confused by the fact that anoxia itself alters elasticity, and therefore the increased efficiency produced by digitalis would in itself be expected to increase diastolic elasticity. Such an indirect effect is a secondary result of the primary action of the drug upon work performance.

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Liver Regeneration in Rats Protected with Xanthine Against Carbon Tetrachloride Poisoning.

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Many investigators have attempted to prevent liver necrosis which may arise from the industrial or medical use of carbon tetrachloride or chloroform. Sato[1] described a liver hormone, "Yakriton," prepared from ox livers. Since that time Sato and his collaborators, in a series of nearly a hundred papers, have reported excellent results from the use of this detoxicating hormone. Among the more important claims for "Yakriton" has been the prevention of chloroform necrosis. Recently, Forbes and Neale[2] and Forbes, Neale, and Scherer[3] prepared a liquid extract of hog livers, which, when administered to albino rats prior to acute poisoning with carbon tetrachloride or chloroform, gave a protective action against these drugs. This protective agent was later crystallized from the crude extract by Forbes and McConnell.[4] Neale and Winter,[5] continuing these investigations, were able to identify as xanthine or sodium xanthine the crystalline material that protected against carbon tetrachloride and chloroform. The protective action of liver extract, as well as of sodium xanthine, has been further confirmed by Barrett, MacLean, and McHenry.[6]

Because of the observations made in this laboratory on calcium

1 Sato, A., *Tohoku J. Exp. Med.*, 1926, **8**, 232.

2 Forbes, J. C., and Neale, R. C., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 319.

3 Forbes, J. C., Neale, R. C., and Scherer, J. H., *J. Pharm. and Exp. Therap.*, 1936, **58**, 402.

4 Forbes, J. C., and McConnell, J. S., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 359.

5 Neale, R. C., and Winter, H. C., *J. Pharm. and Exp. Therap.*, 1938, **62**, 127.

6 Barrett, H. M., MacLean, D. L., and McHenry, E. W., *J. Pharm. and Exp. Therap.*, 1938, **64**, 131.

as a protective agent in carbon tetrachloride intoxication,[7] we decided to study further the action of xanthine in carbon tetrachloride poisoning. The large dose of xanthine required and the insolubility of this substance led us to use a method similar to that of Neale and Winter.[5] During our study of the microscopic sections from the livers of rats protected with xanthine, certain points of interest were noted. The livers of rats protected with xanthine and killed 24 hours after the administration of carbon tetrachloride showed little or no protection in comparison with the controls. At the 48-hour period, however, a marked difference could be seen in the 2 groups of sections. The variation in extent of necrosis in the livers from the xanthine-treated rats killed 24 hours after the administration of carbon tetrachloride prevented any conclusion that repair had occurred between the 24- and the 48-hour periods. Nevertheless, we did see mitotic figures in some liver sections from the xanthine-treated rats killed 48 hours after the administration of carbon tetrachloride. These facts led us to consider the possibility of a stimulated regeneration of the liver cells by xanthine.

Experimental. Xanthine protection. Male albino rats, weighing from 125 to 200 g, were used in all experiments. The diet employed was either the commercially prepared "Purina Chow" or the mixture used by Forbes, Neale, and Scherer[3] of yellow corn meal 35, wheat 12, oats 10, wheat bran 5, wheat germ meal 5, alfalfa 5, dried skim milk 15, dried meat scraps 10, calcium carbonate 1.5, sodium chloride 0.5, and yeast 1%. All animals were fed one of these 2 diets from 10 days to 2 weeks before the experiment began. Several groups of rats were allowed no food for a period of 24 hours prior to the administration of carbon tetrachloride. The relative results were affected neither by the diet nor by the withdrawal of food.

One subcutaneous injection of 100 mg of xanthine per 100 g of body weight was made 24 hours before the administration of carbon tetrachloride. A finely ground, aqueous suspension of xanthine was employed. The carbon tetrachloride was given orally by means of a stomach tube, in doses of 2, 3, or 4 cc per kg of body weight. The rats were killed at intervals of 24 or 48 hours after the administration of carbon tetrachloride. The experimental groups of animals consisted of 157 rats given 4 cc, 103 given 3 cc, and 82 given 2 cc of carbon tetrachloride per kg of body weight (Table I). Small slices of the livers of all rats which survived for the duration of the experiment were fixed in Bouin's fluid. Paraffin sections were cut to 5 microns in thickness and stained with hema-

[7] Minot, A. S., *J. Pharm. and Exp. Therap.*, 1931, **43**, 295.

toxylin and eosin. In all cases the same lobe of the liver was used for microscopic examination to insure uniformity of the sections.

Our experience with the xanthine was essentially the same as that reported by Neale and Winter.[5] It gave a relative protection to the livers against the central necrosis caused by carbon tetrachloride. We were able to observe a difference between the livers of the rats that had had an injection of xanthine prior to the administration of carbon tetrachloride and of those that had received no xanthine. Sections of livers from the control animals killed at 48 hours exhibited the typical central necrosis with its fatty and round cell infiltration extending toward the periphery of the lobule, in some cases involving 50 to 80% of the liver lobule. Although sections of livers from the xanthine-treated rats killed at the corresponding period showed some damage, the extent of the necrosis was significant. In the latter the necrosis, in most cases, was limited to a small area around the central lobular vein, and the remainder of the lobule appeared normal.

