THE SIGNIFICANCE OF THE PLASMA TOCOPHEROL CONCENTRATION AND OF TOCOPHEROL TOLERANCE TESTS IN LIVER DISEASE [1]

By GERALD KLATSKIN AND WILLARD A. KREHL

(From the Department of Internal Medicine and the Nutrition Laboratory of the Department of Physiological Chemistry, Yale University School of Medicine, New Haven, Conn.)

(Submitted for publication April 10, 1950; accepted, August 28, 1950)

Important relationships between vitamin E metabolism and the state of the liver have been recognized in experimental animals, and there is evidence to suggest that alterations in tocopherol metabolism may occur in humans with liver disease.

Diversion of bile from the intestinal tract by chronic biliary fistula produces the typical muscular dystrophy and testicular atrophy of vitamin E deficiency in dogs maintained on normal diets (1). Presumably these effects are due to faulty absorption of the vitamin, since feeding bile results in improvement. Similar observations have been made in the rat (2).

Massive necrosis of the liver, produced in rats by diets low in cystine and high in cod liver oil, or containing alkali-treated casein as the sole source of protein, can be prevented by tocopherol administration (3–7). The mechanism of this protective action is not known, but it does not appear to be related to the usual vitamin E activity of tocopherol, since the protective dose is many times that required to prevent muscular dystrophy and testicular atrophy (4). György has suggested that tocopherol may protect the liver by neutralizing endogenous hepatotoxins which are normally detoxified by methionine and cystine (4). In this connection it is of interest that tocopherol also protects rats against carbon tetrachloride poisoning (8), and recent work by Hove (9) suggests that the toxic action of carbon tetrachloride on the liver and other tissues may be due to an induced vitamin E deficiency. Tocopherol may also have a bearing on hepatic function in connection with its recognized influence on protein (10, 11), fat (7, 12, 13), carbohydrate (14) and vitamin A (15, 16) metabolism.

The possibility of alterations in tocopherol metabolism in patients with liver disease has received little attention in the past. Recently, however, several investigators have reported low plasma tocopherol concentrations (17, 18, 19) and abnormally flat tolerance curves following the oral administration of tocopherol (17, 19) in some cases of cirrhosis. Low concentrations of tocopherol have also been reported in liver tissue in both cirrhosis and obstructive jaundice (19). These changes have been ascribed to faulty absorption of vitamin E.

It is not known whether abnormalities in plasma tocopherol have physiological significance in man. Indeed no clear-cut evidence of vitamin E deficiency has been demonstrated in man, with one possible exception. Pappenheimer and Victor (20) have found "ceroid" pigment, muscular dystrophy and testicular atrophy, suggestive of vitamin E deficiency, in tissues obtained from patients dying of cirrhosis and non-tropical sprue, conditions in which low plasma tocopherol levels have been reported (18, 21).

The following investigation was undertaken as part of a study of endocrine function in chronic liver disease. Testicular atrophy (22, 23) and gynecomastia (24, 25) are well recognized complications of cirrhosis, and are generally considered to be the result of hyperestrinemia (22, 24, 25), a theory based on Glass, Edmundson and Soll's report of increased urinary excretion of estrogen in cirrhosis (24). Efforts to confirm this work have failed in this laboratory (26), so that other causes for testicular atrophy and gynecomastia have been considered. It seemed worthwhile in this connection to explore the possibility that testicular atrophy in cirrhosis might be the result of vitamin E deficiency, and that gynecomastia might be secondary to tubular degeneration, as in Klinefelter's syndrome (27).

[1] Aided by a grant from the Fluid Research Fund, Yale University School of Medicine.

