Further Studies on Toxicity of Thioacetamide in Rats. (17831)

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In a previous study(1) we have shown that weanling albino rats placed on a diet containing 0.032% thioacetamide (TA) for 190 days showed changes in the liver characterized by irregular fibrosis, hyperplasia of the bile ducts, fatty infiltration, necrosis and nodular regeneration of the parenchymal cells. Since it is a well established fact that diffuse hepatic fibrosis is a regular occurrence not only in rats on a diet low in lipotropic factors, but also after the administration of toxic substances which may undergo reversal by lipotropic factors or by dietary means(2,3,4,5,6,7,8), studies were undertaken to determine the effect of lipotropic factors on the course of the lesions in the liver produced by the ingestion of thioacetamide.

The present report is concerned with: (a) studies on spontaneous regression after discontinuance of the hepatotoxic agent, (b) determination of the onset of liver necrosis, and (c) the prophylactic effect of lipotropic factors (choline, methionine, and casein).

Experimental methods and results. Since, as reported previously(1), fatty infiltration and fibrosis with nodular regeneration were found in the livers of rats on a diet containing as little as 0.032% TA, the present studies were conducted at this dietary level of intake. The diet to which TA was added was the same as that previously used (Purina Dog Chow). Both male and female weanling rats were used. The livers from 5 rats maintained on 0.032% TA for 120 days and later placed on the basic diet alone for 170 days showed no spontaneous regression. Grossly the livers of these rats appeared mottled (uniformly nodular) and yellowish green. Since these livers appeared nodular on gross observation, no histological studies were made. Rats maintained on 0.032% TA for 30, 60, and 120 days showed varying degrees of liver damage. The first effect observed in the livers of 3 rats on the diet for 30 days was to bring about cellular hypertrophy with relative increase in size of the nucleus and nucleolus. This effect seemed to be confined to the liver parenchymal cells. At the same time that these hypertrophic changes were occurring in the liver cells a few were apparently succumbing to the effect of TA, since their nuclei were missing and the cytoplasm lacked definition. By the 60th day most of the livers from 4 rats showed some scarring, although collagen was not abundant. In the 6 rats on the diet for 120 days, many, perhaps half, of the cells did not show the hypertrophic change though the nucleoli remained relatively prominent. The hypertrophied cells tended to occur in clusters, as described previously in rats on the diet for 190 days (1).

In the experiments that follow, dietary supplements known to have lipotropic action were added to the basic diet (Purina Dog Chow) containing 0.032% TA as follows: Diet 1, Basic diet + 0.032% TA; Diet 2, Diet 1 + 0.7% DL-methionine + 2% yeast; Diet 3, Diet 1 + 30% casein; Diet 4, Diet 1 + 0.2% choline chloride + 2% yeast; Diet 5, Basic diet alone.

The rats were kept on the respective diets

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TABLE I.
Statistical Comparison of Livers of Rats Receiving Thioacetamide with and without Lipotropic Factors. 5 Rats/Group.

Diet	Liver wt		
	% of bd. wt	S*	e*
1 Basic + 0.032% TA	7.39	.77	.35
2 No. 1 + 0.7% DL-Methionine + 2% Yeast	5.39	.08	.03
3 No. 1 + 30% Casein	5.80	.37	.17
4 No. 1 + 0.2% Choline Chloride + 2% Yeast	5.57	.35	.15
5 Basic (Control)	3.22	.20	.09

* S—Standard deviation. e—Standard error of the mean.

for 78 days, after which time they were weighed, autopsied, and the liver carefully removed and weighed. Grossly the livers from rats on Diets 1, 2, 3, and 4 were indistinguishable, with perhaps the livers from rats on Diet 3 appearing slightly less affected than the livers of rats on the other three diets.

In the table, data are given on the mean percentage of the liver weight to body weight for each group of rats. Based on the relationship of liver weight to body weight, the livers of rats on diets containing no lipotropic agent (Diet 1) other than that not known to be present in the diet, and those on 30% casein (Diet 3) were the heaviest, while those on the diets containing DL-methionine (Diet 2), and choline (Diet 4) were the lightest. However, even these livers were heavier than those of rats on the basic diet (Diet 5) alone. The smaller size of the livers of rats given the active lipotropic agents, DL-methionine and choline (Diets 2 and 4), respectively, is significant and is probably related to the amount of fat present. Histopathological study of the livers of rats on the four different dietary regimens containing 0.032% TA showed that the most severe changes occurred in rats on Diet 2, with the least severe changes in rats on Diet 3. Females on the latter diet showed advanced changes.

