with the isotope dilution method using three different macromolecules (albumin, dextran, and PVP) and by following the hematocrit values during the hardening process, and the results agree with theoretical calculations. The striking difference in the centrifugal packing of hardened vs normal RBC indicates that normal RBC are deformable during centrifugal packing and that the low degree of packing must be considered in any study on hardened RBC.

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Received Nov. 30, 1967. P.S.E.B.M., 1968, Vol. 127.

Thioacetamide Inhibition of Liver and Serum Amylase Production* (32850)

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Administration of thioacetamide will cause centrolobular liver necrosis (1,3) in acute experiments and cirrhosis (2,3) when fed at relatively lower levels for extended periods of time. It is also known that hepatoma (3) may be a result of long term administration. We have shown that liver damage caused by *N*-nitrosodimethylamine or carbon tetrachloride (4) results in lowered liver and serum amylase levels in the rat and would have predicted that damage due to thioacetamide would do likewise. However, Muramatsu and Busch (5) showed that administration of thioacetamide to rats caused an increase in the uptake of radioactive lysine into liver proteins. Also, Kizer *et al.* (6) showed that adenylic acid (AMP) deaminase activity in rat liver was significantly elevated by administration of thioacetamide, both by feeding and intraabdominal injection.

The results presented here show that administration of thioacetamide, as shown by others (1,2,3), causes liver damage in our rats and this damage is accompanied by lowered liver and serum amylase levels.

Methods. The white rats used in these experiments were males, weighing 200–300 gm, of the Sprague-Dawley strain obtained from Rolfsmeyer Laboratory Animals. Thioacetamide used was obtained from Nutritional Biochemicals Corporation. When injected intraperitoneally the drug was dissolved in sterile 0.9% NaCl in a concentration of 20 mg/ml; when administered by stomach tube the concentration was 10 mg/ml.

With each rat used, blood was drawn for serum amylase determination before administration of thioacetamide. After administration of the drug, which was given in varying amounts for different time periods (see Table I), all rats were sacrificed 1 day following the last injection or feeding; and blood, liver, and kidney samples were obtained. Appropriate control animals, to whom only sterile 0.9% NaCl was administered, were included

* This investigation was supported by Grant AM 02610, from National Institute of Arthritis and Metabolic Diseases, National Institutes of Health.

TABLE I. Effect of Thioacetamide on Serum, Liver, and Kidney Amylase Levels.[a]

Amount of drug administered (mg/kg per day)	Serum amylase before adm. (units/100 ml)	Amylase levels following administration of drugs (units/100 ml or 100 gm)		
		Serum	Liver	Kidney
i.p. for 3 days				
50	2800 (2640–3050)	2300 (1940–2690)	1640 (1340–1940)	740 (550–840)
100	3260 (2960–3590)	1880 (1610–2210)	1380 (1220–1460)	570 (470–690)
200	3740 (3420–3940)	1620 (1510–1700)	1560 (1380–1770)	600 (520–700)
controls	3250 (3010–3400)	3350 (3160–3450)	2890 (2600–2990)	1000 (880–1100)
i.p. for 9 days				
50	3200 (2790–3500)	2200 (2100–2260)	1320 (1170–1420)	640 (630–650)
100	3080 (2960–3180)	1330 (1180–1420)	1300 (1250–1370)	530 (470–560)
controls	3470 (3250–3600)	2860 (2610–2900)	2700 (2550–3070)	940 (910–960)
i.p. for 21 days				
50	2980 (2720–3160)	2070 (1850–2200)	1270 (1160–1330)	660 (580–710)
100	3450 (3250–3840)	1600 (1210–1820)	1170 (1010–1250)	520 (410–660)
controls	3550 (3390–3610)	3020 (2930–3100)	2890 (2590–3080)	980 (900–1050)
Fed by stomach tube for 3 days				
50	2810 (2690–2880)	1910 (1780–2100)	1390 (1210–1500)	950 (910–980)
100	2370 (2350–2390)	1630 (1440–1820)	1190 (1080–1240)	530 (520–540)
200	3240 (3170–3320)	1230 (1140–1280)	1360 (1300–1420)	500 (480–510)
controls	2980 (2870–3050)	2910 (2850–3010)	2620 (2510–2700)	920 (900–950)

[a] Each value represents the mean of the determinations on at least three rats, in some cases four. The amylase level on any given sample was always determined in duplicate. The values in parentheses indicate the spread in each category.

in all groups. Determinations of amylase levels of serum, liver and kidney samples were performed by methods previously described (7,8).

Results and Discussion. It is clearly evident from the results in Table I that the administration of thioacetamide is accompanied by lowered liver and serum amylase levels in the rat. It is also evident that only 50 mg/kg per day was a large enough dose to cause acute liver damage and that larger doses, 100 and 200 mg/kg per day, did not cause significant further lowering of liver amylase levels. Serum amylase levels, however, were somewhat lower at the higher doses of the drug. The levels of liver amylase in animals treated with

the drug were only 50% of the controls, and at all doses of the drug they represent significant changes from the normal. Lowered levels of amylase in the kidney seemed to follow the lowerings in serum amylase.

Thioacetamide tested *in vitro* in concentrations of 0.01, 0.02, and 0.05 mg/ml in the reaction mixture did not affect serum amylase activity. These trials included several in which the serum was exposed to the drug overnight before the amylase activity was determined.

Examination of the livers in all rats indicated that damage had occurred even after only 3 days. In contrast to the control livers which were dark red and unmottled, the livers of animals receiving thioacetamide were pale and mottled with yellow spots giving an orange peel-like appearance. No evidence of jaundice was seen, however, until after about a week of drug administration at which time the sera of all drug-treated rats were yellow. Ascitic fluid was present in some of the rats to whom the drug was given orally and enlargement of the spleen was a common finding. Several rats given doses of 200 mg/kg per day died during the course of drug administration. The gross appearance of other organs, i.e., the heart, lungs, and kidneys were not significantly different in control and drug-treated animals. We did not see any evidence of hepatoma in our rats, even those treated with the drug for 3 weeks. The levels of thioacetamide in the serum were not determined routinely but in the rats given 200 mg/kg per day intraperitoneally, the levels in two animals were 15 and 18 mg/100 ml in serum drawn 6 hours after injection on the third day.

We see then, that despite the fact that amino acid incorporation was found to be enhanced (5) in some fractions of rat liver protein when thioacetamide was administered, and that AMP deaminase activity (6) is increased, the drug does damage the liver and such damage is followed by a diminution of liver amylase synthesis as indicated by lowered liver and serum amylase levels. This confirms previous findings from this laboratory that damage to the liver by *N*-nitrosodimethylamine lowered liver amylase levels (4) and decreased ability by the liver to synthesize amylase (9).

Summary. Liver damage was produced in rats by administration of thioacetamide both by intraperitoneal injection and feeding via a stomach tube. Such damage is accompanied by a diminished liver amylase synthesis and leads to lowering of liver and serum amylase levels to 50% of normal or less with the higher doses of drug.

Note added in proof. When thioacetamide was added to the system in which isolated rat livers are perfused with diluted, heparinized rat blood [Arch. Biochem. Biophys. **90**, 319 (1960)], there seemed to be no acute inhibition of amylase synthesis by the liver. In fact, in a few cases the amylase production was slightly enhanced. In experiments with doses added varying from 50 to 200 mg the net amylase production/gm of liver per 4 hours varied from 21.8 to 99.5 in 12 experiments with an average of 37.6. The average for normal livers in control experiments is 38.7 with a range from 27.7 to 73.3.

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Received June 15, 1967. P.S.E.B.M., 1968, Vol. 127.