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Nitrogen and Sulfur Content of Liver Tissue in Relation to Carbon Tetrachloride Exposure.

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Present interest in the use of methionine in the therapy of acute chlorinated solvent poisoning[1, 2] stems to a large extent from the work of Miller and Whipple,[3] who demonstrated that this amino acid, given either shortly before or within 4 hours after the period of inhalation, would protect protein-depleted dogs against the otherwise fatal effects of a chloroform anaesthesia. Coincident with these results were the findings that protein-depleted dogs have livers which are deficient in both nitrogen and sulfur. Sulfur, however, is depleted more than is nitrogen, and therefore the N/S ratio rises. The ratio of nitrogen to sulfur in the liver tissue of their normal adult dogs was 11 or 12 to 1. The protein-depleted dogs which were so susceptible to liver injury showed a nitrogen to sulfur ratio of 14 or 15 to 1, which presumably means a lower content of sulfur-containing amino acids.

That an animal may be rendered inordinately susceptible to the vapors of chlorinated solvents or to hepatotoxic agents in general by manipulation of certain constituents of his diet is unquestioned. The principal point at issue in this investigation is whether inhalation of a chlorinated solvent produces any specific demand for sulfur-containing amino acids upon the animal already receiving an adequate protein intake. If such is the case, one might expect to find a significant depletion of liver sulfur reserves during the course of repeated exposures to the solvent, similar to that found over a period of maintenance on a protein-deficient diet. The effect of repeated inhalation of carbon tetrachloride vapor on the nitrogen and sulfur content of rat liver has been studied with the results presented below.

Experimental. The subjects of this investigation were 40 albino rats averaging about 200 g in weight. They were fed the basal diet described by White and Jackson.[4] The content of sulfur-containing amino acids in this diet is purposely limited; nevertheless, according to these authors, the quantity and nutritional quality of the protein are sufficient to support good growth for relatively long periods of time. After an initial 2-week period on this diet, one-half of the animals were subjected to 14 successive daily exposures, 6 hrs. per day, to 1000 p.p.m. of carbon tetrachloride vapor. The remaining half (controls) were unexposed. As the exposures caused some reduction in food intake, the controls were limited to the average food consumption of the exposed animals of the preceding day.

At the conclusion of the 2-week period of exposures all animals were sacrificed. The livers were removed, blotted free of excess blood, and dried for several days at 76°-78°C to constant weight. The dried livers were crushed to a fine powder, weighed into tared paper thimbles and extracted continuously with ethyl ether for 12 hours in a Bailey-

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[3] Miller, L. L., and Whipple, G. H., *J. Exp. Med.*, 1942, **76**, 421.

[4] White, A., and Jackson, R. W., *J. Biol. Chem.*, 1935, **111**, 507.

TABLE I.
Nitrogen and Sulfur Content of Dry, Fat-Free Liver Tissue from 20 Control Rats and 20 Rats Receiving 14 Successive Daily Exposures to 1000 p.p.m. CCl_4.

Group	% Sulfur		% Nitrogen		N/S	
	Avg	Std. error	Avg	Std. error	Avg	Std. error
Controls	0.903	±0.188	10.672	±0.939	12.281	±1.758
Exposed	1.105	±0.163	12.348	±1.661	11.408	±1.390
Difference	0.202	±0.058	1.676	±0.424	0.873	±0.53

Walker apparatus. The decrease in weight after extraction was calculated as percentage of fat. Total nitrogen content of the dry, fat-free residues was estimated by macro-Kjeldahl, and total sulfur by a wet-ashing method.[5]

Results. As was to be expected, the livers of the exposed animals exhibited gross fatty infiltration. The sulfur content of the fat-free liver tissue from these animals, far from showing a depletion, was actually greater than that of the unexposed animals (Table I). Coincidently, total nitrogen of the former was also elevated, so that the nitrogen to sulfur ratios were not significantly different.

Comment. The failure of repeated inhalation of carbon tetrachloride to effect some diminution in liver sulfur in animals receiving an adequate protein intake argues against a specific role of the sulfur-containing amino acids in protection of the liver from acute chlorinated solvent poisoning. The greater susceptibility of animals receiving protein—and/or methionine—deficient dietaries to these solvents continues to be explained adequately by the observed facts that (a) a deficiency of lipotropic factors creates a specific type of hepatic injury *per se*,[6] and that (b) these dietaries tend to produce livers containing maximal concentrations of fat.[7] These considerations, of course, do not obtain in the normal animal, so that the employment of methionine therapy in his case remains to be justified. This in no way discounts the value of a high-protein diet in the treatment of liver injury, since protein itself is of obvious importance in the repair of structural damage.

Summary. Values for total nitrogen and total sulfur determined on the dry, fat-free liver tissue of rats following repeated exposure to carbon tetrachloride vapor were slightly higher than those of rats maintained on the same diet, but not exposed. The nitrogen to sulfur ratios of the two groups, however, were not statistically different. The significance of these findings is discussed in relation to the general problem of the protection of the liver from toxic agents by sulfur-containing amino acids.

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[6] György, P., *Am. J. Clin. Path.*, 1944, **14**, 67.

[7] Goldschmidt, S., Vars, H. M., and Ravdin, I. S., *J. Clin. Invest.*, 1939, **18**, 277.