Lipotropic Agents in Liver Damage Produced by Selenium or Carbon Tetrachloride (18119)

E. A. SELLERS, ROSEMARY W. YOU, AND C. C. LUCAS. (Introduced by C. H. Best.)

From the Department of Physiology and Banting and Best Department of Medical Research, University of Toronto, Canada.

The severity of liver damage produced by the prolonged ingestion of small amounts of toxic materials is known to be influenced by the nature of the diet. The relationship is complicated by a variety of factors, some of the more important being species, sex, rate of growth, the physiological state of the animal at the time of study, caloric intake, and other complex conditions such as multiple rather than single nutritional deficiencies. Another important consideration is the possibility of chemical or pharmacological interaction between a dietary factor and the poisonous substance.

The purpose of the present study was to examine the effects of giving supplements of choline, of methionine or of cystine to rats which were fed an "adequate" diet and in which hepatic damage was produced by the chronic administration of carbon tetrachloride or of sodium selenate.

Experimental. A basal diet* was adopted in which the amounts of methionine, choline and cystine, although relatively low, were adequate for good growth during the period of most rapid gain in body weight, and were sufficient to prevent the deposition of fat in the liver at this time. Eighty female rats, weighing from 130 to 170 g, were divided into two principal groups. To one group carbon tetrachloride was given by stomach tube (0.2 cc of 20% solution in corn oil) 3 times weekly for a period of 8 weeks. Sodium selenate was added to the diet of the other group in an amount to make the selenium content 20 parts per million. Each of the principal groups was divided into 4 subgroups which received, respectively, the supplements shown in Table I. The animals were kept in individual cages and both food and water were offered *ad libitum*. Food intakes and body weights were recorded.

A protective effect of methionine was observed in the group fed selenium, but was not confirmed in a subsequent experiment of an apparently identical nature. The fact that this second set of diets was prepared less frequently than at our customary weekly intervals and stored without the usual refrigeration suggested that deterioration of a labile component might have occurred. A third experiment (Se3) was therefore performed in which the content of alpha-tocopherol in the diet was varied. Forty female rats weighing from 130 to 160 g were divided into 4 groups which were fed diets containing 20 p.p.m. of selenium. The first group received the same basal diet as was fed in the previous experiment except that no alpha-tocopherol was included. The supplements added to the other groups are shown in Table I. In other respects the procedure remained the same.

At the conclusion of the experiments autopsies and appropriate histological examinations were made. Liver lipids were estimated according to the method of Best, Lucas, Patterson and Ridout(1).

Results. 1. All the groups receiving carbon

* Alcohol-extracted peanut flour 30, casein 2, fibrin 1, salts 4, beef dripping 15, corn oil 5, dextrin 10, cornstarch 10, vitamin powder 1, cod liver oil concentrate 0.015, alpha-tocopherol acetate 0.010, dl-methionine 0.130, choline chloride 0.250, sucrose to 100.

The vitamin powder was of such a composition that the intake per 10 g of diet was as follows: Biotin 3 γ, thiamine hydrochloride 50 γ, riboflavin 25 γ, pyridoxine hydrochloride 20 γ, calcium pantothenate 100 γ, nicotinic acid 100 γ, folic acid 5 γ, 2-methyl 1-4 naphthoquinone 10 γ, inositol 5 mg, para-amino benzoic acid 1 mg.

The cod liver oil concentrate supplied at least 300 i.u. vitamin A and at least 75 i.u. vitamin D per 10 g of diet.

1. Best, C. H., Lucas, C. C., Patterson, J. M., and Ridout, J. H., *Biochem. J.*, 1946, v40, 368.

TABLE I. Effect of Choline, Methionine, Cystine on Toxic Liver Damage.

