output when the fresh yeast was heated to the temperature of boiling water just before ingestion (Table 1). It should be noted that the yeast was fed immediately before each meal to permit the possibility of blending with the food. The capacity for viable yeast to withdraw thiamine from a surrounding

TABLE 1

URINARY THIAMINE* EXCRETION ON YEAST-SUPPLEMENTED DIET

Period	Days of period	Grams yeast ingested		
		15 grams (3 subj.)	150 grams 3 subj.†)	150 grams (3 subj.)
		μg. thiamine/ day	*μg. thiamine/ day*	*μg. thiamine/ day*
Basal	3 or 6 days	374‡	312‡	332‡
Basal plus live yeast	6 or 10 days	217	168	161
		178	124	109
		158	107	99
		163	84	29
		163	52	49
		101	50	33
		...	...	62
		...	...	49
		...	...	42
		...	...	40
Basal	3 days	212	189	80
		227	232	141
		242	257	198
Basal plus boiled yeast	3 days		372	
			712	
			702	

* Thiochrome Assay Method.
† Two subjects, only, ingested boiled yeast.
‡ Values are daily averages for entire period.

medium has been well authenticated (*1*). Fecal thiamine concentrations observed during the various periods showed an inverse relationship to urinary thiamine concentrations, indicating that this is a withholding process by the viable yeast rather than destruction within the digestive tract.

While the low values observed in the present experiment do not approach the zero excretions reported in long-term investigations such as those of Keys (*3*) with acute deprivation of B-vitamins following a long period of mild depletion, they are within the range accepted as denoting "considerable to severe deficiency" (*2*).

This procedure may possibly have an application in certain short-term experiments, in that rapid depletion of thiamine may be achieved without the use of a quantitative or deficient diet, and thiamine stores may be quickly regained by merely discontinuing the yeast from the basal adequate diet.

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Effect of Methionine Supplements on Hepatic Injury Produced by Carbon Tetrachloride[1]

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It was first observed by Hershey and subsequently by Best and collaborators that choline exerts a lipotropic effect. Since then numerous studies have been performed on the relation of choline, methionine, and cystine to fat metabolism with special reference to hepatic changes (*6*). However, little work has been reported on the relationship of dietary choline and methionine to functional changes in the liver. It was demonstrated by Hough and Freeman (*4*) that removal of protein from the diet resulted in an increase in serum phosphatase and a decreased hepatic clearance of dye. They later reported that choline chloride prevented these changes during the first 8 weeks of the deficient diet (*5*). It is also known that such protein-depleted dogs are more susceptible to hepatic poisons. Miller and Whipple (*7*) noted that the toxicity of chloroform in protein-depleted dogs was decreased when methionine was administered either before or up to four hours after the chloroform anesthesia. The methionine-treated animals survived a period of anesthesia lethal to untreated animals. In similar studies Goodell, *et al.* demonstrated that the rise in icterus index following the administration of mapharsen to protein-depleted dogs was lessened when methionine was also given (*2*).

In the above experiments the beneficial effects of methionine were obtained in animals maintained on practically protein-free diets. It is of interest to determine if methionine supplements to a normal protein diet exert any protective action against hepatic toxins. No studies have appeared on this subject, and the following data, pertinent to this question, are reported.

Liver damage was produced by administering small doses of carbon tetrachloride to healthy, adult dogs. The CCl_4 was mixed with an equal volume of corn oil and given by stomach tube before the dogs were fed. Changes in hepatic function were studied by means of the bromsulphalein and serum phosphatase tests, as they have been shown to be sensitive methods in detecting liver damage produced by CCl_4 (*1*). Serum phosphatase was determined by the method of Bodansky (*1*) and bromsulphalein retention by the

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method of Rosenthal and White.[2] The dogs were fed a synthetic diet containing either 41 per cent or 20 per cent of casein.[3] The 20-per cent casein diet is at the lower level of protein intake but is still normal, and either diet will maintain a normal hepatic function in dogs. All animals received the synthetic diet for at least four weeks before CCl_4 was administered. The methionine was injected intravenously, 1.0 gram being dissolved in 20 cc. of distilled water.

