Sholapur, all the other places show variation in mortality for 0.025% concentrations. There is survival in rats beyond 10 days. Individual experiments for Bombay rats shown at Table III indicate that, if any rat was to die, it does this by the 10th day at the maximum and, if it has not died, it will survive. Experiments with alternate food were done for 3 towns, where rats had died in 10 days, and this gave a clear picture where the rats have survived. It thus seems that apart from Bombay, R. rattus from Sholapur, Osmanabad, Murud, Ambejogai, etc., are also indicating some tolerance.

All the rats were dissected after death. In the case of B. bengalensis there was always an extensive hemorrhage not only through nose but all over the viscera. In the case of R. rattus those that died by feeding on the bait for 6 days or more showed hardly any hemorrhage, but those that had actually died by feeding for less than 6 days, showed no hemorrhage at all. In all such cases the liver, heart, lungs, and the subcutaneous tissue showed only a pallor, faint pink color, unlike the deep crimson shown by these tissues in B. bengalensis after death, or in the case of R. rattus killed in normal circumstances. Does this then indicate a tolerance reaction of a poison which must show an internal hemorrhage as a characteristic?

The digestive tract of R. rattus, R. norvegicus, and B. bengalensis was dissected out. The digestive system of these 3 rats was compared to each other. It was noted that the tracts were 34.5", 38.5" and 75" long respectively, but the point of interest in them was that the length of caecum in the first was 2.5" in the second 1.5" and in the third 3.2". Anticoagulant Warfarin is said to act by inhibiting the formation of prothrombin and by causing capillary damage (Hayes and Gaines 1955). W.H.O. Chronical 1966 has indicated the role of Vitamin K in this process and the bacteria that go to form this vitamin in the animal. The role of caecum in such animal is not well established but work in future may show the growth of this organism in certain restricted regions; and caecum may be one of them. Lund (1964), however, has used sulpha drugs to destroy the so-called bacteria, that may be producing Vitamin K, and even then his rats showed resistance. It therefore remains to be seen whether studies on caecum and Vitamin K may throw more light on this phenomenon of tolerance. However, Bentley (1969) has investigated and found that the addition of sulphaquinoxaline or sulphaguanidine to suppress the Vitamin K producing bacteria or the addition of melhyltestosterone was of no avail. He has tried Racumin at 0.05% concentration and mentions that this gave good results in the Warfarin resistant rats. Unfortunately, we have found that the Bombay R. rattus left 40%, 40% and 20% survivors in 0.05%, 0.1% and 1% concentrations of Racumin. This further shows the resistance for allied anticoagulants.

Warfarin has not yet been used on a large scale in India. This phenomenon seen in Bombay R. rattus and indicated in similar species in Marathwada region of Maharashtra are probably suggestive of a natural tolerance by some of these rats in a community. Given a natural population, there will be a variation in susceptibility to a given stimulus. In the B. bengalensis, the population is uniformly susceptible. In R. rattus studied above, there are tolerant individuals who show bait-shyness or refusal, tolerating heavy doses of the anticoagulant without dying and not exhibiting internal hemorrhage. These composite criteria be therefore taken together to test tolerance in rats.

Bentley (1969) has stated that "in fact there is now little doubt that resistance largely or wholly depends on some kind of competition between Warfarin and Vitamin K, possibly for a gene controlled, repressor concerned with the production of blood clotting proteins." Further studies on R. rattus may confirm while the present studies point out a clear tolerance.

SUMMARY

Anticoagulant rat poison "Warfarin" manufactured by the National Chemical Laboratories and currently available in the market was used in the field where on a comparative basis with zinc phosphide it did not give an adequate kill in rats. The majority of these rats were R. rattus. Such rats from Bombay were given 0.005, 0.01, 0.025, 0.05, 0.1 and 0.5% of the anticoagulant in the laboratory for 6 to 10 days. They were kept on 0.025% Warfarin with alternate food for 20 days and individual rats of equal number of sexes were given 0.025% for 2, 4, 6, and 8 days and then shifted to normal food. The experiments have shown that 0.005% is probably the critical dose and that _R. rattus from Bombay even if they take 0.025% Warfarin for 2 days will die within 8 days if susceptible. The individual

Table VII - Anticoagulant (0.025%) trials feeding for 6 days only for Rattus rattus from Marathwada. Held over on normal food up to 20 days.

Locality	Average wt. of rat	Feeding days of Warfarin	Average Warfarin bait consumed per rat in gms. up to death	Mean lethal dose mg/kg of body wt. (dead rat)	Mortality in 6 days No out of	Survived even after 20 days	Mean day of death
Sholapur	111.5	6	47.5	113.4	4/10	-	6.7
Osmanabad	95.5	6	58.4	156.17	4/8	-	6.4
Murud	92.7	6	42.5	125.84	4/7	1(+9)	5.6
Ambejogai	92.3	6	35.2	99.93	6/6	-	5.7
Pali	122.4	6	31.6	65.8	5/5	-	5.0
Gevrai	109.0	6	44.0	84.5	2/5	1(+4)	9.25
Daulatabad	91.0	6	36.7	101.7	4/7	1(+30)	5.8
Pachod	111.6	6	32.7	82.6	2/7	1(+6)	7.6

+ Indicates death due to anticoagulant poisoning.
- Indicates death due to some other causes.
Figures in parenthesis of column 7 indicate the difference in weight when compared with the weight of rat at the start of the experiment.

Table VIII - Trials on R. rattus with Warfarin and normal food offered simultaneously for 10 days and hereafter offered normal food till 20 days.

Locality	Average wt. of rat	Average consumption per rat in gms.		Average day of death	Lethal dose mg/kg	Mortality in days			Survival after 10 days
		Warfarin (10 days)	Normal food			6	10	20	
			(Warfarin 0.025% and normal food.)						
Sholapur	95.0	28.4	50.3	5.7	62.1	1/10	4/10	5/10	50%
Osmanabad	97.1	37.4	64.5	7.66	82.15	2/8	6/8	8/8	-
Ambejogai	120.9	24.6	29.0	5.8	59.9	3/6	3/6	6/6	
			(Warfarin 0.05% and normal food.)						
Sholapur	112.0	54.4	56.9	5.3	30.1	3/9	3/9	3/9	30%
Osmanabad	100.3	57.25	46.5	7.3	29.8	3/8	5/8	8/8	-

survivors with 0.025% concentration have tolerated 22.52 to 149.5 mg/kg, while those that died have taken between 12.19 to 173.1 mg/kg Warfarin. Those that show tolerance do not die even when they have been feeding for 20 days on Warfarin. B. bengalensis which is a much bigger rat dies within 6 days leaving no survivors even with 0.005% Warfarin. R. rattus collected from Sholapur, Osmanabad, Murud, Ambejogai, Pali, Gevrai, Daulatabad and Pachod indicate a similar phenomenon in varying proportions. The rats that died in 10 days took between 10 to a maximum of 231.7 mg/kg of 0.025% Warfarin and there were survivors after 10 days at Murud, Gevrai, Daulatabad and Pachod. With alternate food there are survivors even from Sholapur and Ambejogai. As compared to B. bengalensis, R. rattus in general do not show any profuse hemorrhage after death with Warfarin, but only indicate paleness of organs like liver, heart, spleen, lungs and the viscera.

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