found the benzylidene derivative of 4,4′-diaminodiphenylsulfone to be low in toxicity and to be more effective than the parent compound in pneumococcus infections in mice and monkeys. They reported that mice would tolerate daily repeated doses of approximately 500 mg/kg. In our experiments on tuberculous guinea pigs a favorable therapeutic effect was obtained with doses of 200 mg/kg per day.

Summary. A series of derivatives of diphenyl sulfone, sulfoxide and sulfide were tested for *in vitro* tuberculostatic activity and for antituberculous therapeutic activity in guinea pigs. The following compounds, in addition to the reference materials 4,4′-diaminodiphenylsulfone and its derivative diasone, showed high *in vivo* activity: 4-benzylideneamino,4′aminodiphenylsulfone; 4-nitro,4′acetylaminodiphenylsulfone; 4,4′diaminodiphenylsulfoxide (hypo); 4,4′diacetylaminodiphenylsulfoxide (oral). 4-Chloro, 4′aminodiphenylsulfide and the corresponding iodo derivative were highly tuberculostatic *in vitro* but were devoid of *in vivo* activity.

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Biological Factors Involved in Poisoning Rats with Alpha-Naphthyl Thiourea (ANTU).*

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From modern studies in the fields of nutrition and biochemistry much has been learned regarding the principles involved in the feeding of animals to keep them alive and in good health; but little has been learned regarding the principles involved in feeding animals substances to destroy them. When animals are kept in captivity this latter objective can be readily attained, often unwittingly; when, however, animals are not confined and have full access to all kinds of foods in their natural states it is less readily attained.

The use of poisons for killing animals by feeding involves at least the following considerations: (1) The poisonous compound must be highly toxic to the animals that are to be killed; (2) it must be readily acceptable in lethal amounts; (3) after surviving the ingestion of sublethal amounts of the compound animals must not be able to develop too high or too prolonged a tolerance; (4) the poison must be as free as possible from taste and odors, by means of which animals once having survived poisoning, are able to recognize it on a second exposure and this devolep a "refusal" response.

In the present paper an attempt has been made to place the study of these different qualifications of a poison—toxicity, acceptance, tolerance and refusal—on a controlled experimental basis.

The substance used for this purpose was alpha-naphthyl thiourea (ANTU), a substance which has been shown to be highly toxic for domestic and wild Norway rats, doses of 1-5 mg killing the average-sized rat. On the basis of the results of these and of other similar tests this substance was recommended as a rat poison and is now actually being used as such on a wide scale.[1]

Method. Sixty-four adult hooded and albino male domestic rats, ranging in age from 82 to 228 days and in weight from 227 to 313 g were used for these experi-

* Started under a contract recommended by the Committee on Medical Research between the Office of Scientific Research and Development and The Johns Hopkins University and continued under a contract between the Medical Division, Chemical Corps, U. S. Army, and The Johns Hopkins University.

† With the assistance of Mrs. Romaine Randall.

[1] Richter, C. P., *J. A. M. A.*, 1945, **129**, 927.

ments. At these ages Norway rats have essentially the same high sensitivity to ANTU while younger rats are much less sensitive.[2] The rats came from our own colony. They were the offspring of albino Wistar rats which, approximately 20 years ago, were crossed with hooded and tan rats from the colony of Dr. E. V. McCollum.

To determine the acceptability of ANTU, the rats were kept on our stock diet‡ for a 10-day period, and then were divided into 9 groups of 6 to 10 rats each, which were given the stock diet mixed with ANTU in 9 different concentrations, ranging from 0.0005% to 10.0%, as follows:

Group	% concentration ANTU food	No. of rats
I	10	6
II	2	10
III	1	6
IV	0.1	6
V	0.05	6
VI	0.01	6
VII	0.005	6
VIII	0.001	9
IX	0.0005	9

The rats were kept in individual cages 8″ x 10″ x 12½″, containing a nonspillable food cup and an inverted water bottle. Records were taken daily of the food intake and the rats were weighed weekly and at every time a change was made in their diets. The cups were weighed and refilled to the same weight (100 g) each day. During the time that the rats received the plain stock diet, the food cups were weighed and filled at 11 a.m. every day; while on the day when they were to receive the poisoned diet, the cups were weighed as before at 11 a.m. but were returned empty and were not filled with the poisoned food until 4:30 p.m. Thus, the rats remained without food for 5½ hours. During this time of day, however, rats ordinarily eat little or nothing. The object was, first, to expose to the poisoned food, rats which would be as uniformly hungry as possible, and, further, to avoid at the time of this exposure any disturbing laboratory noises which might be present during the earlier part of the day.

