TABLE I. Free Amino Acids, Total Amino Acid Nitrogen and Glutamine in Brain of Rats, Normal and During Insulin Shock.

Groups	Hr of starving	IU/k	mg/100 g brain tissue: Total amino acid, N A	Total amino acid, N B	Glutamic acid A	Glutamic acid B	Aspartic acid A	Aspartic acid B	γ-amino-butyric acid A	γ-amino-butyric acid B	Glutamine A	Glutamine B
1	24	350	—	—	168.4	127.9	37	110	29.1	20.6	43.7	42.2
2	24	350	—	—	166.6	124.1	52.8	161.7	43.4	32.7	40.5	13.5
3	48	350	39.6	33.9	173.1	127	40	146.3	30.9	17.3	31.6	11.7
4	24	350	—	—	166	77	42.7	84.4	44.8	22.4	33.5	7
5	24	350	37.2	29.9	194.7	136.4	44.8	66	30.1	22.5	41.4	9
6	24	350	45	44.8	146.3	88.9	34.3	138.6	31.7	19.5	34.5	6.5
7	24	350	33	30.5	188	150.7	42.7	55.9	24.7	16.2	—	—
8	0	60	31	30.5	160.9	147.4	41.6	58	38.9	30.6	32.4	17
9	0	60	30.8	30.1	189.2	166.1	40.1	81.2	41.6	25	—	—

A, control rats; B, rats under insulin shock.

tion system in normal brain tissue, has been pointed out by Cohen and Hekhuis(9).

Increased activity in transamination between glutamic and α-ketoglutaric acids explains, furthermore, why the ammonia level remains practically unaffected during insulin hypoglycemia, and makes the lowering of γ-amino-butyric acid and glutamine levels, referable to diminution in available free glutamic acid.

Summary. Brain extracts of rats subjected to insulin shock have, as compared with those from normal animals, smaller amounts of free glutamic acid, γ-amino butyric acid and glutamine, and a greatly increased quantity of free aspartic acid.

1. Dawson, R. M. C., *Biochem. J.*, 1950, v47, 386.
2. Awapara, J., Landua, A. J., Fuerst, R., and Seale, B., *J. Biol. Chem.*, 1950, v187, 35.
3. Roberts, E., and Frankel, S., *J. Biol. Chem.*, 1950, v187, 55.
4. Awapara, J., *Arch. Biochem.*, 1948, v19, 172.
5. Consden, R., Gordon, A. H., and Martin, J. P., *Biochem. J.*, 1947, v41, 590.
6. ———, *Biochem. J.*, 1944, v38, 224.
7. Naftalin, L., *Nature*, 1948, v161, 763.
8. Sahyun, M., *J. Lab. Clin. Med.*, 1938-1939, v24, 548.
9. Cohen, P. P., and G. L. Hekhuis, *J. Biol. Chem.*, 1941, v140, 711.

Received November 19, 1951. P.S.E.B.M., 1951, v78.

Effect of Vitamin E and CCL_4 on Fat, Respiration and Choline Oxidase of Rat Livers.* (19242)

E. L. HOVE AND J. O. HARDIN. (Introduced by W. D. Salmon.)

From the Department of Animal Husbandry and Nutrition of the Alabama Polytechnic Institute, Auburn.

A previous report(1) indicated lower liver fat in young rats deficient in vitamin E as compared with controls receiving this factor. On a diet low in protein and deficient in vitamin E, young rats are known(2,3) to be highly sensitive to acute CCl_4 toxicity; this condition is corrected by supplements of alpha tocopherol. CCl_4 is known to produce an immediate fatty infiltration of the liver, and Ennor(4) has shown an increased rate of respiration of liver slices from guinea pigs poisoned with CCl_4. Therefore, the influence of vitamin E on the response of liver fat and the rate of liver slice respiration after acute CCl_4 intoxication has been investigated in rats.

* Published with the approval of the Director of the Alabama Agricultural Experiment Station. This work was performed under contract No. N9-onr92100 with the Office of Naval Research.

TABLE I. Liver Fat of Rats as Influenced by Vitamin E, Protein Level and CCl_4.

CCl_4/wk, ml	Dietary casein level, %	Dietary α-tocopheryl acetate, %	No. of rats	Avg 8 wk wt gain, g	Liver fat* (dry basis) %	S.E.	t
0	10	0	12†	93	8.7	± .493	5.23§
0	10	.01	12	112	12.7	± .586	
0	18	0	5	213	9.4	± .789	.78
0	18	.01	5	219	8.6	± .618	
.05	10	0	6‡	71	20.1	±1.30	.81
.05	10	.01	8	101	18.2	±2.12	
.05	18	0	5	219	23.8	±2.16	.77
.05	18	.01	5	227	21.2	±2.63	

* Obtained 24 hr after final CCl_4 inj.
† 16 rats started; 4 died with typical vit. E lung hemorrhage syndrome(1).
‡ 12 rats started; 6 died with typical vit. E lung hemorrhage syndrome(1).
§ By the Fisher *t* test with 22 degrees of freedom a P value of 2.4 indicates significance at the 1% level.

