Summary. The inhibition by INH of mammalian catalase and mycobacterial catalase of representative strains was markedly enhanced by divalent copper ions. Pyridoxal hydrochloride completely reversed the inhibitory action of the INH-Cu^{++} complex. This antagonism apparently was due to chemical removal of the inhibitor by precipitation. Pyridoxamine and pyridoxine, which respectively have slight and no effect *in vitro* against the antituberculosis activity of INH, did not significantly affect the INH-metal ion inhibition of mycobacterial catalase.

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Effect of Goldthioglucose Injections on Survival, Organ Damage and Obesity in the Rat.* (27942)

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Brecher and Waxler(1) reported that injection of goldthioglucose (GTG) in some mice would result in hyperphagia and obesity; they found no lesions in the brain or other organs of these animals. Marshall *et al.*(2) described a similar GTG-induced obesity in mice; on autopsy they found extensive ventromedial hypothalamic (VMH) lesions. Mayer and Marshall(3) investigated the specific nature of various gold compounds in production of VMH damage and obesity; they suggested that the glucose component of the GTG molecule accounted for the selective accumulation of gold in the hypothalamus. In the same study it was stated briefly that goldthioglucose administration did not lead to obesity in the rat. It is the purpose of this report to relate the effects of GTG injections to survival, to production of lesions (brain and other organs), and to the incidence of obesity in the rat.

Materials and methods. Male and female rats of the Long-Evans (L-E) or Sprague-Dawley (S-D) strains were used; animals ranged in size from 50 g to 400 g. Freshly prepared solutions of goldthioglucose (Solganol B)§ dissolved in 0.9% saline were injected IP in doses ranging from 0.1 to 0.75 mg/g. Some rats were fasted prior to injection.

Following injection, animals were placed in metabolic cages and given free access to food and water. If the injected dose appeared to be lethal, the rats were perfused just prior to death with 10% formolacacia in saline, their brains removed and stored in the same fixative; kidneys, pancreas, stomach and testes were also removed and stored in Bouin's fixative. Frozen sections of brain were cut at 50 μ and stained with thionin blue; other tissues were paraffin embedded, cut serially at 5-7 micra and stained with he-

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matoxylin-eosin or iron hematoxylin-aniline blue. Pancreatic tissue was stained with aldehyde-fuchsin and Orange-G. Body weights of surviving injected animals were recorded twice weekly.

Results. 1. *Preliminary studies.* A pilot study was carried out on 48 male L-E rats of 50 g, 100 g, and 250 g. GTG doses (IP) ranged from 0.1 to 0.5 mg/g; half of the animals were fasted 24 hours prior to injection. It was found that fasting prior to injection increased survival time; non-fasted rats died more quickly (1.5 to 3.5 days) than did fasted rats (2.0 to 4.5 days). Most fasted 50 g rats survived doses of GTG that were fatal to all older animals. Since the brain histology was of primary interest in this series, the 9 surviving (weanling) rats were sacrificed after 2 weeks; thus, no data on obesity were obtained. Only one animal showed bilateral VMH damage; no instances of unilateral VMH damage were noted in this group, or in subsequent experiments. Occasional necrotic glial cells were found among intact neurons.

To establish a 14-day LD_{50}, 63 male L-E rats, 50 g, 24-hour fasted, were given IP injections of GTG in doses ranging from 0.1 to 0.75 mg/g. The 14-day LD_{50} was found to be 0.25 mg/g. Surviving animals were sacrificed 14 days after injection. Bilateral VMH lesions were found in 3 of 60 brains examined; these were from animals that survived 1½-3 days after injection. Several brains exhibited glial damage (pyknosis, swelling) in presence of normal VMH neurons.

Within 12 hours after injection, all rats exhibited aphagia, adipsia, anuria, evidence of lowered body temperature (*i.e.*, shivering, huddling), increased sensitivity to a variety of stimuli, and mild tremor; the onset of convulsions invariably preceded death in rats given lethal doses of GTG.