The alpha-naphthyl thiourea was used in the form of a fine powder (80-120 micra particle size), which mixes readily with the stock diet. The drug and the diet were mixed for 15 minutes in a kitchen electric mixer to distribute the poison evenly in the food. For the lower concentrations the powder was thoroughly mixed with a small amount of food in a mortar before it was placed in the mixer with the full amount of food.

To determine the tolerance and refusal which develop when the rats have survived a sublethal dose of ANTU, the surviving rats were continued on their various concentrations of ANTU food for a period of 14 days; then they were given ANTU-poisoned food in a concentration (2%) which consistently killed 100% of all previously unpoisoned rats. The rats which survived the first day of this poisoning continued to receive the 2% ANTU food for a total of 5 days. Then they were returned to the unpoisoned stock diet for a period of 16 days, after which they were exposed for one day to both the unpoisoned stock diet and that containing 2% ANTU mixture. The survivors of this test were returned for a 13-day period to the unpoisoned stock diet and then were tested again for one day with 2% ANTU mixture.

The tolerance was measured in terms of the number of toxic units the animals ingested with impunity. Each "toxic unit" was taken to be 5 mg per kg body weight, or the amount of ANTU which, when administered by stomach tube, very consistently kills all rats from our colony.

Results. Acceptance Tests. All of the rats in Groups I, II, III, and IV, which received the 4 highest concentrations of ANTU food (10, 2, 1, and 0.1%) died within the first 24 hours after the poisoned food was placed in the food cups. All of the rats from the other 5 groups survived. Fig. 1A gives a typical record of one of the rats which had access to a 2% concentration of ANTU food. The bottom chart shows the intake

[2] Dieke, S. H., and Richter, C. P., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 22.

‡ Graham flour 72.5%, casein 10.0%, butter 5.0%, skim milk powder 10.0%, calcium carbonate 1.5%, sodium chloride 1.0%.

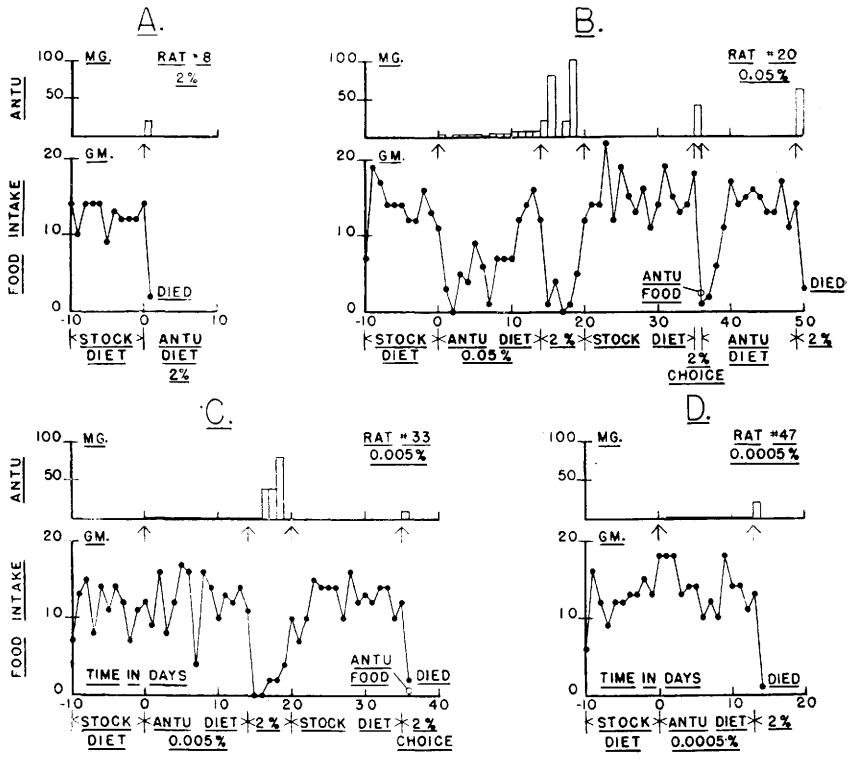

FIG. 1.