Group	Supplement	Avg food intake per day (g)	Avg change body wt (g)	Lipid content % wet liver wt	Liver damage: Gross nodules	Liver damage: Necrosis or hemorrhage	Liver damage: Fibrosis	Remarks
CCl_4								
1	None (basal)	9.1	+31.3	9.32	++	+	++	Typical appearance of CCl_4 damage
2	0.15% choline chloride	9.7	+33.0	12.47	++	+	+++	
3	0.5% DL-methionine	9.4	+23.0	12.58	++	+	++, +++	
4	0.4% cystine	9.7	+27.8	11.30	++	+	++	
Se 1								
1	None (basal)	6.9	+ 3.4	5.17	+	++, +++	+	
2	0.15% choline	7.4	+26.1	5.07	+	± to +++	+	3 of 10 normal grossly
3	0.5% DL-methionine	6.8	+ 8.1	5.06	—	— or ±	—	8 of 10 normal grossly
4	0.4% cystine	7.0	+11.5	5.14	+	++, +++	+	
Se 3								
1	None (basal)	6.2	— 5.1		±	++, +++	±	
2	0.05% alpha-tocopherol	6.6	—11.0		±	+ to +++	±	
3	0.5% methionine	7.7	— 4.4		±	+ to +++	±	
4	0.05% alpha-tocopherol 0.5% methionine	7.8	— 3.1		—	— to ±	—	7 of 10 normal grossly

tetrachloride showed a similar degree of hepatic injury. Fatty and hydropic degeneration, cellular necrosis, fibrosis, and regeneration of parenchymal cells were observed in almost every liver. None of the supplements appeared to protect this organ against damage. Food intakes, survival and gain in body weight were of a similar order in all groups.

2. A considerable degree of protection against damage to the liver was conferred by methionine in the rats that received selenium. The amount of damage did not differ very greatly in the other 3 groups. In the gross the livers were dark red with lighter hemorrhagic areas, often with a yellowish-white "fringe" at the edge of the lobes. Microscopically the type of injury consisted of mild degenerative changes of the parenchymal cells, dilatation of the sinusoids apparently originating in the midzone of the lobule, hemorrhage into the lobule with necrosis and subsequently organization(2). Milder cases (including a few in the methionine-treated group) showed only the first one or two of these changes, while severe cases sometimes had large hemorrhages and extensive focal necrosis. Ascites was commonly associated with the more severely injured livers. Three livers from the group receiving choline showed little gross evidence of damage, although microscopically definite pathological changes existed. This group also showed the largest intake of food and greatest gain in body weight.

3. In a second experiment in which these treatments were repeated all 4 groups showed a similar degree of mild damage. In the third experiment (Se3), rats of the first three groups exhibited a similar degree of liver injury. In the fourth group (basal diet plus methionine plus alpha-tocopherol) little evidence of damage was present.

Results of the 3 experiments described are summarized in Table I.

2. Lillie, R. D., and Smith, M. I., *Am. J. Path.*, 1940, v16, 223.

Discussion. Previous work on the protective effects of lipotropic factors or substances related to them against hepatotoxins has usually been carried out using diets low in protein or other nutrients. Drill and Loomis(3) showed that no extra protection against liver damage due to carbon tetrachloride was given by methionine. This result has been confirmed in the present study. Choline or cystine seems to be equally ineffective. A protective action of methionine against selenium poisoning has been observed by several investigators, notably Smith(4) and Lewis, Schultz and Gortner(5). The latter workers reported that a diet containing 30% casein afforded significantly greater protection against selenium intoxication than did an equicaloric diet containing 6% casein, but that the addition of methionine to the 6% casein diet also afforded protection. They note that the effect was not always so marked with the methionine supplement as with the high protein diet. Cystine did not have the same beneficial effect as did methionine. In these experiments the addition of methionine corrected a deficiency of one essential amino acid, but other deficiencies still existed.

In our experiments the basal diet was not deficient according to current knowledge in any essential amino acid, and judged by the usual criteria was "adequate" in other respects. In spite of this, methionine produced a substantial protective effect. The apparent ineffectiveness of methionine in the second experiment led to the third experiment, in which it was shown that the protective reaction requires the presence of both alpha-tocopherol and methionine.

The significance of this finding is not apparent. Several papers have reported that massive necrosis of the liver, first believed to result from a single deficiency of methionine or of cystine(6-8), occurs only when an associated deficiency of alpha-tocopherol is present(9.10). The apparent preventive action of alpha-tocopherol against hemoglobinuria produced by alloxan(11), and the protective effect of methionine, of cysteine or glutathione against alloxan diabetes(12-14) are further examples of reactions in which a possible relationship between the tocopherols and sulphydryl compounds has been suggested(11). In the instance reported in this communication, as in the report cited(5), cystine did not have the protective effect of methionine. Is it possible that alpha tocopherol enables the potential –SH group of methionine to remain "free" and take part in a detoxification reaction, whereas in the case of cystine the potential –SH groups are less readily available? The observation that choline had little protective effect would appear to exclude the lipotropic activity of methionine as a factor of primary importance. However, an observation that may have a bearing on this is mentioned here because it is not our intention to do further work on selenium poisoning at the present time. The occurrence of 3 livers with only minimal damage in the group fed the choline supplement may indicate that further experimental work is desirable before excluding the lipotropic effect completely.

