diet in which amino-acids completely replace proteins. This finding indicates that the constituent amino-acids are responsible for the conditioning, by dietary proteins, to the above toxic effects of LAP overdosage. Our data also show that the growth, adrenal enlargement and nephrosclerosis produced by LAP were less pronounced on the amino-acid diet than on the Amigen diet of equal nitrogen content. It may incidentally be mentioned that the usual myocarditic changes[5] which accompany this type of hypertension were also less obvious in the amino-acid than in the Amigen groups. This could possibly be explained by the fact that the animals of Groups I and II received an unnatural mixture of amino-acids. Yet the existing difference between Groups I and III is of sufficient magnitude to suggest that equal amounts of amino-N are not necessarily equally conducive to the production of nephrosclerosis by LAP.

Summary. Adrenal enlargement, nephrosclerosis and hypertension were produced by a lyophylized anterior pituitary (LAP) preparation in rats kept on a synthetic diet containing no protein, but an adequate amount of amino-acids. The severity of the lesions was less marked, however, than in rats receiving an equivalent amount of nitrogen in the form of a casein-hydrolysate. It appears that amino-acids suffice to sensitize the organism to the production of hypertensive disease by LAP. This is noteworthy since no such pathologic changes can be produced by LAP on other diets deficient in proteins.

4 Hawk, P. B., Oser, B. L., and Summerson, W. H., *Practical Physiological Chemistry*, The Blakiston Co., Philadelphia, 1947.

5 Selye, H., *J. Clin. Endocrinol.*, 1946, **6**, 117.

16798

Comparative Assays of Rodenticides on Wild Norway Rats. II. Acceptance.*

SALLY H. DIEKE. (Introduced by C. P. Richter.)

From the Psychobiological Laboratory, Phipps Psychiatric Clinic, Johns Hopkins Hospital, Baltimore, Md.

In the previous paper of this series,[1] 8 common rodenticides were compared on the basis of their acute toxicities to recently trapped wild Norway rats. As was pointed out in that paper, a high toxicity does not, however, guarantee effectiveness under field conditions; of at least equal importance are those properties, such as a bitter taste or the lack of it, which influence the voluntary consumption of poisoned baits. It is the purpose of the present work to reexamine the same 8 rodenticides in the light of their acceptability and effectiveness when offered to wild Norway rats in a standard bait under controlled conditions in the laboratory.

Methods. The 565 wild Norway rats on which data are here given were trapped in the city of Baltimore during the period from July 1946 to December 1947. Before use they were kept in the laboratory, in large holding cages, for a few days; this delay proved necessary to allow for occasional deaths or for signs of debility to become evident. They were maintained on Purina fox chow and water during this time. The healthy specimens were then transferred (in almost every instance within 2 weeks after trapping) to individual wire mesh cages provided with a metal food cup and a water bottle. The food cup had fitted over it an aluminum

* This investigation was supported by a research grant from the Division of Research Grants and Fellowships of the National Institute of Health, U. S. Public Health Service.

1 Dieke, S. H., and Richter, C. P., *Pub. Health Rep.*, 1946, **61**, 672.

cover with a small oval opening, designed to minimize spillage, and it rested directly under a matching hole cut in one corner of the wire mesh bottom of the living compartment.

The bait used was a special yellow corn (Funks' Hybrid G-94, grown in McLean County, Illinois†), freshly ground to a uniform fineness for each week's work. The cornmeal was weighed into the food cups and placed in the cages at 4:30 P.M. for the rats to eat overnight. Next morning at 9:30 a.m. the food cups were removed and reweighed. Extra cups containing cornmeal were occasionally weighed in the afternoon and again next morning to make sure that atmospheric conditions did not cause changes in weight which might reduce the accuracy of the observations. No changes of more than 0.2 grams ever occurred, which was considered within the experimental error.