2. *GTG-induced organ damage and obesity.* Twenty-two male and female rats (140 g) of L-E or S-D strains were given 0.4 mg/g GTG (IP) after 48 hours fasting; animals were sacrificed 24 hours after injection. The data (Table I) indicate that female rats of either strain sustained more severe VMH destruction after GTG injection than did males; all animals of this weight injected with 0.4 mg/g after 48 hours fasting showed a high incidence of brain (VMH) lesions (90%) as compared to animals in the preceding experiments (2-5%). An example of the area of neuronal and glial damage is shown in Fig. 1. Lesions of the gastric mucosa were seen in 19 of 20 animals with GTG-induced VMH damage.

TABLE I. Incidence and Degree of VMH Damage and Incidence of Gastric Ulcers in 140 g Rats, 48 Hours Fasted, Injected with 0.4 mg/g GTG and Sacrificed 24 Hours After Injection.

Sex	Strain	Total animals	VMH damage			Gastric Ulcers
			Severe*	Mild†	None‡	
♂	L-E	6	3	2	1	5
	S-D	6	1	4	1	6
♀	L-E	4	4	0	0	4
	S-D	6	5	1	0	5

* Area of destruction clearly visible at 10× magnification (see Fig. 1).

† Damage to neuronal and glial elements requiring 50-100× magnification to verify.

‡ No cellular damage when viewed at 1000× magnification.

Twenty-four 250 g male S-D rats, fasted for 24 hours, were injected IP with 0.2 mg/g GTG; the mortality of this group and body weight gain of surviving animals were compared to a similarly treated group of L-E rats. A strain difference in tolerance and response to GTG was suggested. Five of the S-D animals survived doses that were lethal to all L-E rats; of these 5, two exhibited bilateral VMH destruction with significantly greater body weight gain (Fig. 2) after 4

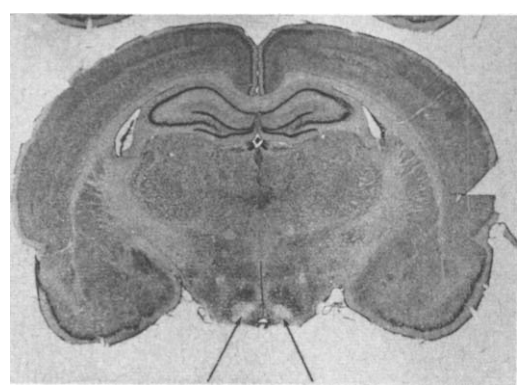
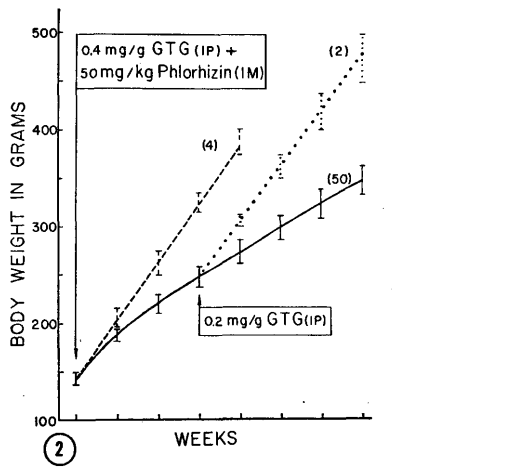


FIG. 1. Photomicrograph of a thionin-stained coronal brain section (10×) from a 140 g L-E rat, 48 hr fasted, injected IP with 0.4 mg/g GTG and sacrificed 24 hr later. Arrows indicate bilateral ventromedial hypothalamic lesions.



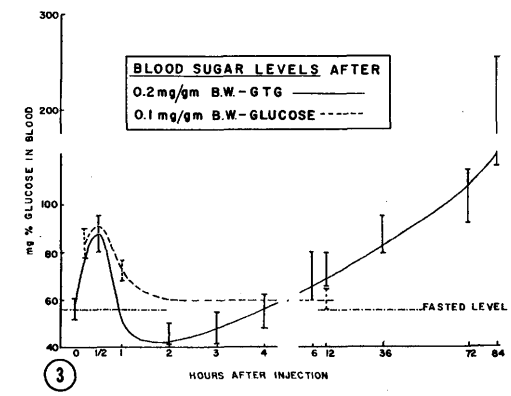


FIG. 2. Body wt changes among surviving GTG-treated rats (broken lines) sustaining VMH lesions as compared to a large control group (solid line). Vertical bars represent weight range in each group.