TABLE I

Relation of the plasma tocopherol level to age and to the serum cholesterol concentration

Controls				Liver disease				
No.	Diagnosis	Age	Tocopherol	No.	Diagnosis	Age	Cholesterol	Tocopherol
		yrs.	*mg. per cent*			*yrs.*	*mg. per cent*	*mg. per cent*
1	Duodenal ulcer	—	0.35	48	Laennec cirrhosis	39	277	0.29
2	Duodenal ulcer	44	0.35	49*	Laennec cirrhosis	47	—	0.54
3	Relapsing pancreatitis	41	0.36	50	Infect. hepatitis	46	280	0.54
4	Psychoneurosis	51	0.37	51	Laennec cirrhosis	59	—	0.58
5	Psychoneurosis	21	0.41	52	Laennec cirrhosis	60	166	0.59
6	Latent syphilis	26	0.46	51	Laennec cirrhosis	59	—	0.60
7	Bleeding peptic ulcer	49	0.57	53	Laennec cirrhosis	31	182	0.62
8	? G.I. malignancy	86	0.58	54	Laennec cirrhosis	38	191	0.67
9*	Bronchial carcinoma	56	0.72	52	Laennec cirrhosis	60	—	0.68
10	Sprue, remission	38	0.75	55	Laennec cirrhosis	31	—	0.69
11*	Myocardial infarction	38	0.75	56	Laennec cirrhosis	32	170	0.70
12	Unknown	—	0.78	57*	Laennec cirrhosis	33	219	0.71
13*	Seborrheic dermatitis	35	0.79	54	Laennec cirrhosis	38	—	0.72
10	Sprue, remission	38	0.79	56	Laennec cirrhosis	32	—	0.76
14	Multiple sclerosis	22	0.79	58	Laennec cirrhosis	65	—	0.78
10	Sprue, remission	38	0.80	59	Infect. hepatitis	55	—	0.79
15	Pulmonary emphysema	71	0.81	60*	Laennec cirrhosis	53	168	0.84
16	Duodenal ulcer	—	0.82	61	Laennec cirrhosis	—	—	0.84
17	Myocardial infarction	—	0.84	62	Laennec cirrhosis	50	—	0.85
18	Pneumonia	37	0.84	59	Infect. hepatitis	55	—	0.88
19	Bleeding peptic ulcer	30	0.87	63	Laennec cirrhosis	48	126	0.88
20	Congenital heart dis.	22	0.94	60*	Laennec cirrhosis	53	88	0.90
21	Cerebral hemorrhage	63	0.94	64	Laennec cirrhosis	43	191	0.95
22	Psychoneurosis	—	0.94	54	Laennec cirrhosis	38	—	0.96
75	Epilepsy	—	0.94	63	Laennec cirrhosis	48	172	1.01
76*	Hypertension	—	0.96	65*	Laennec cirrhosis	27	212	1.02
77	Heart disease	—	0.97	66*	Infect. hepatitis	38	242	1.02
23	Multiple sclerosis	—	0.97	67	Laennec cirrhosis	48	170	1.05
24	Myocardial infarction	—	0.98	53	Laennec cirrhosis	31	—	1.13
25	Syphilis	29	1.02	68	Subacute hep. necros.	75	113	1.13
26	Unknown	—	1.02	69*	Laennec cirrhosis	43	195	1.16
27*	Asthma	36	1.03	70	Laennec cirrhosis	36	162	1.16
28	Pneumonia	51	1.05	71	Obstr. jaund. (stone)	47	288	1.17
29	Syphilis	28	1.05	58	Laennec cirrhosis	65	—	1.19
30	Diabetes, hypertension	71	1.09	69*	Laennec cirrhosis	43	—	1.20
31*	Thrombophlebitis	43	1.12	72	Laennec cirrhosis	55	360	1.22
32*	Myocardial infarction	50	1.12	70	Laennec cirrhosis	36	—	1.23
33	Psychoneurosis	42	1.13	73	Laennec cirrhosis	35	—	1.29
34	Duodenal ulcer	31	1.17	74*	Laennec cirrhosis	44	255	1.40
78	Multiple sclerosis	—	1.18	70	Laennec cirrhosis	36	190	1.41
35	Neurosyphilis	64	1.20	74*	Laennec cirrhosis	44	257	1.52
36	Pneumonia	—	1.21	62	Laennec cirrhosis	50	—	1.60
37	Cerebral hemorrhage	—	1.24	69*	Laennec cirrhosis	43	—	1.70
38	Duodenal ulcer	38	1.32					
39*	? Brain tumor	44	1.35					
40	Myocardial infarction	64	1.35					
41	Rheumatic fever	35	1.40					
42	Unknown	—	1.42					
43	Syphilis	54	1.42					
44*	Latent syphilis	41	1.46					
79	Multiple sclerosis	38	1.47					
40	Myocardial infarction	64	1.48					
45	Congenital syphilis	28	1.48					
46	Myocardial infarction	54	1.60					
80	Paraplegia, post-polio.	17	1.64					
35	Neurosyphilis	64	1.73					
47	Spont. pneumothorax	24	2.01					

† Mean tocopherol = 1.02 ± s.d. 0.37 mg. per cent.