Typical individual food and ANTU intake records for rats from 4 different groups which received 2%, .05%, .005%, and .0005% concentrations of ANTU food respectively. The ordinates give the intake of food in grams and of ANTU in milligrams; and the abscissæ give the time in days.

of the stock diet for the 10-day preliminary period and of the ANTU-poisoned food for the first day. The top chart shows the intake of ANTU in milligrams. The food intake dropped from a daily average of 13 g for the 10-day period on the stock diet to 2 g for the first day on the poisoned food.

Table I summarizes the results, giving for each group the average age and body weight; the average daily intake of unpoisoned food for the 10-day preliminary period; the average intake of poisoned food for the first day. For the 10-day period the intake of stock diet ranged from 12-15 g for the different groups of rats. On the first day of the poisoning the rats of the first 6 groups ate much less of the food than they had previously eaten of the unpoisoned food. With these amounts the rats of the first 4 groups ingested lethal doses (from 1.4 to 62 toxic units on the average). The rats of the 5 other groups ingested an average of 1.2 toxic units or less and survived.

Evidence at hand indicates that when exposed to the higher concentrations of ANTU-poisoned food, these previously unpoisoned rats ate less, not because they disliked the food, but because the effects of the poisoning appeared before they could eat their usual amounts. Observations made on many rats while they were eating the poisoned food showed that when first exposed they ate the poisoned food freely in all concentrations as high as 20%. They gave no evidence of refusing to eat it freely, until suddenly, usually after less than 10 minutes, they stopped eating, the ingested poison apparently having made them sick. On unpoisoned stock diet rats ate for from 30 to 60 minutes and ingested 4 to 5 g, definitely more than was eaten by the rats on the poisoned diets.

TABLE I.
First Day of Poisoning.

Groups	Conc. of ANTU in food, %	Avg age at start, days	Avg wt at start, g	Avg daily food intake last 10 days on stock diet, g	Avg intake of ANTU food g	Avg intake of ANTU mg	mg/kg	Toxic units*	Results
I	10	228	313	13	1.0	100	311.3	62	All 6 died.
II	2	—	272	15	2.1	42	153.8	31	" 10 "
III	1	220	293	13	1.2	12	40.2	8	" 6 "
IV	0.1	205	313	13	2.2	2.2	7.2	1.4	" 6 "
V	0.05	100	265	13	3	1.5	5.8	1.2	" 6 survived
VI	0.01	101	255	13	9	0.9	3.5	0.7	" 6 "
VII	0.005	103	268	13	10	0.5	1.8	0.36	" 6 "
VIII	0.001	82	232	12	12	0.13	0.6	0.12	" 9 "
IX	0.0005	89	227	12	12	0.06	0.3	0.05	" 9 "

*5 mg per kilo body wt.

It is likely that the effects appeared no sooner when rats ingested the 10% concentration than when they ingested the 0.1% concentration, since 5 to 10 minutes appears to be the minimum time required for the poison to become absorbed and to take effect. This explains why the rats ate approximately the same amounts of each of the 4 highest concentrations of ANTU-poisoned food. The amount of ANTU derived from the food with the 0.05% concentration may have made the rats ill in approximately the same time, but the effect probably was not severe enough to stop them from eating at once, as was true with the 4 higher concentrations. Undoubtedly, therefore, the rats exposed to the 5 highest concentrations ate only one meal, while the rats on the lower concentrations, with a food intake which ranged from 9 to 12 g per day, must have eaten at least 3 meals during the 18-hour period.