FIG. 3. Blood glucose levels (Triphasic pattern, 7 determinations per point) in adult L-E rats after GTG injections (solid line) and after equivalent glucose injections (dashed line). Vertical bars represent range of values.

weeks. Autopsy showed excessive subcutaneous and intra-abdominal fat deposits.

A constant finding in all GTG-injected rats was extensive glomerular and tubular degeneration. In an effort to reduce the toxic effects of GTG on the kidneys, 20 of 60 150 g male S-D rats, 48 hours fasted, were injected IP with 0.4 mg/g GTG, followed 6 hours later by IP injections of phlorhizin dissolved in normal saline; phlorhizin administration was repeated 24 and 48 hours after GTG injection to a total of 50-100 mg/kg. The remaining 40 rats were treated with dimercaprol (BAL) in sesame oil administered IM to a total of 0.15 mg/g on the same schedule as for phlorhizin injections. A slight increase in survival rate was noted after phlorhizin administration; the 4 survivors showed increased fat deposition and body weight gains (Fig. 2) within 4 weeks and VMH damage at autopsy. Without protective measures, death occurred within 24 hours after 0.4 mg/g GTG injections; when GTG was followed by BAL, animals lived from 48-72 hours. Two rats survived 6 weeks after treatment with BAL; they showed normal weight gains and intact VMH areas. Treatment with phlorhizin or BAL reduced, but did not eliminate, the renal damage produced by GTG injection.

Microscopic study of pancreatic tissue from all groups of GTG-injected animals revealed variable amounts of degranulation within the beta cells of the islets. There was no apparent correlation between this response and dose of GTG, survival or strain differences in the groups studied.

The glycemic condition in response to injections of GTG was examined. Seven adult male L-E rats (350 g-400 g) bearing permanently implanted (jugular) venous catheters, 24-hour fasted, were injected IP with 0.2 mg/g GTG. Blood glucose levels were determined (enzyme method of Marks[4]) until death occurred. These data were compared with glucose levels from 10 rats which received IP injections of glucose (in normal saline) equivalent in volume and concentration to the GTG solution used (Fig. 3).

Sections of testes appeared normal in all GTG-injected rats.

Discussion. Mayer and Marshall(3) noted that rats were more susceptible to the toxicity of gold compounds than were mice at dose levels of 1.0 mg/g (GTG). The present study indicates that less than half of this dose is capable of producing VMH damage and obesity similar to that described by these authors. However, the minimum effective obesogenous dose of GTG in rats is difficult to distinguish from lethal or toxic doses. Liebelt and Perry(5) reported differences in susceptibility to GTG-induced obesity in genetically unrelated inbred strains of mice; a similar difference has been demonstrated among rats in this study. Although obesity

was not seen in any of the L-E adult rats in this short-term experiment, further investigation is needed to determine whether or not it may be induced in younger animals of this strain; the increased tolerance seen in 50 g rats suggests this possibility.

A triphasic blood glucose curve similar to that following GTG injection has been described for rats after alloxan injection(6). Its significance in the total response to GTG is not clear; the terminal hyperglycemia and the observed degranulation of pancreatic islets indicate a possible action of this compound as a diabetogenic agent.

Debons *et al.*(7) using autoradiographic localization methods detected the earliest evidence of gold deposition in the hypothalamus 6 hours after GTG injection in mice. In the present study, BAL was withheld for 6 hours after GTG injection to permit entrance of goldthioglucose into the VMH; the finding of well-formed lesions in these animals suggests that the effective gold-binding action of BAL was delayed for a considerable period thereafter. Administration of this protective agent at an earlier time was contraindicated by the observation(8) that GTG and BAL combined before injection in mice reduced the incidence of obesity to zero and increased survival to 100%.

Histological study of the kidneys from GTG-injected rats revealed glomerular and tubular destruction comparable to the kidney damage reported by Brobeck, Tepperman and Long(9) in rats with hypothalamic lesion-induced obesity. It should be noted, however, that renal damage was observed among GTG-injected rats without detectable hypothalamic lesions; thus, the relationship between VMH destruction and kidney damage remains obscure.

That the gastric ulcers observed may be a consequence of (GTG-induced) VMH lesions is suggested from Deter's recent finding (10) that such ulcers were formed only *after* VMH damage appeared.