† Mean tocopherol = 0.95 ± s.d. 0.32 mg. per cent.

* Females.

† Difference between means not statistically significant (t = 1.00, P > 0.20).

Normal healthy adults had the following distribution of values:
0.75–0.99 mg. = 4, 1.00–1.24 mg. = 11, 1.25–1.49 mg. = 3, 1.50–1.74 mg. = 3, 1.75–1.99 mg. = 1, and 2.00–2.24 mg. = 1.
Mean—1.23 ± 0.31 mg. per cent.

Comparison of frequency distributions based on three groupings (0.24–0.74, 0.75–1.49 and 1.50–2.25 mg. per cent); normals differed significantly from hospitalized controls (chi-square = 7.37, P = >0.02, <0.05), and from liver disease subjects (chi-square = 10.17, P = <0.01); liver disease subjects did not differ significantly from hospitalized controls (chi-square = 3.04, P = >0.10).

The objectives of this preliminary investigation were: *1*) to study the effects of liver disease on the plasma tocopherol level, and *2*) to determine whether there is a defect in tocopherol absorption or utilization in liver disease.

METHODS

Subjects. Three groups of subjects were studied: *a*) 23 normal healthy adults (physicians, nurses and technicians between the ages of 20 and 40), *b*) 57 hospitalized patient-controls, who were convalescent from a variety of diseases, and *c*) patients with liver disease (Table I).

Individuals with a history of liver disease in the past, or of any recent illness, were excluded from the normal healthy group. Subjects exhibiting abnormalities of hepatic function or any degree of hepatomegaly were excluded from the hospitalized control group. In the liver disease group the diagnosis was confirmed by needle biopsy, and a series of liver function studies were carried out within a few days of each tocopherol tolerance test. In a few instances the serum cholesterol determination was delayed up to a week.

Diets. The normal healthy adults were on uncontrolled, self-chosen diets. All the hospitalized controls were on the routine hospital diet at the time of investigation. In the liver disease group, the cirrhotics were on a previously described experimental, maintenance diet of 1 gm. of protein and 30 calories per kg. of body weight without vitamin or lipotropic supplements (28), while the subjects with hepatitis and obstructive jaundice received a high-protein, high-caloric diet, which, in some instances, was poorly eaten.

Experimental procedure. Plasma tocopherol concentration was determined on oxalated venous blood by a modification of the Quaife and Biehler method (29, 30).

The tests of hepatic function employed have been described elsewhere (28).

Tocopherol tolerance tests were carried out as follows: a fasting blood specimen was drawn in the early morning, the appropriate dose of tocopherol was then administered by the oral or intramuscular route, and the subject was allowed to eat his regular meals. Blood samples were drawn 3, 6, 9, 12, 24, 36, 48, and in some instances, 72 and 96 hours later. The 24, 48, 72 and 96-hour specimens were also drawn in the fasting state.

To measure the spontaneous fluctuations and those due to the diet, plasma tocopherol concentrations were determined at intervals for a period of 24 hours in subjects who had not received tocopherol.

The following forms of tocopherol were administered: *a*) dl-alpha-tocopheryl acetate,[2] 500 mg. *orally, b*) d-alpha-tocopheryl di-sodium phosphate, 500 mg. *orally* (714 mg. of a preparation[3] containing 70 per cent pure d-alpha-tocopheryl di-sodium phosphate), *c*) dl-alpha-tocopherol,[4] 500 mg. in 1.0 cc. sesame oil *intramuscularly,* and *d*) dl-alpha-tocopheryl acetate in an aqueous emulsion[5] (stabilized with a mixture of Tweens and containing dispersed particles, 93 per cent of which were less than 14 microns in size), 500 mg. in 2.5 cc. of water *intramuscularly.* Taking the differences in molecular weight into account the acetate ester contained 457 mg., and the phosphate ester 388 mg. of tocopherol per dose.