Experiment 1. In this initial study the dogs received the 41-per cent casein diet. One-half cc. of CCl_4 per kilo of body weight was administered on days 1 and 5 of the study and liver function tests performed on the eighth day (Table 1). The methionine-treated animals received 1.0 gram on days 1, 2, 5, and 6. No protective effect of the methionine on liver function was observed (Table 1).

Experiment 2. It was thought that methionine may not exert any protective effect when supplementing a 41-per cent casein diet. Therefore, in this study the amount of casein was reduced to 20 per cent. The dose of CCl_4 was also reduced to 0.25 cc. per kilo of body weight and administered on days 1, 3, and 5. Hepatic function tests were performed two days after each preceding dose of CCl_4 (Table 1). Of the methionine-treated dogs, No's 11 and 12 received 1.0 gram of methionine morning and afternoon each day, starting with the first administration of CCl_4. Animal No. 10 received only 1.0 gram of methionine every day. No significant protective effect of the methionine on hepatic function was noted (Table 1).

Experiment 3. It was possible that the dose of CCl_4 in the previous experiment was too high and might overshadow any effect of the methionine. Therefore in this study the amount of casein was reduced to 20 per cent. The dose of CCl_4 was also lowered to 0.125 cc. per kilo of body weight and administered on days 1, 3, 5, and 7. Hepatic function tests were performed on days 3, 5, 7, and 9. Two grams of methionine were injected each day as in Experiment 2. The methionine was not observed to exert any protective effect on the hepatic functions tested (Table 1).

TABLE 1

EFFECT OF CARBON TETRACHLORIDE AND METHIONINE ON BROMSULPHALEIN RETENTION AND SERUM PHOSPHATASE

Days of Exp.		Bromsulphalein retention						Serum phosphatase					
		CCl_4				CCl_4 + methionine		CCl_4				CCl_4 + methionine	
		Exp. 1. 41% casein diet, 0.5 cc. CCl_4/kg.											
	Dog No.	1	2	3	4	5	6	1	2	3	4	5	6
Control		2	3		5	3	2	4.40	2.67		4.50	3.15	5.13
Control		2	2	4	4	2	2	4.38	3.63	4.50	4.99	1.45	2.10
8		20	38	14	14	40	38	23.75	4.90	4.70	5.62	20.48	20.00
		Exp. 2. 20% casein diet, 0.25 cc. CCl_4/kg.											
	Dog No.	7	8	9		10	11	12	7	8	9	10	11	12
Control		2	4	3		3	4	2	5.75	3.95		4.55	2.40	5.25
Control		3	5	3		4	4	4	5.25	3.38	6.75	4.65	4.15	4.50
3		3	6	40		8	2	3	4.00	1.88	10.71	6.50	3.00	9.67
5		24	60	60		8	60	40	6.36	4.88	16.75	5.00	3.58	9.50
7		95	90	95		95	95	60	16.70	7.50	26.80	17.50	10.50	18.15
		Exp. 3. 20% casein diet, 0.125 cc. CCl_4/kg.											
	Dog No.	13	14	15		16	17	18	13	14	15	16	17	18
Control		3	2	3		3	2	2	4.25	3.88	5.00	4.23	5.64	5.75
3		4	3	5		6	5	6	3.95	3.68	5.08	5.03	4.50	5.38
5		22	6	5		38	45	6	8.43	3.00	5.60	6.75	8.00	4.00
7		45	10	4		30	50	18	6.25	2.75	3.75	9.50	9.88	6.25
9		95	18	25		24	100	60	6.75	4.00	3.57	6.63	8.18	8.50

It has been definitely shown that the reduced hepatic resistance to chloroform and mapharsen in dogs fed a protein-free diet can be counteracted by the administration of methionine (*2, 7*). Under the conditions of the above experiments methionine did not exert any demonstrable protective effect on the hepatic damage produced by CCl_4 in dogs fed a normal amount of protein, either 20-per cent or 41-per cent casein. This can be seen with both the bromsulphalein and serum phosphatase tests. Generally the bromsulphalein test became abnormal before or at the same time as the serum phosphatase values, confirming previous results with these two tests. In only one dog (No. 12) did the phosphatase values become abnormal before the dye test. Some variation is present in that one methionine-treated dog (No. 10) maintained a normal liver function longer than un-

[2] The customary dose of 5 mg./kilo of body weight was injected instead of the original 2-mg. dose. A single blood sample was taken one-half hour later, and the concentration of dye present divided by $2\frac{1}{2}$.