Development of Tolerance and Refusal. After having survived the first day of poisoning, no rat from any of the 5 remaining groups died during the rest of the 14-day period. At the end of this period, however, when the rats of all groups were offered the 2% ANTU food, only those of Groups VIII and IX, which had previously received the 2 lowest concentrations of ANTU, 0.001% and 0.0005% respectively, died. All of the rats of the other 3 groups survived. Fig. 1D shows a typical record of one of the rats of Group IX. During the 14-day period on the poisoned food this rat continued to eat just as much as it had eaten during the previous 10-day period on the unpoisoned stock diet. When placed on the 2% ANTU diet it ate only 1 g and died. That this amount was smaller than the average intake of previously unpoisoned rats of Group II, which received the 2% ANTU diet, indicates that the rat may have developed a slight refusal; that it died indicated that it had not developed a definite tolerance.

Fig. 1C gives a record which is typical for the rats of the 2 groups, VI and VII, which received diets with the 0.01 and 0.005% concentrations of ANTU respectively. During the 14-day period on the poisoned food this rat continued to eat almost as much as

it had previously eaten of the stock diet. When given access to the 2% ANTU food it showed a total refusal for 2 days. Then on the 3rd, 4th, and 5th days, it ate large amounts (over 65 toxic units on one day) and survived. This rat had developed a refusal and also a very marked tolerance during the 14-day period on the poisoned food. At the end of the following 16-day period on the unpoisoned stock diet when the rat was given a choice for only one day of the 2% ANTU diet and of the unpoisoned stock diet, it ate less than 1 g of the poisoned food and died. This indicates that a slight refusal may have remained at this time, but that most of the tolerance had disappeared.

Fig. 1B gives a typical record of the rats of Group V, which received the 0.05% ANTU food for the 14-day period. On the first day of the 14-day period this animal ate 3 g of the poisoned food, which apparently sufficed to make it sick but not to kill it, for on the next day it did not eat any food at all. Each day thereafter, however, it ate progressively more until, toward the end of the period, its intake reached its normal level again, showing that during this period the rat had developed a definite tolerance. When on the 15th day it was given the 2% ANTU food it ate 1 g (20 toxic units of ANTU) and on the 4 succeeding days ate fairly liberal amounts with impunity. Thus this rat appeared to have developed a slight refusal and a very great tolerance.

Its tolerance continued for a longer time than did the tolerance of the rat whose chart is shown in Fig. 1C. Fig. 1B shows further that after 16 days on the unpoisoned stock diet, this rat ate 2 g of the unpoisoned food and 1 g of the poisoned food but showed no ill effects. It was returned to the unpoisoned stock diet for a 13-day period. At the end of this time it ate 3 g, which is more than the average intake of the same ANTU food of previously unpoisoned rats. Thus at this time it showed no definite refusal or tolerance, and died.

Table II summarizes for the 5 surviving groups of rats the results of the observations made for the 14 days during which they re-

TABLE II.
Fourteen Days on ANTU Diet.

Group	0-14 day period on ANTU diet					15th day—(2% with diet)			Results
	Conc. of ANTU in food %	Avg daily intake of ANTU food g	Avg daily intake of ANTU		Avg total intake of ANTU		Avg ANTU food intake g	Avg ANTU intake		
			mg	Toxic units	mg	Toxic units		mg	Toxic units	
V	0.05	7.0	3.40	2.82	48.70	39.6	1.0 (5 rats)*	20.0	18.0	All survived
VI	0.01	10.7	1.04	0.82	14.58	12.0	1.0 (2 rats)†	20.0	16.5	" "
VII	0.005	12.9	0.63	0.46	8.93	7.3	1.0 (2 rats)‡	20.0	13.1	" "
VIII	0.001	13.2	0.13	0.10	1.58	1.28	2.4	48.8	36.9	" 9 died
IX	0.0005	12.6	0.06	0.05	0.75	0.62	1.2	24.4	19.3	" 9 "

* One rat did not eat a measurable amount.
† Four rats did not eat measurable amounts.
‡ Four rats did not eat measurable amounts.

TABLE III.
2% Concentration of ANTU—5-Day Period (15th to 19th Days Inclusive).

Group	Avg daily intake of ANTU food g	Avg daily intake of ANTU mg	Avg daily intake of ANTU Toxic units	Total amt of ANTU in 5-day period Toxic units	Results
V	2.2	41.3	39.5	197.5	All 6 survived
VI	0.6	12.6	11.1	55.4	" 6 "
VII	1.4	28.0	20.1	108.0	" 6 "

ceived their varying concentrations of ANTU food; it also gives the records for the 15th day, on which they received the 2% ANTU food.

