Brucella after prolonged cultivation has involved reversions to virulence in mutants originating from relatively avirulent nonsmooth types. Thus, it would appear that in most instances the potentiality for virulence is not really lost* but its manifestation is merely suppressed and may be reestablished by various types of mutational change. As frequently suspected in the past(3), this ability of relatively avirulent cells to yield new mutants with high virulence, capable of establishing themselves under proper environmental conditions, may be of significance to problems of pathogenesis and epidemiology.

Summary. Following prolonged incubation of originally smooth *Brucella abortus* broth cultures, relatively avirulent nonsmooth variants established themselves but were eventually replaced by a smooth type, labelled S′, in turn replaced later by still another smooth type, labelled dS. These three smooth types were found to be similar in many characteristics, including virulence, but differed significantly in their resistance to alanine, an inhibitory metabolite which accumulates in the culture fluid. These differences in alanine resistance permitted the S′ and dS mutants to establish themselves at periods when alanine levels of the cultures were high. Therefore, what superficially appeared to be an establishment of reverse mutants actually involved the establishment of novel mutants which simulated the original smooth type in many characteristics.

* Strain 19 of *Br. abortus* seems to be one exception.

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Influence of Alpha-Naphthyl Thiourea on Gastric Evacuation.* (18634)

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In the course of earlier experiments performed in this laboratory(1), it was noted that the stomachs of rats dying from acute poisoning with alpha-naphthyl thiourea (ANTU) were frequently hugely distended with food, and since the deaths occurred some hours after the rats had eaten, this suggested the possibility that the ANTU had in some way interfered with the normal process of gastric evacuation. The experiments described in the present paper were, therefore, planned to investigate the possible effect of various doses of ANTU on gastric emptying time.

Methods. The experiments were performed with adult albino rats, both male and female, of the Wistar strain or a strain derived from it. The acute median lethal dose (LD_{50}) of ANTU, as administered to these rats, was roughly 5 mg/kg of body weight; doses of 2 mg/kg killed none, while 10 mg/kg was almost invariably fatal within 7 to 17 hours (2). The rats were trained to eat a sizable meal in one hour or less; ANTU suspended in olive oil was then administered by intraperitoneal injection, and the weights of the stomach contents at varying intervals afterwards were then compared with those of control rats that received only olive oil. In one experiment banthine† was given to a group of rats to compare the effect of this drug with that of ANTU in delaying gastric evacuation. Data on a few rats receiving epinephrine‡ were also obtained. The rats were trained to eat a substantial amount of food in a short time by offering food for only a stated 2-hour

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1. Richter, C. P., *J.A.M.A.*, 1945, v129, 927.

2. Unpublished data from this laboratory.

† Diethylaminoethyl xanthine-9-carboxylate methobromide, provided through the courtesy of Dr. Irwin C. Winter of G. D. Searle & Co.

‡ 1:500 suspension in peanut oil, from Abbott Laboratories.

period of each day during a week or 10 days' training period. Water was available at all times. The food used was our regular stock diet.§ Frequently a rat did not eat on the first or even the second day, and occasionally rats ate so little as to die of inanition, but by the end of a week the majority were avid eaters, waiting for the food to be put in their cages and consuming it rapidly. In one typical group of 24 rats on this regimen, 1 died on the 7th day, while the food intake of the 23 survivors on the 10th day averaged 12.2 g, ranging from 7.5 to 29.3 g. These rats thus ate in 2 hours about as much food as they normally would eat in 24 hours. The average loss of body weight during the training period was 17 g, or 4.7% of the average initial body weight (363 g).

On the day of poisoning, the drinking water was removed and the rats were offered food that they were allowed to eat for one hour. Occasionally rats stopped eating before the hour was up. Immediately after this meal, (or just before, in one experiment) the rats received a dose of ANTU, banthine or epinephrine. The doses were graduated to the rats' body weights, and were given by intraperitoneal injection rather than by mouth to avoid any direct effect on the gastro-intestinal tract. Control rats received only olive oil, also by intraperitoneal injection. Groups of drugged and control rats were always carried through in parallel on the same days. At hourly intervals thereafter, the rats were sacrificed (by crushing the cervical spine with pliers) and autopsied. The stomach was exposed, separated from adjacent organs, tied off with silk thread at the cardiac and pyloric sphincters, and weighed; it was then split lengthwise, emptied and reweighed, the difference giving the weight of the contents. In those experiments in which dry weights were also determined, the contents were placed in individual evaporating dishes and dried in an oven at 110°C to constant weight loss, together with control dishes containing equivalent amounts of food.