Summary. 1. The addition of supplementary methionine, choline or cystine to an "adequate" basal diet failed to protect the livers of rats against damage produced by the

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6. Hock, A., and Fink, H., *Z. physiol. Chem.*, 1943, v279, 187.

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8. Himsworth, H. P., and Glynn, L. E., *The Lancet*, 1944, v1, 457.

9. Shwartz K., *Z. physiol. Chem.*, 1944, v281, 101, 109.

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11. Gyorgy, P., Biological Antioxidants, Tr. of the Third Conference Josiah Macy Jr. Foundation, 1948.

12. Lazarow, A., PROC. SOC. EXP. BIOL. AND MED., 1946, v61, 441.

13. Lazarow, A., Patterson, J. W., and Levey, S., *Science*, 1948, v108, 308.

14. Houssay, B. A., and Martinez, S., *Science*, 1947, v105, 548.

chronic oral administration of carbon tetrachloride. 2. A supplement of methionine, but not of cystine or of choline, showed a protective effect against damage produced by feeding sodium selenate (selenium 20 p.p.m.). 3. This action was demonstrated only in the presence of alpha-tocopherol. The relationship is discussed briefly.

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Experimental Histoplasmosis. Susceptibility of Male DBA Line 1 Mice By Various Routes of Injection. (18120)

ARDEN HOWELL, Jr., AND G. F. KIPKIE. (Introduced by N. F. Conant.)

From the Field Studies Branch, Division of Tuberculosis, Public Health Service and the Departments of Pathology and Bacteriology, Duke University School of Medicine.

It has recently been shown that, by intracerebral injection, male dba line 1 mice, 4-5 weeks of age, are uniformly susceptible to *Histoplasma capsulatum* and are apparently more so, by this route of injection, than several other strains(1,2). In the present report it will be shown that this strain of mice is more susceptible to infection with *Histoplasma* by this route of injection than by any other route.

Procedure. The mice used in these experiments were primarily dba line 1. In addition, a few Bar Harbor C 57 Black mice, lines 6 and 10, also were employed. All mice were obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, and were 4-5 weeks of age, weighing 10-18 g at time of injection. Altogether, in the experiments in which routes of injection were compared, 137 male and 60 female dba line 1 mice, 35 male and 34 female Bar Harbor C 57 Black, line 10, and 23 male and 23 female Bar Harbor C 57 Black mice, line 6 were injected with *Histoplasma*. In addition to the above animals, a few mice in each group were injected with saline alone to serve as controls. However, since it was found that, in general, male mice of both strains were more susceptible than females, the present report will deal only with the results obtained with male animals. The strain of *Histoplasma*, No. 155, and the method of preparation of the inoculum were the same as that used in previous studies of this series(1). Three routes of injection were employed; for intracerebral injection, each mouse was injected with 0.02 ml of a given dilution of a saline suspension of the yeast phase of the fungus or 0.02 ml of saline alone; for intravenous injection, each animal was given 0.1 ml of the saline suspension of the fungus or 0.1 ml of saline alone; for intraperitoneal injection, each animal was given 0.5 ml of the saline suspension of the fungus or 0.5 ml of a suspension of the fungus in 5% mucin, or 0.5 ml of saline or mucin alone. The mucin suspension was prepared according to the method of Milzer and Levine(3). Equal parts of a 1-25 or a 1-50 saline suspension of the yeast phase of the fungus and the 5% mucin suspension were then mixed to give a 1-50 or a 1-100 dilution of the fungus in mucin. Serial dilutions were then prepared from this suspension with sterile physiological saline.

The estimation of the number of viable organisms actually injected, cultures of tissues obtained at autopsy, and other procedures followed were the same as those previously reported(1). A description of the gross lesions present at autopsy and the results of

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3. Milzer, A., and Levine, E. R., PROC. SOC. EXP. BIOL. AND MED., 1948, v69, 16.