The cups were marked and always returned to the same cage. The rats were purposely left without food during the day in order to prevent them from starting to eat before dusk, which is their normal feeding time under field conditions.

After 2 nights of prebaiting with plain corn, according to the above schedule, poison was added to the cornmeal for the third night. To ensure a uniformly small particle size, each sample was first ground in a mortar for 2 or 3 minutes; it was then added to the appropriate amount of cornmeal and blended in a mechanical mixer for 15 to 20 minutes. The poisoned baits were always freshly prepared on the day the rats received them.

Next morning the poisoned food was removed and weighed, and any rats alive at this time were fed fox chow until they died or were discarded (4 to 10 days after poisoning, depending on the poison and the condition of the rat). A large number of rats were autopsied, including all those not dying promptly, in order to exclude data on rats not succumbing to the poison alone. No surviving rat was ever used again.

Most of the experiments were performed during the autumn, winter and spring months. No seasonal variation in toxicity was found in previous work, and since the present work likewise gave no indication of any, the data have been considered as a whole. A difference in response attributable to sex was previously found to exist for none of the poisons except red squill; so a separation of data on male and female rats is here made only for that poison. And although data on young rats are included in the table giving nightly consumptions of plain corn, no sexually immature rats were used in the poison experiments, thus obviating any variation attributable to age.

The poisons used came from the same containers as in the preceding work, with the exception of sodium fluoroacetate (1080) and arsenic trioxide, supplies of which had been exhausted. The new samples of these two poisons were reassayed by stomach tube, according to the method previously described,[1] and 52 additional wild Norway rats were used to check the toxicity figures previously obtained for the other poisons. No substantial changes in toxicity were detected and accordingly the LD50 figures previously obtained for strychnine sulfate, alpha-naphthyl thiourea (ANTU), thallium sulfate, fortified red squill and barium carbonate were allowed to stand.

Results. Plain Cornmeal. The average amounts of unpoisoned cornmeal eaten by the rats during the prebait period are shown in Table I. They have been broken down according to size of rat and sex, to show that the average consumption increased from 6 to 10 g for immature rats up to more than 18 g for full grown males. Individual large rats had intakes as high as 30 g on the second night, and a few ate more than 25 g both nights.

In each weight range the females seem to have eaten somewhat less than the males, although not significantly less in the young adult classes (100 to 299 g body weight). On the average all but the small rats ate more the second night than they did the first, indicating a certain hesitation to eat what was presumably an unfamiliar food. Only 24 rats (not included in the tabulation) refused

† Provided through the courtesy of Funk Brothers Seed Company, Bloomington, Ill.

TABLE I.
Consumption of Cornmeal on 2 Successive Nights by 565 Wild Norway Rats.*

Wt range	No. rats	Avg body wt, g	Avg consumption, g 1st night	2nd night
Below 50 g	4 ♂	47.0	8.0	7.1
	3 ♀	47.0	6.3	6.0
50- 99	49 ♂	80.0	10.3	10.0
	63 ♀	73.8	9.7	9.4
100-199	51 ♂	140.5	10.7	11.1
	86 ♀	144.5	10.3	11.2
200-299	74 ♂	249.0	12.7	14.6
	77 ♀	251.5	13.5	14.5
300-399	63 ♂	342.1	15.6	17.8
	60 ♀	343.4	13.9	16.4
400-499	19 ♂	435.5	16.4	18.4
	13 ♀	427.4	14.3	16.4
>500	3 ♂	543.0	11.8	17.0
	0 ♀	—	—	—

* Not including data on 23 additional rats which refused to eat the first night, and 1 rat which refused both nights.

to eat any cornmeal on the first night, a ratio of approximately one refusing rat in 24, or 4%, and of these all but one ate well the second night.