TABLE I.
Mortality of Rats Given Carbon Tetrachloride and Xanthine.

Dose of CCl_4 (cc/kilo body wt)	Dose of Xanthine (mg/100 g body wt)	Time observed after CCl_4 (hr)	No. dead	No. alive	% survived
4	100	24	22	16	42.1
	0	24	24	14	36.8
	100	48	9	5	35.7
	0	48	44	23	34.3
3	100	24	1	22	95.6
	0	24	11	15	57.2
	100	48	5	10	66.7
	0	48	19	20	51.3
2	100	24	0	20	100.0
	0	24	0	15	100.0
	100	48	1	30	96.8
	0	48	1	15	93.8

The mortality rates of the controls and the xanthine-treated animals were not materially different except in those that received 3 cc of carbon tetrachloride per kg (Table I). In this group 30 from a total of 65 rats used as controls died, and of the 38 that received xanthine only 6 died. The mortality of the rats receiving 4 cc of carbon tetrachloride was more than 50% in both cases, whereas of those that received 2 cc only one died in each group. From a mortality standpoint, the xanthine may have protected the rats that received a moderately toxic dose. A larger amount of xanthine

was not administered because much of the 100 mg per 100 g of body weight remained unabsorbed at the site of injection.

Cell regeneration. Since colchicine has been shown to stop cell division in the metaphase,[8] we used this substance to determine the growth rate of the liver cells. The rats were given the moderately toxic dose of 3 cc carbon tetrachloride per kg of body weight. Colchicine, in the amount of 0.15 mg per 100 g of body weight was injected subcutaneously 9 hours before the time of sacrificing the animals. The xanthine was administered in the manner described previously. Animals were killed 24, 36, 48, 72, and 96 hours from the time of carbon tetrachloride administration. Livers from 96 rats, about equally divided between xanthine-treated and controls, were studied. Microscopic slides of the livers were prepared with the same technic that was used with the first series.

TABLE II.
Percentage of Hepatic Cells in Mitosis.

Time after CCl_4 administration (hr)	No. of rats	Cells in Mitosis (%)	
		Xanthine-treated	Controls
24	18	0.21	0.20
36	25	0.32	0.29
48	22	4.70	6.60
72	16	1.30	1.71
96	15	0.25	0.20

Mitoses were counted under the oil immersion objective and over an area containing approximately 1000 hepatic cell nuclei. At least 500 nuclei were actually counted, and, in sections of even thickness, other measured areas of the same size were assumed to contain the same number of cells. Only areas with normal-appearing cell nuclei were selected for counting. The number of cells in mitosis is expressed in Table II as the percent of hepatic cell nuclei dividing in a given area. It is assumed (within the reasonable limits of error) the same number of cell nuclei would be dividing in other selected areas.

No difference in the number of mitotic figures in the least damaged areas of the livers could be observed. The necrotic areas had no cells in division. Fewer areas appeared normal in the controls; consequently, numerically there were more mitotic figures in the liver sections from the rats that had received xanthine. However, in like areas the number of cells appearing in the metaphase was not significantly different.

[8] Ludford, R. J., *Arch. f. exp. Zellforsch.*, 1936, **18**, 411.

Mitotic figures in the livers of the xanthine-treated rats and of the controls for the 24- and 36-hour periods averaged only from 2 to 4 per thousand hepatic cells. This was, however, a greater number than has been observed in normal rat livers.[9] The greatest cell division occurred at the 48-hour period. Mitoses at this time averaged 47 and 66 per thousand hepatic cells for the livers from the xanthine-treated and from the controls, respectively. However, there was extreme variability in the number of mitotic figures at the 48-hour period. In the livers from the xanthine-treated rats the mitotic rate varied from 6 to 127 per thousand, and in the livers from the controls the rate likewise varied from 11 to 124 per thousand.

The rate of cell regeneration in the livers of the rats killed 72 hours after the administration of carbon tetrachloride was less than one-third the rate of those at the 48-hour period. At the 72-hour period the extent of the damaged areas had become considerably decreased, and there was no longer the extreme need for so rapid repair. Although complete recovery in the liver lobule had not occurred in 96 hours, mitosis had returned to a low rate.

Summary and Conclusions. Our experiments confirm the observations of Neale and Winter[5] that xanthine has a protective action against carbon tetrachloride poisoning. The extent of the necrosis in the liver lobule is decreased by xanthine. Liver protection was observed best in rats killed 48 hours after the administration of carbon tetrachloride. At this time the damage in the livers from the xanthine-treated animals was limited to a small area around the central lobular vein, whereas in the controls the necrotic area involved from 50 to 80% of the liver lobule.

Xanthine given prior to a moderately toxic dose of carbon tetrachloride lowered the mortality rate. When large doses of carbon tetrachloride were administered, no difference in the mortality rate was observed.

We observed no evidence that xanthine stimulated the regeneration of cells in the damaged livers. The number of mitotic figures in the livers of the xanthine-treated rats did not differ significantly from the number in the livers of the controls.

Mitosis increased in rate from the 24-hour period to the 48-hour period. At the 48-hour period it was occurring rapidly in the livers of both the controls and the xanthine-treated rats. After 48 hours the number of mitotic figures per thousand hepatic cells decreased. By 96 hours relatively few cells were in mitosis.

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[9] Brues, A. M., and Marble, B. B., *J. Exp. Med.*, 1937, **65**, 15.