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

Data on 137 rats poisoned with ANTU, 10 rats receiving banthine, 13 rats receiving epinephrine, and 71 control rats are included here. The observations cover the 24 hours subsequent to poisoning. ANTU was given at 3 dosage levels: 15 rats received 2 mg/kg, a sublethal dose; 52 had 5 mg/kg, from which dose approximately 50% of the rats might be expected to survive, but which customarily produces no casualties in the first 18 to 20 hours(2); and 70 received 10 mg/kg, 16 just before and 54 just after feeding. Casualties from this last dose are usually 100%, with the rats beginning to die at 7 hours, and in fact the data between 7 and 16½ hours were obtained at death, and no data for intervals longer than this could be got.

Results. Fig. 1 shows that ANTU caused a delay in gastric evacuation, the degree of which depended on the amount of ANTU administered. Average values of the ratio of stomach contents to food eaten are plotted against the time elapsed after feeding ended. It will be seen that for the control rats the ratio of stomach contents to food eaten ranged from 1.30 to 1.60 directly after eating¶. The ratio then decreased gradually for the controls, as the stomach emptied, reaching an average 0.39 at 12 hours and becoming 0.02 at 24 hours.‖ In marked contrast to the controls, the values obtained in rats receiving 10 mg/kg of ANTU never fell below 1.29 and at 16½ hours a value of 1.75 was obtained, indicating minimal discharge of food from the stomach. At all times these values were well above the highest individual values obtained with control rats.

The ratios of stomach contents to food

¶ This is above 1.00 on account of the oral and gastric secretions—see below for dry weights.

‖ According to x-ray studies on the rat's gastro-intestinal tract(3), a barium meal is normally evacuated from the stomach in about 2 hours, with a range of 1 to 3 hours. Since such a meal is liquid and of high specific gravity, it might be expected to leave the stomach more quickly than the solid food eaten by the rats in the present experiment.

3. Gershon-Cohen, J., and Shay, H., in *The Rat in Laboratory Investigation,* edited by E. J. Farris, and J. Q. Griffith, Jr., 2nd ed., *J. B. Lippincott Co*, 1949, page 431.

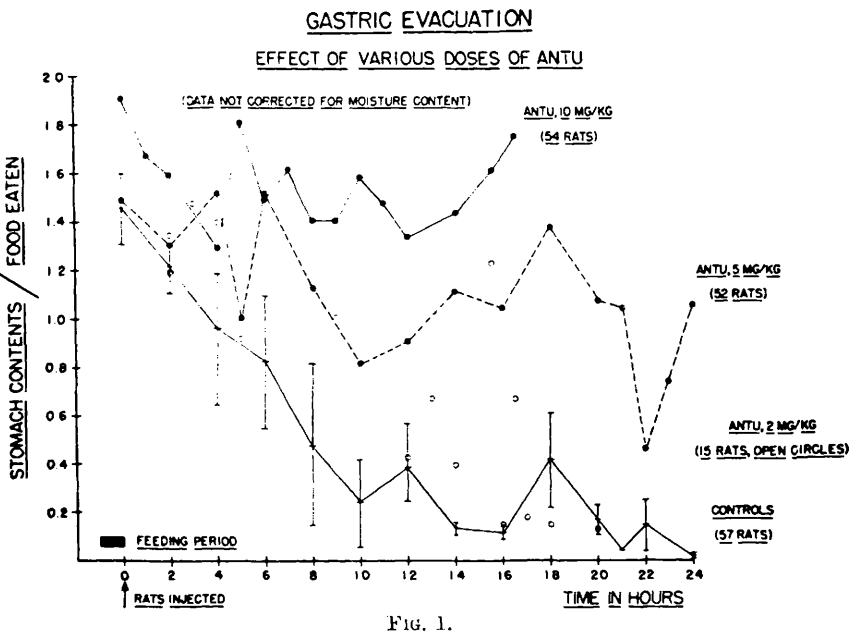

FIG. 1.