Poisons. The kills obtained with various percentages of each poison are given in Table II, which also lists for comparison the acute median lethal doses (LD50's) of the poisons as determined by stomach tube administration. The lowest poison concentrations at which complete kills were obtained are seen to range from 0.1% for thallium sulfate to 5% for female rats receiving squill. No bait containing strychnine sulfate or barium carbonate killed all the rats receiving it, nor was any concentration of red squill completely successful against both male and female rats.

In general, the higher the concentration of any poison, the more rats died from eating it. This was, however, not true for strychnine sulfate and squill: for these, some fatalities occurred throughout the range but increasing the concentration did not increase the efficiency of the bait.

The reduced consumptions of bait resulting from the addition of the various poisons to the cornmeal are shown in Table III, which gives data on the same rats as Table II. This shows that the amount of bait consumed decreased fairly regularly with increasing content of poison. At the levels marked with asterisks, which were the lowest concentrations killing all the rats used, the average intakes were reduced to 14% of prebait for 1080, 21% for female rats receiving squill, 28% for ANTU, and 30% for zinc phosphide. No reduction at all followed the introduction of thallium sulfate into the bait at the equivalently lethal concentration. Reduced intakes are seen also for strychnine and barium carbonate, but even with these poisons a total refusal to eat never occurred.

Discussion. From a practical standpoint, experiments such as these serve only to indicate what will happen under field conditions. Just as toxicity figures based on administration by stomach tube represent the irreducible minimum of poison which is likely to kill, so results with trapped and individually caged wild Norways merely show what percentages of poison in bait can be expected to kill under the most favorable conditions in the field. Captive wild rats have been removed from their normal environment and may well be suspicious of any food offered them; on the other hand they are not subject to distrac-

TABLE II.
Comparative Killing Concentrations.
(Voluntary consumption of poisons in cornmeal bait by wild Norway rats; mortality ratios (rats dying/rats used) for each poison are listed opposite the appropriate concentrations. The median lethal doses and their standard errors are added for comparison at the bottom of the table.)

Conc. in bait	1080 (36 rats)	Strychnine SO_4 (20 rats)	ANTU (36 rats)	Tl_2SO_4 (36 rats)	Zn_3P_2 (40 rats)	As_2O_3 (24 rats)	Squill (24 ♀, 24 ♂) ♀	Squill ♂	$BaCO_3$ (20 rats)
.01%	2/ 4								
.02	1/ 4		0/4	0/ 4					
.05	10/12	1/4	4/8	11/12	2/8				
.1	7/ 8	0/4	1/4	8/ 8	1/4				
.2	8/ 8	5/8	5/8		8/8				
.5		0/4	4/4	8/ 8		0/4			
1			8/8	4/ 4	4/4	1/4			
2					7/8	6/8	5/8	1/8	
4						4/4			
5					8/8		4/4	1/4	
6						4/4			
10							2/4	0/4	0/4
15									3/4
20							2/4	0/4	3/4
50							2/4	2/4	7/8
Lowest conc. killing all rats, %	0.2	—	0.5	0.1	0.2	4	5	—	—
LD_{50} Acute,* mg/kg (± S.E.)	0.44 ±0.19	4.8 ±0.4	6.9 ±0.5	15.8 ±0.9	40.5 ±2.9	57.5 ±7.0	133 ±10	276 ±29	1480 ±340

* Values for 1080 and As_2O_3 from Table IV, the rest from reference [1].

TABLE III.
Average Voluntary Intakes of Poisoned Cornmeal Showing Lowered Consumptions with Increasing Concentrations of Poisons.*

Conc. in bait, %	1080 g	Strychnine SO_4 g	ANTU g	Tl_2SO_4 g	Zn_3P_2 g	As_2O_3 g	Squill ♀, g	Squill ♂, g	$BaCO_3$ g
0 (prebait)	15.4	16.0	14.5	15.2	13.8	15.6	16.4	17.4	14.7
.01	9.1								
.02	2.6		9.0	11.0					
.05	3.3	7.7	8.0	15.7	10.3				
.1	2.4	6.1	5.7	15.5*	6.4				
.2	2.2*	4.1	2.7		4.1*				
.5		2.8	4.1*	8.4		2.9			
1			1.2	6.9	2.5	0.9			
2					1.7	1.7	9.7	8.7	
4						1.4*			
5					1.3		3.4*	4.9	
6						0.6			
10							0.4	3.5	1.5
15									4.0
20							1.0	0.2	2.4
50							0.8	1.3	3.4