Since liver fibrosis, nuclear and cytoplasmic inclusion, and cell variability were observed in all of the rat livers, except those on Diet 5, it cannot be said that any of the dietary lipotropic supplements were effective in preventing liver damage, although there appears to have been produced a quantitative alteration in the character of the lesion.

Histological changes of tissues were not remarkable except for the thyroids. In all animals, other than the controls, the thyroids appeared grossly atrophic. On histological examination, the thyroid alveoli contained dense colloid and the lining epithelial cells were flattened, suggesting inactivity.

Conclusions. As has been reported by Orr (6) and by Sellers *et al.*(7) spontaneous regression of liver cirrhosis may occur on discontinuance of the hepatotoxic agent when damage to the liver is not too severe. In our studies rats maintained on 0.032% TA for 120 days and then placed on the basic diet alone for 120 days showed no regression in the amount of liver damage produced by TA. This is in agreement with the findings of Cameron and Karunaratne(8) that the cirrhosis produced after the prolonged administration of carbon tetrachloride is irreversible.

Rats on 0.032% TA for 30 days all showed some changes in the liver which became progressively worse after 60 and 120 days on the diet. The addition of the active lipotropic agents (choline, DL-methionine, or casein) to the diet containing 0.032% TA did not prevent liver damage.

Summary. Scarring and nodular hyperplasia of the liver were produced in rats maintained on an otherwise adequate diet containing 0.032% thioacetamide (TA). Administration of lipotropic factors (choline or DL-methionine and casein) in conjunction with 0.032% TA significantly decreased liver weight when compared to body weight. On the other hand histological examination of the livers from rats on the diet containing 30% casein in conjunction with 0.032% TA

(Diet 3) showed the least severe changes, while the livers of rats on Diet 2 (DL-methionine) showed the most severe changes. Since fibrosis and various degrees of parenchymal lesions were observed in all rats on 0.032% TA with and without the added lipotropic factors, it cannot be said that any of the lipotropic factors studied were effective in preventing liver damage, although there appears to have been a quantitative alteration in the character of the lesion.

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Failure of Malononitrile in Therapy of Experimental Poliomyelitis.* (17832)

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Recently Szanto and Felsenfeld(1) reported malononitrile to be effective in protecting more than fifty per cent of mice infected with a low concentration of the Lansing strain of poliomyelitis and prolonging the life span of mice infected with a larger dose of the virus. They also reported that mice treated with malononitrile after onset of paralysis showed a markedly prolonged survival time, about one-third of which recovered completely or partially. The purpose of this communication is to report the failure of malononitrile to protect mice against intracerebral inoculation of the Lansing strain of poliomyelitis. It was also found that this drug had no therapeutic effect when administered to mice after the onset of paralysis.

Methods. The Lansing strain of poliomyelitis virus was used in all therapy experiments. The technic of preparation and storage of mouse brain suspensions of this virus for intracerebral inoculation is described elsewhere(2). The identity of the Lansing strain used in this laboratory is periodically verified by production of typical paralysis in rhesus monkeys following intracerebral inoculation, histopathologic findings and neutralization tests with hyper-immune Lansing antiserum. Two strains of Swiss mice (Webster & Beyer) weighing 13 to 15 g were employed in the present studies. Groups of mice were inoculated with either 10 or 100 LD_{50} units of virus intracerebrally in chemotherapy experiments. The LD_{50} titer of the virus was approximately $10^{-3.5}$. Two types of experiments were done. In the first, mice were inoculated daily with malononitrile $CH_2(CN)_2$† beginning 24 hours before virus inoculation and therapy was continued until onset of paralysis. In the second, mice were treated with malononitrile after onset of paralysis, and the survival time was compared with that of untreated, paralyzed mice. The survival time of the paralyzed mice was recorded as the time when the mice were found dead. In most instances the actual time of death was observed.

Results. Mice were inoculated intraperitoneally with 2.5 to 3 mg per kg body weight daily in therapy experiments. The dosage of 3 mg per kg of body weight was given in most experiments, and we also found that this amount of drug was well tolerated by the mice. Combined results obtained in 3 experiments with groups of treated and control mice challenged with approximately 10 and

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† Obtained from the Schwarz Laboratories, N.Y.