The rats of Group V, which received the 0.05% ANTU diet, ate an average of 7 g, or only slightly more than half of the amount that they had previously eaten of the stock diet. Most of the animals of this group ate very small amounts during the first few days, and almost normal amounts during the last few days of the 14-day period. For the entire period the total amount of ANTU ingested averaged 48.7 mg or 39.6 toxic units, which indicated that a high tolerance had been developed. When given access to the 2% ANTU food, one rat did not eat a measurable amount on the first day while the other 5 ate an average of 1 g, less than half as much as the previously unpoisoned rats ate of this concentration. Their ANTU intake averaged 18.0 toxic units. All 6 rats survived.

During the initial 14-day period the rats of Groups VI and VII, which received the 0.01 and 0.005% concentrations of ANTU, ate only slightly less than they had previously eaten of the plain diet. When given access to the 2% ANTU food most of these rats showed a very strong refusal. Four of the 6 rats of Group VI and 4 of the 6 rats of Group VII completely refused the diet on the first day. The ANTU intake on the first day for the 2 remaining rats of each of the 2 groups averaged 16.5 and 13.1 toxic units respectively. All of the rats of these 2 groups survived.

During the initial 14-day period the rats of Groups VIII and IX ate just as much of the ANTU food as they had previously eaten of the plain diet. When given the 2% ANTU diet they ate almost as much as previously unpoisoned rats ate of the same concentration. Thus, they showed little or no refusal. They ingested 36.9 and 19.3 toxic units respectively and died.

These results show that the daily ingestion over a 14-day period of ANTU in amounts as small as 0.63 mg or 0.46 toxic units sufficed to establish a definite tolerance and refusal.

The high tolerance established in these rats is shown further in Table III, which gives for the 3 remaining groups of rats, V, VI, and VII, the intake records for the 5-day period on 2% food. This daily intake of ANTU averaged 39.5, 11.1 and 20.1 toxic units respectively, while the total intake for the 5-day period averaged 197.5, 55.4 and 108.0 toxic units. None of the rats died. Thus, the rats of these 3 groups developed a very strong tolerance during the 14-day period on their respective concentrations of ANTU food.

Duration of Tolerance and Refusal. Returning the rats of the 3 remaining groups, V, VI, and VII, to the plain stock diet for a 16-day period caused the tolerance and refusal of some of the rats to disappear. Table IV, which summarizes the results, gives the intake of poisoned and of unpoisoned food on the day following the end of the 16-day period; also a record of the mortality. The intake of the plain stock diet averaged 2.0, 1.3 and 2.5 g, respectively, while the intake of the poisoned food averaged 1.6, 1.3 and 2.0 g. One rat in Group V and 2 in Group VII did not eat measurable amounts of the ANTU-poisoned food. The intake of ANTU averaged 25.6, 19.5 and 27.4 toxic units respectively for the 3 groups. All of the rats of Group V survived, while only one of Group VI and 2 of Group VII survived. Thus, the rats which previously had been on the 0.01 and 0.005% concentrations of

TABLE IV.
2% Concentration of ANTU—36th Day (16 Days after Last ANTU).

Group	Avg intake of unpoisoned food g	Avg intake of ANTU food g	Avg intake of ANTU mg	mg/kg	Toxic units	Results
V	2.0	1.6 (5 rats)*	32.0	128.3	25.6	All 6 survived
VI	1.3	1.3	26.6	97.4	19.5	5 died, 1 survived
VII	2.5	2.0 (4 rats)†	40.0	138.0	27.4	4 " 2 "

* One rat did not take a measurable amount.
† Two rats did not take measurable amounts.

TABLE V.
2% ANTU—50th Day.

Group	No. of rats still alive	Avg intake of ANTU food g	Avg intake of ANTU mg	mg/kg	Toxic units	Results
V	6	0.18	6.6	24.8	5.0	All 6 died
VI	1	1.0	20.0	69.9	14.0	Died
VII	1	0.75	15.0	52.8	10.6	"
	1	0.5	10.0	33.3	6.6	Survived

ANTU had lost much of their tolerance as well as their refusal. The rats of Group V which had previously received the 0.05% concentration still maintained a high tolerance.