RESULTS

Fasting plasma tocopherol level. The mean plasma tocopherol concentration was 1.23 ± 0.31 mg. per cent in the normal healthy adults, 1.02 ± 0.37 mg. per cent in the hospitalized controls and 0.95 ± 0.32 mg. per cent in the liver disease group. The concentration in the liver disease group was significantly lower than in the normal healthy adults, but it did not differ significantly from that in the hospitalized controls (Table I).

The range of values observed in all three groups was wide: 0.89 to 2.00 mg. per cent in the healthy adults, 0.35 to 2.01 mg. per cent in the hospitalized controls, and 0.29 to 1.70 mg. per cent in the subjects with liver disease. However, the range and distribution of values in the liver disease group were significantly wider than in the normal healthy adults, but they did not differ significantly from those in the hospitalized control group (Table I).

Almost identical values for normal adults have been reported by Quaife and Harris (29). Slightly lower concentrations have been found by others (17, 21, 31). It may be of significance in this connection that Engel (32) found the average plasma tocopherol concentration in Holland to be 0.80 mg. per cent. Following the continuous administration of doses up to 120 mg. of alpha-tocopheryl acetate daily the plasma concentration rose to 1.20 mg. per cent, but did not exceed this level, suggesting that lower levels may reflect a low dietary intake of tocopherol. The differences observed between young healthy adults and randomly selected hospital patients confirm the findings of Lemley and her associates (33).

The plasma tocopherol level was reduced as inconstantly in infectious hepatitis as it was in cirrhosis (Table I), in accord with the report of

[2] Ephynal acetate, obtained through the courtesy of Dr. R. J. Floody, Hoffmann-LaRoche, Inc., Nutley, N. J.

[3] Obtained through the courtesy of Dr. P. L. Harris, Distillation Products, Inc., Rochester, N. Y.

[4] Obtained from Merck and Company, Rahway, N. J.

[5] Obtained through the courtesy of Dr. A. E. Osterberg, Abbott Laboratories, N. Chicago, Ill.

