tween taking the fumarates and the first bowel movement was about 6 hours.

The 3 salts, magnesium fumarate, calcium fumarate, and sodium fumarate, are free from toxic effects in the doses which were used.

The data obtained in this study are strikingly similar to those reported by Gold and Zahm, and indicate that the fumarates provide a satisfactory substitute for the tartrates as laxative agents.

Summary. The laxative action of sodium, magnesium, and calcium fumarates has been compared with that of sodium potassium tartrate in 143 chronically constipated patients. The fumarates have been found as satisfactory as Rochelle Salts as a laxative agent.

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A Method for Consistent Induction of Chronic Hyperglycemia with Alloxan.

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The capacity of alloxan for inducing in the experimental animal a state resembling diabetes mellitus[1,2,3] has opened a new and promising approach to the study of the disease. One of the major problems involved in the use of alloxan lies in the inconstant and variable responses of experimental animals to its administration.[1,4,5] Large doses of alloxan frequently cause toxic deaths with hepatic or renal lesions, while smaller doses inconstantly produce chronic hyperglycemia. The experience of Lackey *et al.*[6] is a common one; they observed that 60% of rats injected intraabdominally with 200 mg of alloxan per kg of body weight developed moderate to severe diabetes, while 40% either died during the first 72 hours or failed to become diabetic.

In view of the manifest desirability of standardizing the experimental approach to the study of the chronic hyperglycemic state in rats, means were investigated whereby a high percentage of rats treated with alloxan would develop hyperglycemia, with relatively few deaths and with relatively little tendency to become comatose on ordinary laboratory diets. We have found, in substance, that withdrawal of all food from adult animals for periods of 48 to 60 hours will render them almost uniformly susceptible to the subsequent subcutaneous injection of 175 mg of alloxan per kg of body weight. The chronic hyperglycemia so produced leads to few fatalities in the rat and the tendency toward spontaneous recovery of normal blood sugar levels is greatly reduced as compared with animals made diabetic under other conditions.

The animals used were white rats of the Sprague-Dawley strain which were fed on ordinary pellet ration throughout except where otherwise indicated. The dose of alloxan was 175 mg per kg after correction for the molecule of water in alloxan monohydrate. Alloxan samples from two different commercial sources were used, with no discernible differences in their effects. The final solution used contained 19.9 mg of alloxan per ml and was always made just before use; one ml of this solution for each 100 g of body weight was then administered subcutaneously. In most of the experiments the animals used weighed 200-280 g, and dosages were measured to the

[1] Dunn, J. S., and McLetchie, N. G. B., *Lancet*, 1943, **2**, 384.

[2] Brunschwig, A., Allen, J. G., Goldner, M. G., and Gomori, G., *J. A. M. A.*, 1943, **122**, 966.

[3] Bailey, C. C., and Bailey, O. T., *J. A. M. A.*, 1943, **122**, 1185.

[4] Gomori, G., and Goldner, M. G., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 287.

[5] Bailey, C. C., Bailey, O. T., and Leech, R. S., *N. Eng. J. Med.*, 1944, **230**, 533.

[6] Lackey, R. W., Brinde, C. A., Gill, A. J., and Harris, L. C., PROC. SOC. EXP. BIOL. AND MED., 1944, **57**, 191.

TABLE I.
Effect of Starvation on Susceptibility of Rats to Alloxan.

Starvation period (days)	Total No. rats	Blood sugar (mg%)			
		> 250	180-250	< 180	Deaths
		No. of rats			
3	20	14	1	1	4
2	60	49	6	3	2
1	44	21	6	17	0
1*	16	3	1	12	0
0	40	5	5	28	2

* Fed *ad lib.* 6 hours before injection of alloxan.

nearest 10 g of body weight. When comparisons were made using different methods of treatment, the attempt was made to keep the ages of the rats in comparable groups as nearly alike as possible. Blood sugar levels were routinely determined before injection of alloxan as well as 5-7 days thereafter.[7]