Table V shows that after another period (13 days) on the stock diet the rats of Group V died when they were placed on the 2% ANTU diet. They had lost most of their tolerance. The rats of this group ingested 5.0 toxic units on the average. The small food intake of these rats, 0.18 g, showed that they still had a strong refusal response. The one surviving rat of Group VI ate 1 g, or 14.0 toxic units of ANTU and died. One of the 2 surviving rats of Group VII ate 0.75 g or 10.6 toxic units and died, while the other ate 0.5 g or 6.6 toxic units and survived.

This last rat was returned to the stock diet and exposed again to the 2% ANTU food for one day. It ate scarcely a measurable amount. That the failure to kill this animal depended largely on the presence of a strong refusal response rather than on the presence of a marked tolerance is shown by the fact that forced feeding with a stomach tube a few days later with 30 mg per kg body weight, or 6 toxic units, promptly killed it.

Discussion. The results of these experiments show that the domestic Norway rat readily accepts ANTU-poisoned food in concentrations at least as high as 10%. They show further that a sharply defined boundary exists between the concentrations of ANTU-poisoned food that are lethal and sublethal. All animals that were offered food poisoned with concentrations of 0.1% died, while all animals that ate concentrations of 0.05% survived. The minimum lethal concentration must lie between these 2 concentrations.

The results brought out an equally sharply defined boundary between concentrations of ANTU-poisoned food which did and did not produce a tolerance or refusal. Thus, all of the rats which had received ANTU-poisoned food in a concentration of 0.0005% survived, while all of the rats which received the next lower concentration (0.001%) died when exposed to the 2% concentration of ANTU food. The lowest concentration at which ANTU-poisoned food will produce a tolerance or refusal, or both, must, therefore, lie between these 2 concentrations.

These experiments show that in the 14-day period the rats on the 0.05% concentration of ANTU-poisoned food developed a very great tolerance. The frequent eating of small amounts of poisoned food during the day may account for the ability of the rats to withstand such great amounts of poison. These experiments show also that the tolerance disappeared quite rapidly; in many instances

in 16 days; in all instances in 30 days.

The physiological basis for the development of this tolerance remains unknown. ANTU poisoning increases the permeability of the lung capillaries, and as a consequence produces a great pulmonary edema and pleural effusion. The lung edema is apparently the cause of death.[1] The development of tolerance must therefore produce some changes in the effects produced by the poison in the lung capillaries.

It is noteworthy that some animals have a natural tolerance to very large amounts of alpha-naphthyl thiourea.[2] Thus, rhesus monkeys have survived doses of 3500 mg per kg body weight by stomach tube, which is 700 times the amount required to kill domestic Norway rats; likewise chickens have survived doses of 2500 mg per kg body weight. Guinea pigs, rabbits and most wild rodents, including Alexandrine rats, have a high tolerance. Of the animals tested so far dogs and pigs[3] are the only animals besides Norway rats which do not show this tolerance. Their LD_{50} lies between 25-35 mg per kg body weight which is still much higher than that for the strain of rats used in the present studies.

Domestic Norway rats from colonies in some laboratories and wild Norway rats, recently trapped from the streets, show a high resistance to the parent compound thiourea, while the rats from our colony die after the ingestion of minute doses.[4] It was found that while 640 mg per kg body weight was required to kill the rats of the Long-Evans strain from Dr. Astwood's colony in Boston, 1830 mg per kg body weight was required to kill recently trapped wild Norway rats. Recently trapped wild Alexandrine rats *(Rattus rattus alexandrinus)* showed the same high resistance.

The resistance to thiourea poisoning does not seem to be a natural tolerance since it is markedly influenced by diet. Thus feeding our stock diet for 1-6 months reduced the LD_{50} of the rats from Dr. Astwood's colony to 44 mg per kg body weight,[4] and feeding experimental rats of the hooded (Rowett) strain a straight oatmeal diet for 4 weeks reduced the amount of thiourea at which a 100% mortality was obtained from 200 mg to 20 mg.[5] Likewise feeding adult wild Norways on our stock diet reduced the LD_{50} from 1830 mg per kg body weight to below 500 mg per kg body weight in 9 months.[6]

These experiments show also that after eating the ANTU-poisoned food, even in a very low concentration, the rats developed a marked refusal to eat food that was poisoned with definitely lethal concentrations. The lower concentrations, which apparently did not produce such a marked tolerance, developed a greater refusal. In some animals the refusal was much reduced after 16 days and practically absent after 30 days. However, in one animal it remained very strong after 36 days and gave no sign of diminishing.

