The amount of thrombin formed during this period was measured by the addition of fibrinogen and by observing the time required for a clot to form (vertical axis, Fig. 1). This observed time is an inverse measure of the amount of thrombin formed in the first reaction. The rate of thrombin production is proportional to the concentration of the Ac-globulin in the reacting medium. Thus, it may be seen that there is a definite decrease in serum Ac-globulin activity from the time that the blood was drawn. Within 300 minutes after blood was drawn less than one-third of the Ac-globulin activity remained. On standing at room temperature the loss of serum Ac-globulin activity was considerably more pronounced (not shown in Fig. 1).

One would naturally like to offer an explanation for these observed variations, but sufficient information is not available at this time. It has been shown that relatively large amounts of thrombin (30 units per cc or more) partially destroy the Ac-globulin activity of oxalated bovine plasma.[1] It is therefore possible that the instability of serum Ac-globulin in certain species may be due to the action of thrombin. Since the amount of thrombin present during and immediately following the clotting reaction is determined by the rate of thrombin formation and by the effective concentration of antithrombin, variations of these factors from one species to another may account for the differences in the stability of Ac-globulin activity. The stability of serum Ac-globulin may also be an inherent characteristic of the substance for each species.

The similarity in the stability of human serum Ac-globulin and that of Factor VI described by Owren[4] tends to confirm the suggestion that these two substances are identical.

16674

Effects of Diethylcholine and Ethionine on Growth Rate, Liver Fat, and Kidney Degeneration of Rats.

VIRGINIA L. HARDWICK* AND RICHARD J. WINZLER.

From the Department of Biochemistry and Nutrition, University of Southern California School of Medicine, Los Angeles.

It is now well established that a dietary deficiency of choline and methionine leads to an accumulation of fat in the liver, to the development of hemorrhagic degeneration of the kidney, and to a failure of young animals to grow on diets containing homocystine but lacking in labile methyl groups. The lipotropic action and kidney protection have been ascribed to the action of intact choline or choline-like molecules or their precursors, whereas growth on a low methionine diet containing homocystine is dependent upon the availability of labile methyl groups.

Diethylmethylhydroxyethyl ammonium chloride (diethyl choline)[1] and ethionine[2] have been reported to be toxic to rats on low methionine diets. These compounds are ethyl homologs of choline and methionine respectively, and their toxicity raised interesting questions as to the mechanisms by which they may interfere either with fat metabolism or with transmethylation. The present experiments were devised to investigate in the rat the influence of these two compounds on the three criteria of inadequate choline or methyl group intake-growth on a diet containing

* The data are taken from a thesis presented by Virginia L. Hardwick to the Graduate School of the University of Southern California in partial fulfillment of the requirements for the degree of Master of Science.

1 Moyer, A. W., and du Vigneaud, V., *J. Biol. Chem.*, 1942, **143**, 373.

2 Dyer, H. M., *J. Biol. Chem.*, 1938, **124**, 519.

TABLE I.
Constitution of the Basic Diet.

Constituent	%
Arachin	18
Cerelose	65.2
Osborne and Mendel salts	4
Crisco and Lard*	10
Vitamin mixture†	2
Supplement	0.8

* The Crisco and lard was fortified with 6,000,000 U.S.P. units of vitamin A, 85,000 units of vitamin D, and 0.5 mg of alpha tocopherol per g of fat.

† The vitamin mixture had the following composition: cerelose, 95 g; riboflavin, 50 mg; thiamin hydrochloride, 25 mg; pyridoxine, 25 mg; nicotinic acid, 100 mg; calcium pantothenate, 50 mg; and inositol, 5 mg.

homocystine, deposition of liver fat, and hemorrhagic degeneration of the kidneys.

Methods. Diethyl choline was prepared by treating diethylaminoethanol in ether with one equivalent of methyl chloride at −50°C and allowing the condensation to proceed at room temperature at high pressure. Homocystine was prepared by the method of Patterson and du Vigneaud.[3] Ethionine was prepared from S-benzylhomocysteine according to the method of Dyer.[2]

The basic diet used had the composition shown in Table I. Arachin was selected for the protein of the basic diet since it has been shown to contain limited amounts of methionine. It was prepared from peanut meal by the method of Johns and Jones[4] and was found to contain 0.5% methionine. All diets were supplemented with 0.8% homocystine, ethionine, or methionine as indicated in the experimental protocols. Choline or diethylcholine were administered daily by mouth with a 0.25 ml syringe and a rounded needle. The animals objected to the administration of the supplements, but with some practice this could be achieved without loss. Rats of the University of Southern California strain weighing between 50 and 70 g were employed throughout. The experiments were continued for 12 days with the food consumption and weight changes being determined every second day.