TABLE II

Relation of the plasma tocopherol level to the functional status of the liver

Subject	Diagnosis	Fasting plasma tocopherol	Max. rise plasma tocopherol	Type of tocopherol administered	1-minute serum bilirubin	Total serum bilirubin	BSP retention	Cephalin-cholesterol flocculation	Thymol turbidity	Serum alkaline phosphatase	Total serum protein	Serum albumin	Serum globulin	Serum cholesterol	Free serum cholesterol
		mg. per cent	*mg. per cent*		*mg. per cent*	*mg. per cent*	*per cent*	*24 hr./48 hr.*	*units*	*Bodansky units*	*gm. per cent*	*gm. per cent*	*gm. per cent*	*mg. per cent*	*per cent of total*
48	Cirrhosis	0.29	0.44	Oral phosph.	—	—	—	—	—	—	—	—	—	277	—
50	Hepatitis	0.54	—	—	3.57	7.21	—	4/4	11.0	11.9	6.89	3.26	3.63	280	52
51	Cirrhosis	0.58	0.00	Oral acetate	7.00	11.00	—	—	—	—	—	—	—	—	—
52	Cirrhosis	0.59	0.63	Oral acetate	0.37	3.06	36.9	4/4	6.0	5.7	6.79	3.58*	3.21	166	40
51	Cirrhosis	0.60	0.44	Oral phosph.	0.20	0.85	—	—	—	—	—	—	—	—	—
53	Cirrhosis	0.62	0.38	I.M. emulsion	0.67	1.83	33.6	0/0	1.0	11.4	5.95	2.17	3.78	182	35
54	Cirrhosis	0.67	1.21	Oral phosph.	4.60	6.75	62.0	–/4	4.0	4.9	—	—	—	191	—
55	Cirrhosis	0.69	0.12	I.M. in oil	0.20	0.40	25.0	–/4	2.0	3.5	—	—	—	—	—
56	Cirrhosis	0.70	0.65	Oral phosph.	0.20	0.60	—	—	—	—	—	—	—	170	—
57	Cirrhosis	0.71	—	—	2.30	4.37	21.0	3/4	4.5	11.3	6.19	2.43	3.76	219	82
54	Cirrhosis	0.72	0.22	I.M. in oil	0.10	0.30	—	—	—	—	—	—	—	—	—
56	Cirrhosis	0.76	1.86	Oral acetate	0.20	1.60	—	—	—	—	—	—	—	—	—
58	Cirrhosis	0.78	0.72	I.M. in oil	1.05	1.90	28.0	3/3	5.0	—	6.77	1.37	5.40	—	—
59	Hepatitis	0.79	0.35	I.M. emulsion	2.11	4.28	22.2	3/4	14.0	7.7	6.80	3.41	3.39	—	—
60	Cirrhosis	0.84	0.17	I.M. in oil	0.14	0.88	7.8†	0/0	3.0	2.2	6.98	2.15	4.83	168	30
61	Cirrhosis	0.84	0.28	I.M. in oil	1.15	2.55	—	—	—	—	—	—	—	—	—
62	Cirrhosis	0.85	1.45	Oral acetate	0.34	1.09	20.0	4/4	—	—	—	—	—	—	—
59	Hepatitis	0.88	0.32	I.M. in oil	4.46	8.93	57.9†	4/4	20.0	7.7	6.80	3.41	3.39	—	—
63	Cirrhosis	0.88	0.53	Oral acetate	0.10	0.49	—	1/3	1.0	—	5.69	2.92	2.77	126	30
60	Cirrhosis	0.90	0.30	Oral acetate	0.41	1.22	7.8†	0/0	3.5	2.2	7.29	2.40	4.89	88	39
64	Cirrhosis	0.95	0.27	Oral phosph.	0.20	0.80	0.0	0/1	3.0	3.3	—	—	—	191	—
54	Cirrhosis	0.96	1.13	Oral acetate	1.35	2.95	42.5	–/2	1.5	4.2	5.50	—	—	—	—
63	Cirrhosis	1.01	0.99	Oral acetate	0.14	0.27	4.0†	1/2	1.0	—	6.00	3.07	2.93	172	31
65	Cirrhosis	1.02	—	—	0.41	0.89	5.1	0/0	3.5	5.9	7.87	3.60	4.27	212	71
66	Hepatitis	1.02	0.03	Oral acetate	12.69	21.42	—	3/4	3.0	21.6	6.53	3.25	3.28	242	59
67	Cirrhosis	1.05	1.04	Oral acetate	0.70	1.90	45.0	3/4	10.0	—	6.03	1.48	4.55	170	33
68	Hep. necrosis	1.13	0.85	Oral acetate	7.30	21.01	63.5	3/4	4.5	8.0	6.05	2.62	3.43	113	74
69	Cirrhosis	1.16	0.32	Oral acetate	0.07	0.58	36.0	0/0	2.0	11.4	7.02	2.27	4.75	195	32
70	Cirrhosis	1.16	0.04	I.M. in oil	0.85	4.15	40.2†	0/0	5.0	3.9	5.75	2.17	3.58	162	42
71	Obst. jaundice	1.17	0.33	Oral acetate	8.80	16.25	—	2/3	1.0	36.7	7.52	3.33	4.19	288	52
58	Cirrhosis	1.19	0.77	Oral acetate	1.77	3.74	40.0†	3/4	6.5	—	5.51	1.77	3.74	—	—
72	Cirrhosis	1.22	0.40	Oral acetate	1.25	2.70	16.0	–/2	4.7	4.7	7.80	—	—	360	—
73	Cirrhosis	1.29	0.29	Oral acetate	1.25	2.00	0.0	—	1.0	6.9	6.90	4.70	2.20	—	—
74	Cirrhosis	1.40	0.20	Oral phosph.	1.94	4.56	—	3/4	3.0	—	5.73	2.04	3.69	255	51
70	Cirrhosis	1.41	—	—	2.38	11.56	35.4	—	—	9.4	5.84	2.77	3.07	190	45
74	Cirrhosis	1.52	1.13	Oral acetate	0.49	1.94	26.1	—	—	5.6	6.17	2.40	3.77	257	38
62	Cirrhosis	1.60	0.50	I.M. in oil	0.61	2.99	53.4	3/4	1.0	—	—	—	—	—	—
69	Cirrhosis	1.70	0.00	I.M. in oil	0.24	0.68	21.6†	0/0	2.0	17.0	6.20	2.58	3.62	—	—

* After serum albumin intravenously for several days.