Ratios of stomach contents to food eaten, obtained at various times after feeding, when rats were injected with ANTU: 2 mg/kg (open circles, individual values), 5 mg/kg (crossed circles and dotted line, average values), and 10 mg/kg (black circles, average values). All injections intraperitoneal in olive oil. Data *not* corrected for moisture content. Data on control rats (injected with olive oil only) are presented with both average and range.

eaten obtained after 5 mg/kg of ANTU, on the other hand, were found to fall into 2 groups from 5 hours on; one quarter of the rats gave values falling along the control curve, while for the remainder, gastric evacuation was greatly delayed. The irregularity of the 5 mg kg curve reflects the averaging of disparate values. Rats receiving this dose survived longer than those receiving 10 mg/kg of ANTU, and accordingly the data extend over the full 24-hour period, with an average ratio of 1.06 being obtained at 24 hours. This is comparable to values obtained with control rats at 4 hours after feeding. After 2 mg kg of ANTU no long-lasting delay in gastric emptying occurred, although it can be seen that the individual values usually fell on the high side of the control curve, but were never as high as ratios obtained after the larger doses of ANTU.

It would seem then that abnormal retention of food in the stomach occurs characteristically after a fatal dose of ANTU. Some of the values obtained at 20 or more hours after poisoning were truly surprising: one poisoned rat ate 12.7 g of food and when sacrificed at 22 hours had 16.4 g in its stomach, as compared to a control that ate 12.2 g and had only 0.2 g remaining at 22 hours. Another poisoned rat ate 29.3 g of food and had a stomach containing 27.6 g 20 hours later.

To determine what fraction of the stomach contents, as measured above, was food and what secretion, an experiment was performed in which dry as well as wet weights of stomach contents were obtained, using 35 additional rats: There were 14 controls, while 11 received 10 mg/kg ANTU, and 10 received 10 mg/kg banthine (a non-fatal dose: 12 additional rats received 10 or 20 mg/kg of banthine and showed no toxic symptoms during the following 48 hours). These injections were again all intraperitoneal, with olive oil as the vehicle. Fig. 2A gives the average values obtained, while the ratios plotted are now for food and for stomach contents after drying.**

** The food itself was found to contain 9.1% moisture, and this fact was taken into account in computing dry weights of food to compare to the dried stomach contents.

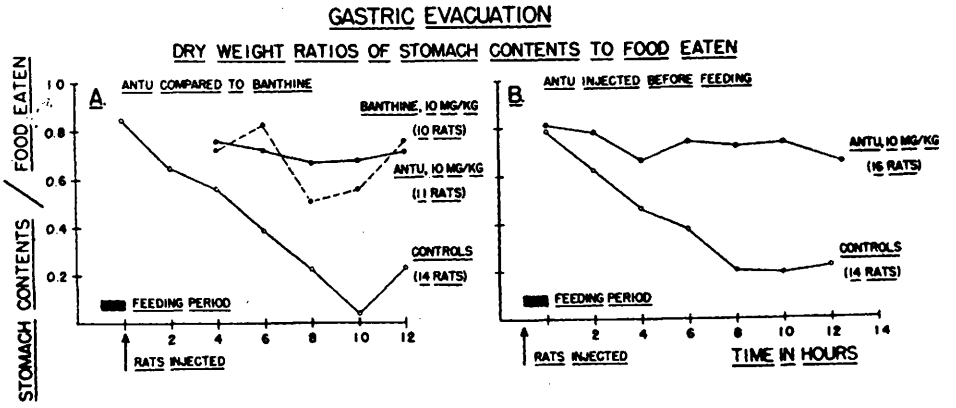

FIG. 2.

Ratios of stomach contents to food eaten, obtained at various times after feeding. Data all corrected for moisture in food and stomach. All injections intraperitoneal in olive oil. A—Comparison of delay produced in control rats, in rats receiving 10 mg/kg of banthine (dotted circles) and in rats receiving 10 mg/kg of ANTU (black circles). B—Effect of ANTU given just before rather than just after feeding.

It will be noted that ANTU and banthine both delayed gastric evacuation, and to an equivalent degree if allowance is made for individual variation.