Liver lipids were determined by extracting the dried samples with diethyl ether in continuous extractors, the total lipids being determined gravimetrically. The choline in the liver lipids were determined by the Reinecke salt method of Engle[5] after hydrolysis with barium hydroxide. This method does not distinguish between choline and its monoethyl, diethyl or triethyl analogues. The kidneys were decapsulated and fixed in Zenkers solution, and sections embedded, sectioned and stained with iron hematoxylin.

Results. The results of the experiments are given in Table I. The rats on the unsupplemented homocystine diet (I) showed the expected weight loss, high liver fat content, and extensive kidney hemorrhage. Supplementation of this diet with choline (II) or substitution of methionine for homocystine (V) resulted in growth, lowered liver fat levels and minimal kidney pathology as anticipated from many previous studies. Growth with the methionine diet was somewhat superior to that with homocystine plus choline, but was considerably inferior to that obtained with the stock diet (VIII). This is no doubt to be attributed to deficiencies in amino acids other than methionine in arachin. Supplementation of the homocystine diet with diethyl choline did not permit growth, but a strong lipotropic action and a prevention of kidney hemorrhage were apparent. This is in accordance with previous observations.[1] However, the toxicity on growing rats reported for this compound[1] was not apparent in the present studies. The animals receiving both choline and diethyl choline (Group IV) did not differ significantly from those receiving choline alone.

The animals receiving ethionine (VI) lost weight more rapidly than did those receiving homocystine. This toxic effect of ethionine was partially prevented by addition of methionine. The toxic effect of ethionine on rats and the protection by methionine has already been reported by Dyer.[2] It is of utmost interest, however, that the accumulation of liver fat and the development of kidney hemorrhage did not develop on the ethionine diet.

[3] Patterson, W. I., and du Vigneaud, V., *J. Biol. Chem.*, 1935, **111**, 393.

[4] Johns, C. O., and Jones, D. B., *J. Biol. Chem.*, 1916, **28**, 77.

[5] Engel, R. W., *J. Biol. Chem.*, 1942, **144**, 701.

A most outstanding result apparent from Table II is the fact that the total liver choline (or its ethyl analogues) is very markedly reduced in the animals on homocystine plus diethyl choline (Group III) and on ethionine (Group VI).

Discussion. It is of special interest that ethionine should show a lipotropic action and a protection against kidney hemorrhage, since it has no methyl groups and does not have a structure which can reasonably be considered to substitute for choline in phospholipids. It does not seem likely that the lipotropic and antihemorrhagic action of ethionine can be explained on the basis of a transethylation to ethanolamine to form an ethyl analogue of choline, since such choline analogues were not found in the livers of the animals of Group VI.

The possibility that diethyl choline exerts its lipotropic action by virtue of its structural relationship to choline and consequent incorporation into phospholipid would seem to be supported by the observation of McArthur,[6] who showed the presence of administered triethyl choline in the liver phospholipids of rats. However, in our experiments we found very much lower than normal amounts of choline or its analogues in the livers of animals fed homocystine plus diethyl choline (Group III).

It is therefore concluded that the action of ethionine and diethyl choline in preventing fatty livers and kidney hemorrhage of rats on a low choline and low methionine diet probably does not involve their incorporation in whole or in part into phospholipids.

Summary. The toxic actions of ethionine on the growth of rats and the protective action of methionine reported by Dyer[2] has been confirmed. It has been shown that ethionine prevents the accumulation of liver fat and the hemorrhagic kidney degeneration on low methionine, low choline diets. The diethyl analogue of choline also prevents the fatty livers and kidney lesions of rats on similar diets. The action of these compounds does not appear to involve their direct participation in phospholipid formation.

[6] McArthur, C. S., *Science*, 1946, **104**, 222.

TABLE II.
Weight Changes, Liver Fat Content, Liver Choline Content and Kidney Hemorrhage in Rats.

Group	Diet	No. of animals	Avg initial wt (g)	Avg food eaten per day (g)	Wt gain per day (g)	Liver fat % dry wt*	Liver choline† mg/g	Kidney damage
I	Homocystine	14	59	6.2	—1.0	41.0 ± 2.5	2.1	++++
II	Homocystine + choline	13	61	8.1	1.5	18.8 ± 1.4	2.8	+—
III	Homocystine + diethylcholine	10	57	5.0	—0.7	8.4 ± 1.0	0.1	+—
IV	Homocystine + choline + diethylcholine	10	68	7.7	1.0	13.8 ± 1.1	2.9	+—
V	Methionine	25	63	9.4	1.6	14.3 ± 1.1	1.8	+—
VI	Ethionine	6	66	2.8	—2.6	9.8 ± 1.4	0.2	+—
VII	Ethionine + methionine	5	67	7.2	1.1	23.4 ± 1.4	3.6	+—
VIII	Stock	10	59	11.0	3.3	7.4 ± 0.3	2.3	—

* Including the standard error of the mean.
† Including the monoethyl, diethyl, and triethyl analogues.