† Retention at 30 minutes following dye injection (5 mg. per kg.), other values determined at 45 minutes.

TABLE III

Relation of the plasma tocopherol level and the response following orally administered tocopherol to changes in hepatic function

Subject	Interval between tests	Functional status of liver	Fasting plasma tocopherol level*	Max. rise plasma tocopherol following 500 mg. tocopherol orally*	Fasting plasma tocopherol	Max. rise plasma tocopherol	Total serum bilirubin	BSP retention	Cephalin-cholesterol flocculation	Thymol turbidity	Serum alkaline phosphatase	Total serum protein	Serum albumin	Serum globulin	Serum cholesterol	Free cholesterol
	days				*mg. per cent*	*mg. per cent*	*mg. per cent*	*per cent*	*24 hr./48 hr.*	*units*	*Bodansky units*	*gm. per cent*	*gm. per cent*	*gm. per cent*	*mg. per cent*	*per cent of total*
51	120	Improved	Unchanged	Increased	0.58	0.00	11.00									
					0.60	0.44	0.85									
54	9	Improved	Increased	Unchanged	0.67	1.21	6.75	62.0	−/4	4.0	4.9					
					0.96	1.13	2.95	42.5	−/2	1.5	4.2					
56	9	Worse	Unchanged	Increased	0.70	0.65	0.60									
					0.76	1.86	1.60									
59	7	Improved	Unchanged	—	0.88	—	8.93	57.9†	4/4	20.0	7.7					
					0.79	—	4.28	22.2	3/4	14.0	7.7					
63	33	Improved	Increased	Increased	0.88	0.53	0.49	—	1/3	1.0	—	5.69	2.92	2.77	126	30
					1.01	0.99	0.27	—	1/2	1.0	—	6.00	3.07	2.93	172	31
60	9	Improved	Unchanged	—	0.90	—	1.22	—	0/0	3.5	—	7.29	2.40	4.89	88	39
					0.84	—	0.88	—	0/0	3.0	—	6.98	2.15	4.83	168	30
54	150	Improved	Decreased	—	0.96	—	2.95									
					0.72	—	0.30									
69	15	Improved	Increased	—	1.16	—	0.58	36.0	0/0	2.0	11.4	7.02	2.27	4.75		
					1.70	—	0.68	21.6†	0/0	2.0	17.0	6.20	2.58	3.62		
58	7	Improved	Decreased	—	1.19	—	3.74	40.0†	3/4	6.5	—	5.51	1.77	3.74		
					0.78	—	1.90	28.0	3/3	5.0	—	6.77	1.37	5.40		
74	19	Improved	Unchanged	Increased	1.40	0.20	4.56	—	—	—	—	5.73	2.04	3.69	255	51
					1.52	1.13	1.94	—	—	—	—	6.17	2.40	3.77	257	38
70	32	Improved	Decreased	—	1.41	—	11.56	35.4	—	—	9.4	5.84	2.77	3.07	190	45
					1.16	—	4.15	40.2†	—	—	3.9	5.75	2.17	3.58	162	42
62	10	Improved	Decreased	—	1.60	—	2.99	53.4	3/4							
					0.85	—	1.09	20.0	4/4							

* Changes were regarded as significant when they were greater than twice the standard deviation of the means of the group not receiving tocopherol.
† Retention at 30 minutes following dye injection (5 mg. per kg.), other values determined at 45 minutes.

Popper and his associates (19). The only observation on a patient with obstructive jaundice in this series fell within the normal range. There are conflicting reports of both high (19) and low (21) values in this condition.

Factors which may influence the plasma tocopherol level. No significant correlation could be demonstrated between the plasma tocopherol concentration and the functional status of the liver, judged by the results of liver function tests (Table II). Similarly, in serial observations on individual subjects, alterations in hepatic function were not uniformly reflected in plasma tocopherol changes (Table III), further emphasizing the independence of these two variables.

Popper and his associates (19) found that the presence of jaundice, either as complication of cirrhosis or as a manifestation of biliary obstruction, resulted in an increase in plasma tocopherol. In the present study there appeared to be no relationship between the presence of jaundice and the plasma tocopherol level. It is evident from the data in Table II that the serum bilirubin and plasma tocopherol concentrations varied independently, and that if the serum alkaline phosphatase level can be taken as an index of intrahepatic obstruction or regurgitation (34), there was no correlation between the plasma tocopherol level and the degree of biliary obstruction.