Data on the effect of dietary vit. B_{12} have been included since, following acute CCl_4 poisoning, Popper, Koch-Weser and Szento (5) reported that this factor protected against liver damage and Hove and Hardin(3) reported protection against fatality in rats. Choline oxidase has been estimated on homogenates from livers of rats receiving various dietary supplements as well as CCl_4, since this enzyme system possibly is related to fat metabolism. Other work from this laboratory (6) showed that CCl_4 poisoning inhibited the methylase system in liver as measured by creatine synthesis. It is of interest to determine whether this was a general effect on liver enzymes.

Methods and results. The basal diet contained water-washed casein(7) 10%, sucrose 76%, lard 9%, cod liver oil 1%, and salt mixture(7) 4%. Sufficient dry vitamins were ground with a portion of sucrose and added to the diets to furnish the following levels per gram: thiamine, riboflavin and pyridoxine, 5 μg each; calcium pantothenate, 25 μg; niacin, 4 μg; choline chloride, 2 mg; i-inositol, 0.2 mg; and 2-methyl 1-4 naphthoquinone, 2 μg. Variations in this diet were made in a few cases in which the casein was raised to 18%, or the lard was raised to 19%, or both, at the expense of the sucrose level. Supplements to the diets were as follows: none; alpha tocopherol acetate 0.01%; vitamin B_{12} 30 μg/kg (as a concentrate); or both of these factors. Male albino rats (Sprague-Dawley) were placed on the diets at weaning (40 to 50 g), housed individually, and food and water supplied, *ad libitum*. For the animals reported in Table I, the time on experiment was 8 weeks. During this time about half of the animals on each treatment received weekly subcutaneous injections of 0.05 ml of CCl_4 diluted 1:1 in olive oil. Twenty-four hours after the last injection, these, as well as the uninjected rats, were killed for liver fat determination. After removal, the livers were dried overnight in an air oven at 105°C, ground, and analyzed for ether extractable material under code numbers.

Liver fat of rats on the 10% casein diet and not receiving vit. E was distinctly and significantly lower than that of controls receiving this factor (Table I). However, no difference was noted due to the vitamin E supplement when the diet contained 18% casein. Following the CCl_4 injections, the liver fat increased sharply to about the same degree on all diet treatments.

The rats reported in Fig. 1 had been on the diets for 5 weeks prior to injection with 1 ml of CCl_4/kg body weight. At this time they weighed 95 ± 11 g. Two rats from each diet treatment were removed at time intervals of 0, 1, 2, 4, and 8 days after the injections. The livers were analyzed for fat, and respiration of liver slices was determined as follows: About 200 mg of fresh liver slices obtained with the Stadie slicer were dropped into Warburg vessels containing 3 ml of glucose-Ringers phosphate solution(8). The rate of respira-

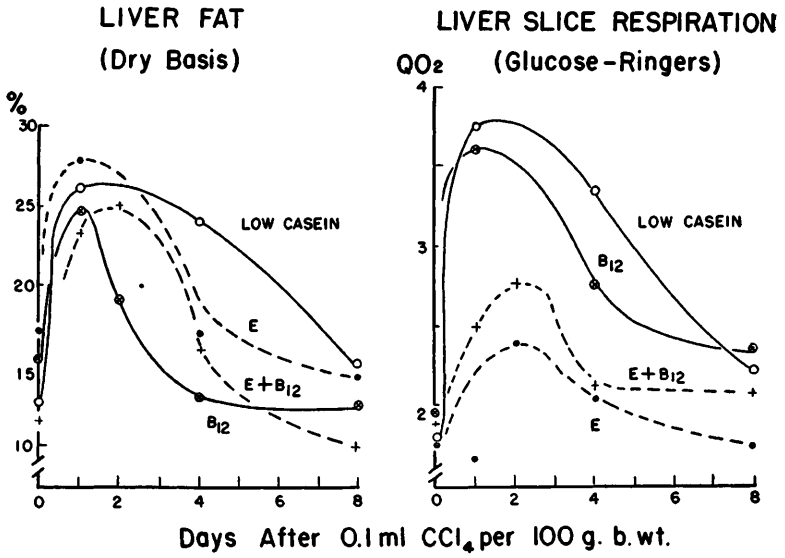

FIG. 1. Effect of single CCl_4 injections on liver fat and on rate of respiration of liver slice as influenced by dietary supplements to the 10% casein diet (low casein) of α-tocopheryl acetate .01% (E), vit. B_{12} 30 μg/kg (B_{12}), and the combination of these vitamins (E + B_{12}). Each value represents average for 2 rats that had been on the diets for 5 weeks prior to injection. Fat values are reported on the dry basis and respiration values as mm^3 O_2 uptake per hr per mg of dry fat-free liver, with 2 mg glucose in 3 ml of Ringer's phosphate.

tion at 37.5°C was expressed as the oxygen consumed per hour per mg of dry, fat-free tissue.

From the data in Fig. 1, it is evident that the vit. E and B_{12} supplements had no marked influence on the course of fatty changes in the livers of rats 24 hours after the injections of CCl_4. There was indication that the supplements, especially vit. B_{12}, allowed a more rapid return to normal liver fat values.