Table I illustrates the effect of starvation and feeding on the susceptibility of rats to alloxan. The animals are grouped according to the severity of the hyperglycemia, using 180 mg % as the upper limit of normality. The incidence of hyperglycemia in the animals which had been starved for 2 days prior to injection of alloxan was 92% (55 of 60 rats) whereas the incidence of hyperglycemia was but 25% (10 of 40 rats) in animals whose food had not been withheld. Furthermore, those animals which did respond to the drug tended to attain higher blood sugar levels if they had previously been starved. It is of interest to note that only one animal in the group of previously starved animals had normal blood sugar values three months after the onset of hyperglycemia, whereas 4 of 6 rats from the group which had not been starved previous to the injection of alloxan had normal blood sugar levels when tested 3 months after they had been shown to be hyperglycemic. The hyperglycemic animals typically lost weight slowly, and showed pronounced polydipsia, polyuria, and polyphagia. Very few deaths occurred during the observation period of 3-4 months subsequent to the onset of hyperglycemia, even though the ordinary pellet diet was used; despite the prolonged hyperglycemia, insulin was not necessary to keep the animals alive.

We have confirmed the observation in the literature that animals which have not responded to one injection are frequently refractory to reinjection of a similar dose of alloxan.[4,8] In order to determine the relation of this refractory state to the state of nutrition of the animal, 26 rats were chosen at random from animals which had previously failed to respond to alloxan. These animals were all in groups which had not been starved prior to the injection, and at least one month had elapsed since the injection of the first dose of alloxan. They were then starved for 60 hours, given the standard dose of alloxan and examined one week later. Twenty-three of the 26 showed marked hyperglycemia, one animal failed to respond and 2 died. The 23 hyperglycemic animals showed no diminution in blood sugar levels 3 months later.

While it is likely that truly refractory animals do exist, it appears that most of the "refractory" rats so far encountered can be made susceptible to alloxan by withholding food for a suitable period of time.

The duration of the starvation period is not a constant factor; in one experience in which 20 larger, older rats (290-340 g) were used, alloxan did not induce hyperglycemia despite previous starvation for 48 hours. After a week's rest, however, the animals were starved for 72 hours, reinjected with alloxan, and all became markedly hyperglycemic.

Since food administered 6 hours before the injection of alloxan conferred a significant degree of protection against the effects of the drug, certain aspects of this phenomenon were investigated further. Groups of rats were

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[8] Hard, W. L., and Carr, C. J., PROC. SOC. EXP. BIOL. AND MED., 1944, **55**, 214.

TABLE II.
Effect of Vitamins, Glucose, and Epinephrine on Production of Alloxan Diabetes in Starved Rats.

Treatment	Blood sugar (mg%) > 250	180-250	< 180	Deaths
	No. of rats			
None	7	2	1	0
Vit. (B comp. + C)	7	0	0	3
Glucose*	5	4	1	0
Glucose†	3	0	7	0
Epinephrine	2	2	6	0

* Alloxan injected 1 hr after administration of glucose.
† Alloxan injected 6 hr after administration of glucose.

starved for 48 hours and then given vitamin mixtures, glucose, or epinephrine before injection of alloxan. A typical experiment is summarized in Table II. The rats receiving vitamins each received 200 γ of thiamine chloride, 400 γ riboflavin, 400 γ pyridoxine, 400 γ nicotinic acid, 400 γ *p*-aminobenzoic acid, 400 γ inositol, 1 mg calcium pantothenate and 35 mg choline; other groups of rats received 10 mg ascorbic acid in addition, in a separate injection. The vitamins were administered intraperitoneally 6 hours before the alloxan and, as can be seen, did not protect the animals against the alloxan. Despite the apparent relationship between the B vitamins and other types of experimental diabetes,[9] the vitamins did not protect against the effects of alloxan. This indicates, further, that the increased susceptibility of starved animals to alloxan is not directly related to depletion of vitamin stores.