[3] Diet consisted of: casein, 41.2 per cent; sucrose, 33.4 per cent; lard, 21.5 per cent; bone ash, 2.6 per cent; salt mixture (Karr), 1.3 per cent. In the 20-per cent casein diet the sucrose was increased to 54.6 per cent. Four grams of brewer's yeast concentrate was given each day to supply the B vitamins, and 10 drops of oleum percomorphum were added to each kilo of diet.

treated controls. However, the variation was such that in the other two experiments the dogs receiving methionine showed a somewhat earlier and greater degree of liver damage.

This failure of supplementary methionine in a normal protein diet to protect against CCl_4 liver damage may be likened to the failure of supplementary thiamine to produce any added effect in the presence of an adequate vitamin intake. From the present experimental studies one would expect a beneficial effect of methionine or choline on an abnormal hepatic function only in the face of a previous history of protein deficiency. To date three studies have failed to find any beneficial effect of methionine or choline in homologous serum jaundice (*11*) or in infectious hepatitis (*3, 12*). On the other hand, choline, in combination with high protein and high vitamin diets, has been reported to be of value in the treatment of hepatic cirrhosis (*8, 10*), but not all are in agreement with this point (*10*). Experimentally, a protective effect of supplements of methionine added to a normal diet might later be demonstrated with another type of liver damage. The above experiments are fairly acute, and when larger amounts of methionine are available some effect may be demonstrated on a more chronic liver damage. Studies on hepatic repair are also needed as previous studies are all prophylactic in nature.

Summary. At the present time the experimental evidence demonstrates that supplements of methionine will decrease the degree of liver damage produced by toxic agents in protein-depleted animals. In animals receiving a normal protein intake of 20-per cent or 41-per cent casein, methionine supplements did not decrease the degree of hepatic damage produced by carbon tetrachloride as judged by serum phosphatase values or bromsulphalein retention.

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In the Laboratory

Inhibition of Oxidation of Ascorbic Acid by Certain Vegetable Extracts

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Aqueous extracts of a number of vegetables exert an inhibitory effect on the oxidation of ascorbic acid. Evidence of this effect was first obtained when, to avoid errors in sampling, an attempt was made to use an extract of cabbage, rather than the vegetable itself, in a study of the effects of various compounds on the oxidation of ascorbic acid during boiling. When an aqueous extract of cabbage containing added ascorbic acid was boiled, it was observed that only a small percentage of the vitamin was oxidized. The inhibition of oxidation was found not to be caused by a hydrogen-ion concentration unfavorable to the oxidation, although it was influenced by this factor; nor was it an apparent effect resulting from the formation or release of ascorbic acid or other reducing substances during boiling. Similar aqueous extracts of other vegetables were found to differ widely in their capacity to inhibit oxidation.

Both fresh and previously boiled extracts produced inhibition; the latter were used in the present experiments. The extracts were prepared by grinding a weighed amount of the fresh vegetable with water in a Waring blendor; the filtrate from the blended mixture was boiled for 30 minutes, chilled, filtered, and made to volume. One milliliter of extract was equivalent to 0.25 gram of fresh material.

Inhibition of oxidation was determined by the difference between the amounts of ascorbic acid oxidized, during the same boiling period, in equal volumes (40 ml.) of two solutions (buffered or unbuffered) containing the same amount of ascorbic acid, but differing in that one solution contained a known amount of vegetable extract. The reaction mixtures, complete except for the ascorbic acid, were brought to boiling in uncovered 150-ml. beakers containing glass beads,