* The prebait figure in each case is the average intake of all rats used for that poison. The intakes of poisoned bait at the lowest concentrations giving complete kills are marked with asterisks. (Mortality data on these same rats are given in Table II.)

tions such as other food supplies, or disturbances resulting from attacks by other rats or natural enemies, which could influence their food consumption. It is, for instance, unlikely that every unwanted rat would have the opportunity or even the inclination to eat 15 or 20 g of a 0.1% thallium sulfate bait at one sitting, and therefore poisoning with so low a concentration, while very efficient in the laboratory, might easily prove futile for

TABLE IV.
Reassays of 1080 and Arsenic Trioxide.
(Administered by stomach tube, in water containing 10% acacia, to recently trapped wild Norway rats (methods described in Ref. 1).

Poison and source	Dose, mg/kg BW	Mortality	$LD_{50} \pm$ S.E. mg/kg BW
1080	0.2	1/4	0.44 ± 0.19
(Monsanto Chemical Co.)	0.3	2/4	
	0.5	4/7	
	1	4/6	
	2	7/8	
As_2O_3	25	0/4	57.5 ± 7.0
(Mallinkrodt, analytical)	50	3/8	
	75	3/4	
	100	1/1	

practical purposes. But it can be said that any poison not effective in laboratory feeding tests such as these will undoubtedly kill far fewer rats in the field.

On the basis of the above results, then, 1080, ANTU, thallium sulfate, and zinc phosphide should be good and efficient rodenticides; arsenic trioxide (of good grade and with small particle size) slightly less so; while perfect control of Norway rat populations should not be obtainable through the use of red squill, barium carbonate, or strychnine sulfate.

The value of prebaiting is indicated by the fact that rats did not entirely refuse to eat baits containing the very bitter strychnine sulfate, or even concentrations of red squill as high as 50%, after they had had access to the same (unpoisoned) bait for 2 nights previously.

The reduced intakes of bait after adding poison may be ascribed either to early detection of the poison by the rats and subsequent refusal, or to the rapid onset of toxic reactions making the rats too ill to do more than sample the bait. In the case of 1080, which rats do not taste,[2] the second is probably the determining factor. For strychnine sulfate, the bitter taste is undoubtedly the deterrent. For most of the others it is more difficult to distinguish between the two possibilities. Thallium sulfate appears to be entirely undetected by the rats, and, were it not for the hazard to humans involved in its use, would seem to be the ideal rat poison.

Summary. The relative efficiencies of 8 common rodenticides were determined by offering the poisons to recently trapped wild Norway rats in a standard bait (freshly ground cornmeal) under standard conditions. The voluntary consumption of baits containing sodium fluoroacetate (1080), alpha-naphthyl thiourea (ANTU), thallium sulfate and zinc phosphide resulted in complete kills at concentrations of 0.5% or lower. A sample of arsenic trioxide of relatively high toxicity required a concentration of 4% for a complete kill. All the female rats offered 5% of a good grade of fortified red squill died, but higher concentrations, and all concentrations offered to male rats, gave only partial kills. Barium carbonate gave good but not complete kills at concentrations up to 50%, while strychnine sulfate killed few rats in the range tested.

The amounts of unpoisoned cornmeal consumed on 2 successive nights, by male and female wild Norway rats in various weight groups were determined. The addition of every poison except thallium sulfate to the cornmeal caused a considerable reduction in intake, owing either to recognition of the poison or to a rapid onset of toxic effects.

2 Richter, C. P., in press.