Glucose was administered as a 25% solution, each rat receiving 4 ml intraperitoneally. Those rats which received alloxan one hour after administration of glucose were not protected against the drug, whereas those which received alloxan 6 hours after injection of the sugar were significantly protected. Blood sugar values of rats in both groups were determined just prior to the injection of alloxan and values of 200-400 mg % were obtained in all cases, indicating that the blood sugar level itself is not the factor determining resistance to alloxan.

The experiments with epinephrine are of particular interest. Each starved rat received 0.1 ml of 1-1000 dilution of epinephrine intraperitoneally, blood samples were immediately drawn, and the alloxan injected. None of the blood sugar levels was higher than 132 mg % yet there was marked protection against the effects of the alloxan. We have repeated this interesting observation many times and have never failed to demonstrate the protective effect of epinephrine despite the fact that the alloxan was administered before any appreciable alteration in blood sugar levels had occurred.

The uniform susceptibility to alloxan evidenced by rats after starvation does not appear to be due to alterations in the liver glycogen, since it has been demonstrated that liver glycogen values in the rat are greater during the second day of starvation than during the first day, probably as a result of increased adrenal cortical activity.[10,11] This increased cortical activity may explain the greater tendency of starved animals to respond to alloxan. Certainly the susceptibility to alloxan bears no simple relationship to blood sugar levels, as is borne out by the experiments in which starved animals were treated with the drug after feeding or after administration of glucose or epinephrine. Detailed histological studies of these effects are in progress.

The action of epinephrine in inhibiting the effect of alloxan was unexpected since the hyperglycemia produced by the epinephrine was never so great as that produced following injection of glucose. Glucose, on the other hand, did not protect against alloxan unless a

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[10] Britton, S. W., and Silvette, H., *Am. J. Physiol.*, 1932, **100**, 693.

[11] Long, C. N. H., Katzin, B., and Fry, E. G., *Endocrinol.*, 1940, **26**, 309.

period of some hours had elapsed before injection of the drug. While the possibility of direct union between epinephrine and alloxan exists, it hardly seems likely since the drugs were given by different routes of injection, using different syringes. Although a few of the substances which react with alloxan *in vitro* exert some protective effect *in vivo,* the amounts of the protective substances are much greater than the amount of epinephrine which protected against alloxan.[12]

Although Best *et al.*[13] have shown that starvation reduces markedly the insulin content of the pancreas, and this may explain the increased susceptibility of the starved animal to alloxan, the failure of insulin to protect against alloxan when the two are injected simultaneously[14] would indicate the inadequacy of such an explanation.

Summary. 1. Chronic hyperglycemia was produced in 90-95% of adult white rats which had been starved for 48-60 hours and then injected subcutaneously with 175 mg of alloxan per kg. Only 25% of rats which had not been starved became hyperglycemic after injection of a similar dose. The hyperglycemia is persistent and relatively benign; few spontaneous recoveries or fatalities occurred within 3-4 months after injection of alloxan.

2. Fed animals which had not developed hyperglycemia following injection of alloxan became susceptible to subsequent injection of another dose if they were previously starved.

3. Feeding or injecting glucose 6 hours before alloxan diminished the susceptibility of the starved animals to alloxan. Glucose administered one hour before alloxan had no protective effect. Epinephrine, administered immediately before alloxan, protected the starved animals. These effects are not directly related to the blood sugar levels existing at the time alloxan is injected.

4. Mixtures of vitamins of the B complex, and ascorbic acid given 6 hours before injecting alloxan failed to protect starved rats from becoming hyperglycemic.

5. Heavier rats require longer periods of starvation in order to achieve a high degree of susceptibility to alloxan.

[12] Weinglass, A. R., Frame, E. G., and Williams, R. H., PROC. SOC. EXP. BIOL. AND MED., 1945, **58**, 216.

[13] Best, C. H., Haist, R. E., and Ridout, J. H., *J. Physiol.*, 1939, **97**, 107.

[14] Goldner, M. G., and Gomori, G., *Endocrinol.*, 1944, **35**, 241.