The respiration of liver slices was increased following the injections of CCl_4, especially when vit. E was not included in the diet. Dietary supplements of vit. B_{12} had no effect on this phenomenon (Fig. 1).

The choline oxidase values reported in Table II were determined on livers of rats that had been on the indicated diets for periods of 6 to 12 weeks. The livers were removed 24 hours after the rats were injected, i.p., with 2 ml CCl_4 per kg body weight. One ml of the homogenized liver (1:3 in phosphate buffer, pH 7.4) was added to Warburg vessels containing 2 ml of buffer, or 2 ml of buffer with 4 mg of choline chloride. The difference in oxygen uptake between the flasks with and without choline was considered to be a measure of the choline oxidase activity of the liver.

The data in Table II indicate that the choline oxidase activity of the liver was not influenced by dietary tocopherol supplements or by CCl_4 poisoning. However, an increased level of fat in the diet appeared to increase the choline oxidase activity.

Discussion. Liver fat of young rats on a vit. E deficient 10% casein diet was increased 4 percentage points, from 8.7 to 12.7, by inclusion of alpha tocopheryl acetate in the diet, a highly significant difference statistically. This result confirms previous data(1) in which the average liver fat of rats, similarly treated except for a higher fat level in the diet, increased from 12.3% to 14.2% (dry basis), and is in accord with the general conclusion of Menschik(9) that a vit. E deficiency in mice resulted in a marked disappearance of both liver and body fat. The level of dietary fat may have a bearing on the absolute values of liver or body fat found since in the previous work, and for the data of Fig. 1, the diet contained 20% fat as compared with 10% in the diet of rats reported in Table I.

TABLE II. Choline Oxidase of Rat Liver Homogenates. Rats had been on diets 6 to 12 weeks from weaning.

Single CCl_4 inj., ml/kg	Levels/100 g diet			No. of rats	mm³ O_2/hr/mg dry liver*		
	Casein, g	Fat, g	α-tocopheryl acetate, g		Choline/flask: None	Choline/flask: 4 mg	Increase due to choline
0	10	10	0	8	.46	1.92	1.46
0	10	10	.01	7	.32	1.76	1.44
0	10	20	0	8	.90	3.45	2.55
0	10	20	.01	4	.61	3.34	2.73
0	20	20	0	5	.95	3.89	2.94
0	20	20	.01	3	1.33	3.47	2.14
2	10	10	0	2	1.10	2.67	1.57
2	10	10	.01	2	.80	2.18	1.37
2	20	20	0	4	.63	3.38	2.75

* 24 hr after CCl_4.

The increased liver slice respiration after CCl_4 treatment confirms Ennor's work(4) on guinea pigs. Since in the present study the increased rate of respiration parallels the increased fat content of the liver, it is possible that this fat is being catalytically oxidased, and that the tocopherol when fed, inhibits this oxidation by simple antioxidant action.

It is clear that the striking sensitivity to acute CCl_4 toxicity displayed by young rats on 10% protein diets deficient in vitamin E, and preventable by vitamin E, vit. B_{12} or a higher casein level(2,3,10), cannot be attributed to an altered course of fatty changes in the liver following CCl_4.

Summary. (1) Liver fat of young rats on a vit. E-deficient, 10% casein diet was increased 4 percentage points, from 8.7 to 12.7, by the inclusion of alpha tocopheryl acetate in the diet, a highly significant difference statistically. When the diet contained 18% casein, no difference was noted due to supplements of alpha tocopheryl acetate. (2) Dietary treatments did not influence the increase in liver fat 24 hours after CCl_4 injections, though vit. B_{12} supplements resulted in more rapid return to normal values. The rate of respiration of liver slice in glucose-Ringers was doubled following CCl_4 injections. This was substantially inhibited by dietary supplements of vit. E. (3) Choline oxidase activity of the liver was not influenced by dietary vit. E or by CCl_4 injections into rats, although increased dietary fat level increased the activity of this enzyme system.

1. Hove, E. L., Copeland, D. H., and Salmon, W. D., *J. Nutrition,* 1949, v39, 397.

2. Hove, E. L., *Arch. Biochem.,* 1948, v17, 467.

3. Hove, E. L., and Hardin, J. O., PROC. SOC. EXP. BIOL. AND MED., 1951, v77, 502.

4. Ennor, A. H., *Australian J. Exp. Biol. Med. Sci.,* 1942, v20, 73.

5. Popper, H., Koch-Weser, D., and Szento, P. B., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 688.

6. Hove, E. L., and Hardin, J. O., *Fed. Proc.,* 1951, v10, 385.

7. Salmon, W. D., *J. Nutrition,* 1947, v33, 155.

8. Umbreit, W. W., Burris, R. H., and Stauffer, J. F., *Manometric Techniques,* 1945, p194.

9. Menschik, Z., *Edinburgh Med. J.,* 1944, v51, 486.

10. Hove, E. L., *Ann. N. Y. Acad. Sci.,* 1949, v52, 217.

Received November 19, 1951. P.S.E.B.M., 1951, v78.