It is possible from the available data to make some inference concerning the mechanism whereby GTG may influence the neural substrates involved. The electron microscope studies of Luse *et al.*(11) showed that neuronal degeneration was secondary to oligodendroglial damage in the hypothalamus of GTG-injected mice. This is consistent with the present observation that rats which received doses of GTG insufficient to produce distinct VMH lesions, did exhibit varying degrees of glial damage; moreover, extensive glial destruction accompanied all visible VMH lesions. Thus as previously stated (11), GTG appears to interfere with the glial route between capillary and neuron. Evidence as to whether or not the entry of GTG into the brain is dependent on variation in blood-brain barrier permeability(12) or on a "glucoreceptor" mechanism(2) has been inconclusive in studies on mice.

Summary. Preliminary studies showed that weanling Long-Evans rats are less susceptible to goldthioglucose than are adults; the LD_{50} for 50 g animals was 0.25 mg/g. Some adult Sprague-Dawley rats were found to withstand doses of GTG that were lethal to adult Long-Evans rats, and to demonstrate a developing obesity. More severe VMH lesions were seen in female than in male rats following GTG injection. Glial damage without neuronal destruction appeared in several GTG-injected rats. Treatment with phlorhizin and BAL reduced renal damage produced by GTG injection. GTG injection, in adult rats, produced a triphasic blood-glucose pattern.

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Circulatory Responses to Cardioaccelerator Nerve Stimulation after Induction of Tyramine Tachyphylaxis. (27943)

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It is now clear that norepinephrine (NE) can be released from sympathetic nerve endings not only by nerve stimulation, but also by a number of aromatic amines, including tyramine(1). It has recently been shown that after a prolonged infusion of tyramine, the physiologic response to subsequent injection of this amine is virtually abolished, in spite of the finding that substantial quantities of NE remain in the tissues(2). If the physiologic response to neural stimulation could be shown to remain unimpaired despite loss of responsiveness to tyramine, this would suggest that the NE released by these two stimuli is not derived from a homogeneous pool.

Methods. The chronotropic and inotropic responses to stimulation of the cardioaccelerator nerve were compared before and after an infusion of tyramine. Six dogs weighing 10.2 to 12.8 kg were lightly anesthetized with a chloralose-urethane mixture given intravenously and, following a right thoracotomy, right ventricular contractile force was determined with a Walton-Brodie strain gauge arch. Supramaximal stimulation of the right cardioaccelerator nerve at a frequency 10/second was carried out with a Grass stimulator. Tyramine was injected intravenously at a dose of 60 μg/kg before and 1000 μg/kg after a one-hour infusion of 200 μg/kg/min of this substance.

Results. Prior to the tyramine infusion cardioaccelerator nerve stimulation produced an average increase in heart rate of 75 $\pm$ 11 beats/min and of right ventricular contractile force of 58% $\pm$ 25%. Administration of 60 μg/kg tyramine at this time resulted in an average increase in heart rate of 61 $\pm$ 8 beats/min and of contractile force of 108% $\pm$ 19%. Immediately following the one-hour infusion of tyramine, the response to stimulation of the cardioaccelerator nerve was essentially unchanged, with an average increase in heart rate of 67 $\pm$ 10 beats/min and in contractile force of 54% $\pm$ 26%. At this time, 1000 μg/kg of tyramine produced an average increase in heart rate of only 13 $\pm$ 4 beats/min and in contractile force of 13% $\pm$ 9% (Fig. 1).

Discussion. A number of investigators have suggested that the NE pool at the sympathetic nerve endings is not a homogeneous one. Trendelenburg has proposed a distinction between NE which is "available" for release by tyramine and nerve stimulation and "bound" NE(3). In support of this hypothesis of 2 compartments of NE stores in the tissues, it has recently been shown that following depletion of myocardial NE stores by reserpine, the physiologic response to tyramine is dependent upon the presence of only a small fraction of the normal tissue NE concentration(4,5). In addition, Potter and associates have demonstrated that isotopically labelled NE remaining in the heart for long periods of time is mainly confined to a tightly bound store and unavailable for release by tyramine, which also suggests the presence of more than one tissue store of NE(6).

It was shown previously that an infusion of 200 μg/kg/min of tyramine for one hour reduced atrial NE content by an average value of 43% of the control, and abolished the cardiovascular responses to further injec-