Neither age nor the serum cholesterol concentration appeared to be a significant factor in determining the plasma tocopherol level (Table I). These results differ from those of Lemley and her associates (33) who found a significant positive correlation between plasma tocopherol and age, and from those of Darby and coworkers (21) who found a similar correlation between plasma tocopherol and serum cholesterol concentration. However, the small number of subjects in the present series whose age fell outside the 30 to 60 year range and whose serum cholesterol concentration was significantly elevated may have masked the slight degree of correlation reported by others.

Fluctuations in the plasma tocopherol level. The spontaneous fluctuations throughout a 24-hour period for a group of hospitalized controls and liver disease subjects are recorded in Table IV. The diets employed appeared to have little effect on the plasma tocopherol concentration, and the two successive fasting specimens (the initial and the 24-hour) in each subject remained remarkably constant. The standard deviation of observations within the group as a whole was ± 0.09 mg. per cent. In the following experiments fluctuations in plasma tocopherol concentration smaller than twice the standard deviation were regarded as insignificant.

TABLE IV

Fluctuations in the plasma tocopherol level related to the ingestion of food *

Subject	8 a.m.	11 a.m.	2 p.m.	5 p.m.	8 p.m.	8 a.m.	Mean
			Controls				
	mg. per cent	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*
8	0.58	0.74	0.62	0.60	0.71	0.62	0.65
12	0.78	0.76	0.76	0.72	—	—	0.76
17	0.84	0.82	0.94	0.91	0.86	0.94	0.89
24	0.98	0.88	0.92	0.74	0.84	1.00	0.89
25	1.02	0.96	0.97	0.91	0.90	0.95	0.95
26	1.02	0.68	0.70	0.82	—	—	0.81
36	1.21	1.28	1.42	1.41	1.30	1.20	1.30
37	1.24	1.20	1.04	1.28	1.31	1.20	1.21
42	1.42	1.50	1.63	1.63	—	—	1.55
43	1.42	1.24	1.40	1.02	1.04	1.21	1.22
			Liver disease				
49	0.54	0.58	0.62	0.60	0.61	0.57	0.59
50	0.54	0.49	0.42	0.48	0.54	0.60	0.51
57	0.71	0.74	0.80	0.85	0.77	0.78	0.78
65	1.02	1.10	1.14	1.12	1.04	0.99	1.07

Standard deviation of the observations in the group = ±0.09 mg. per cent.†

* Diets served at 8 a.m., 12 noon, and 5 p.m.

† $\sqrt{\frac{\text{Sum of (deviations from the mean of each subject)}^2}{\text{Sum of (n − 1 for each subject)}}}$.

Similar increases in plasma tocopherol have been reported in normals following single large doses of *orally* administered tocopherol (17, 19, 29), and, in general, the curves obtained have resembled the control curve illustrated in Figure 1.

Relatively few tocopherol tolerance curves have been reported in liver disease. The curve obtained by Steinberg (17) in a cirrhotic coincides with that illustrated in Figure 1. The responses in the five cirrhotics studied by Popper and his associates (19) were very irregular, two subjects showing a relatively normal and three a definitely diminished rise in plasma tocopherol concentration. The number of observations on each subject, however, was too small to warrant any comparisons based on the contour of the curves. In the one subject with obstructive jaundice that these investigators

TABLE V

The plasma tocopherol level following the oral administration of 500 mg. of dl-alpha-tocopheryl acetate