When the individual dry and wet ratios were compared and then averaged, it was found that the reduction obtained by drying amounted to 54 ± 3% for the controls, 50 ± 4% for the ANTU-treated rats, and 52 ± 5% for those receiving banthine. This variation is sufficiently small to indicate that a rat's stomach usually contains roughly equal amounts of food and secreted fluids, and that division of all the data of Fig. 1 by a factor 2 will correct fairly adequately for moisture content. It is noteworthy that data corrected for moisture content (Fig. 2) give much smoother curves than uncorrected data (Fig. 1).

Fig. 2A also shows that rats sacrificed directly after eating have already discharged about 15% of the ingested food from the stomach. Since ANTU is a relatively insoluble substance which requires several hours after injection before it builds up to toxic levels in the body, it was thought that by injecting ANTU before feeding somewhat higher amounts of food might be retained in the stomach. Accordingly, an experiment was performed in which the rats were injected just before feeding; 14 rats receiving only olive oil served as controls, while 16 received 10 mg/kg of ANTU. The results are shown in Fig. 2B, where the ratio of dried stomach contents to dried food is again plotted against time after feeding ended. Comparison with Fig. 2A shows that no substantially increased retention of food in the stomach occurred, despite the earlier administration of the ANTU.

The experiment using epinephrine (in peanut oil, nearly fatal doses of 3-4 mg/kg injected intraperitoneally) showed that this drug also delayed gastric emptying for the first 12 hours after feeding, giving average "wet" ratios of stomach contents to food eaten ranging from 1.80 to 1.48, but at 16 to 21 hours the effect had worn off and the values obtained (0.17 to 0.09) were equivalent to those of the corresponding controls.

An attempt to repeat these experiments with dogs was unsuccessful, owing to the emetic properties ANTU has even when given intraperitoneally. (The technic was satisfactory for rats because they cannot vomit).

Discussion. The explanation for this action of ANTU in prolonging the sojourn of food in the stomach is as yet unknown. The magnitude of the effect appears from the above results to be related to the acute toxicity, but the relation of this finding to other acute effects of ANTU poisoning, namely the production of pulmonary edema and pleural

effusion(4), the disturbance of carbohydrate metabolism as evidenced by a high blood sugar and depleted stores of glycogen in the liver (5), and the rapid drop in body temperature (6), is not clear. It might be mentioned that the data reported above on the moisture content of the rats' stomachs after eating are so nearly identical in rats poisoned with ANTU and in the controls as to indicate that no part of the liquid in the poisoned rats' stomachs was edema fluid. Nor was edema of the stomach wall specifically noted by Latta in his histological studies on ANTU-poisoned rats(4). Sections which might show edema of the pylorus were, however, not included in his material.

It is possible that this delayed gastric evacuation is the result of a direct central action of ANTU, although evidence for a vomiting center in rats is lacking(7). Or it may be mediated reflexly through the autonomic innervation of the stomach, as are the equivalent effects of banthine(8) and epinephrine

When administered chronically, ANTU affects the thyroid, causing hyperplasia: it presumably interferes with the production of thyroid hormone just as do the thiouracils and other related anti-thyroid drugs(9). This effect also occurs after a single dose(10); the delayed gastric emptying may therefore be mediated through the thyroid. In this connection it is interesting to consider the work of Northrup and Van Liere(11), who found that sulfapyridine had a statistically significant influence in delaying the emptying of the normal human stomach.

Another possibility is that with the development of pulmonary edema, the rats suffered from anoxia and that this was what delayed gastric evacuation. Van Liere, Cresler and Robinson(12) have found (as later confirmed by Van Liere)(13) that gastric emptying was delayed in dogs subjected to reduced pressures, the more so the less oxygen available. A normal emptying time of 7 to 7.5 hours at atmospheric pressure was increased to more than 24 hours in 2 of their dogs held at somewhat less than half that pressure. This is the only instance a search of the literature has provided of delays in gastric evacuation equal to those reported in this paper.

Summary. Intraperitoneal injection of fatal or near-fatal doses of alpha-naphthyl thiourea (ANTU) markedly lengthened gastric emptying time in rats, as determined by comparing the weights of stomach contents in ANTU-poisoned rats to those of controls. In some rats only minimal evacuation of the stomach had occurred after 24 hours. The chemical vagotomizing agent banthine, in sublethal doses, and epinephrine, in near fatal doses, also delayed gastric evacuation, although the effect of epinephrine wore off before that of ANTU. The explanation for this finding and its relation to other effects produced by ANTU are still unknown.

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