The basis for this refusal response has not been determined. It could depend on association of ill effects with the taste of ANTU, or with the smell. ANTU has a moderately bitter taste to some human subjects and therefore it probably has a bitter taste to rats also. Observations made on rats as they ate ANTU mixtures in concentrations as high as 20% demonstrated that its taste cannot be extremely unpleasant and bitter to them. The slight bitterness of the ANTU may however suffice as a cue for the refusal. In the form used in these experiments ANTU had an extremely faint odor. This odor, scarcely detectable by human subjects, may be readily detected by rats, since their olfactory sensitivity probably is much greater than that of human beings.§ The rats may detect it in the surrounding air since it floats readily in air, and can then be detected by its taste rather than by its smell. Sectioning of taste nerves and removal of the olfactory bulb in rats with a strong

[3] Anderson, W. A., and Richter, C. P., *Vet. Med.*, 1946, **41**, 302.

[4] Dieke, S. H., and Richter, C. P., *J. Pharmacol.*, 1945, **83**, 195.

[5] Landgrebe, F. W., and Morgan, T. N., *Nature*, 1946, **157**, 22.

[6] Dieke, S. H., and Richter, C. P., unpublished data.

§ Wild Norway rats may have an even greater sensitivity than domestic rats.

refusal reaction should differentiate the part played by taste and smell in the refusal response.

These experiments show that when rats are exposed to ANTU, which they probably taste only faintly, they are able to protect themselves by a rapid development of both a tolerance and a refusal. It is unlikely that rats ever encounter ANTU or closely allied thiourea compounds in the wild state, but the principle involved in the development of tolerance and refusal probably holds equally true for other poisons.

Summary. 1. Adult domestic Norway rats were found to accept ANTU when mixed with the stock diet in concentrations at least as high as 10%. In all concentrations from 0.1% to 10% inclusive the ANTU-poisoned food killed 100% of the rats. Lower concentrations of ANTU-poisoned food gave a 100% survival.

2. When fed sublethal concentrations of ANTU food from 0.05% to 0.005% inclusive for 14 days, the rats developed a marked tolerance and refusal. Lower concentrations failed to produce either.

3. In some rats the tolerance disappeared at the end of 16 days and was almost imperceptible in all of the rats at the end of 30 days.

4. The refusal decreased after 16 days, though in some animals it was definitely present even at the end of 30 days.

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Further Studies on Relationship Between Activity and Constitution in the Cardiac Glycoside Series.*

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In a recent report[1] various glycosides and genins were compared with respect to their ability to produce atrioventricular block in the isolated embryonic chick heart, and it was possible to suggest certain conclusions as to the relationship between chemical structure and cardiac activity. The technic has been described in detail and a procedure for estimating the precision of the results has been proposed.[2] A number of additional glycosides and related compounds have now been studied and the results are given in Table I. In general, each compound was compared directly with a closely related reference material and indirect comparisons were computed from previously published activity ratios.

For practical reasons it is difficult to study the interrelationships of the glycosides in man. Hence, in comparing these drugs on an artificial preparation it is necessary to inquire into the significance of the results with respect to the observed clinical potencies. Comparison of the oral therapeutic dose for 3 well known drugs with the results obtained by bio-assay showed a direct correlation with the embryonic heart data but divergent results with respect to the cat assay.[1] Thus, the ratios of the human daily maintenance doses for digitoxin, digoxin, and lanatoside C were 1:3:7; the concentration ratios for atrioventricular block in the isolated heart, 1:3:4.5; while for the intravenous lethal doses in the cat the ratios were 1:0.7:0.45. Each of these ratios has been computed on

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1 DeGraff, A. C., Paff, G. H., and Lehman, R. A., *J. Pharmacol.*, 1941, **72**, 211.

2 Lehman, R. A., and Paff, G. H., *J. Pharmacol.*, 1942, **75**, 207.