Subject	Hours										Maximum rise	
	0	3	6	9	12	24	36	48	72	96		
Controls												
	mg. per cent	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*hours*
2	0.35	0.87	0.69	0.37	0.62	0.58	0.59	0.43	0.56	0.73	0.52	3
3	0.36	1.29	2.15	0.86	0.39	0.16	0.01	0.60	0.35	0.21	1.79	6
4	0.37	0.34	1.90	2.13	0.87	0.66	1.54	1.40	1.17	1.13	1.76	9
5	0.41	0.69	1.53	—	1.61	0.72	0.95	—	0.49	0.79	1.20	12
6	0.46	0.64	0.82	0.77	0.63	0.56	0.90	0.67	0.41	—	0.44	36
7	0.57	1.17	0.75	0.68	0.59	0.69	0.81	0.71	0.93	—	0.60	3
9	0.72	1.77	1.62	1.62	2.51	2.60	1.67	1.53	1.58	—	1.88	24
13	0.79	1.98	1.74	1.78	1.93	—	1.71	1.66	1.65	—	1.19	3
14	0.79	0.88	1.26	—	1.50	1.35	1.23	1.46	0.90	—	0.71	12
15	0.81	0.96	1.29	2.25	2.10	1.89	2.14	1.16	1.07	—	1.44	9
19	0.87	1.40	0.53	0.83	1.28	0.26	0.31	—	0.83	0.09	0.53	3
27	1.03	1.33	1.73	2.70	2.07	1.93	1.42	1.35	—	—	1.67	9
28	1.05	1.07	1.65	2.26	3.09	4.30	—	—	—	—	3.25	24
31	1.12	1.16	1.50	1.62	1.40	1.32	1.17	1.12	—	—	0.50	9
33	1.13	1.63	2.55	2.46	1.53	1.55	1.66	1.28	1.31	1.78	1.42	6
34	1.17	1.08	1.73	1.49	1.05	0.96	1.32	0.68	1.08	—	0.56	6
40	1.35	1.28	2.35	3.20	—	4.98	—	—	—	—	3.63	24
41	1.40	1.21	1.18	1.43	1.06	1.44	1.35	1.19	1.21	—	0.04	24
45	1.48	1.47	2.96	3.14	2.70	1.48	1.63	1.82	1.98	—	1.66	9
46	1.60	1.72	2.81	2.38	2.25	1.95	2.10	2.18	2.25	—	1.21	6
35	1.73	2.25	2.66	—	—	3.24	—	—	—	—	1.51	24
Mean	0.93	1.25	1.69	1.78	1.54	1.63	1.25	1.20	1.11	0.79	1.31*	
±s.d.	0.41	0.45	0.69	1.05	0.76	1.26	0.56	0.47	0.53	0.47	0.87	
Liver disease												
51	0.58	0.56	0.56	0.54	0.39	0.51	0.51	0.51	0.45	—	0.00	—
52	0.59	0.85	—	1.20	1.22	1.04	0.66	0.77	1.20	—	0.63	12
56	0.76	1.11	2.62	2.19	2.14	1.91	1.92	1.67	1.40	1.05	1.86	6
62	0.85	0.95	2.30	1.87	1.60	—	—	—	—	—	1.45	6
63	0.88	0.91	1.41	1.18	1.07	1.07	—	—	1.01	—	0.53	6
60	0.90	0.78	0.90	—	0.95	1.20	—	—	—	—	0.30	24
54	0.96	1.25	1.04	0.92	0.98	0.87	2.09	—	0.68	0.87	1.13	36
63	1.01	1.35	2.00	1.82	1.60	1.54	1.18	1.50	1.48	—	0.99	6
66	1.02	0.86	0.84	0.84	—	0.79	—	1.05	1.05	—	0.03	48
67	1.05	1.30	1.44	1.50	1.43	2.09	1.21	1.64	0.81	—	1.04	24
68	1.13	1.42	1.98	1.44	1.63	1.65	—	—	—	—	0.85	6
53	1.13	0.90	0.79	0.88	0.90	0.85	0.89	0.99	1.05	—	0.00	—
69	1.16	1.44	0.72	—	1.48	1.24	—	—	—	—	0.32	12
71	1.17	1.50	1.50	1.41	—	1.35	1.35	1.28	1.12	—	0.33	3
58	1.19	1.02	1.50	—	1.41	1.96	—	—	—	—	0.77	24
72	1.22	1.24	1.18	1.35	1.43	1.62	1.22	1.35	1.53	—	0.40	24
70	1.23	1.31	1.44	1.56	1.93	2.12	—	—	—	—	0.89	24
73	1.29	1.35	1.40	1.22	1.35	1.35	1.35	1.35	1.58	—	0.11	6
74	1.52	—	—	2.11	2.55	2.65	2.37	—	—	—	1.13	24
Mean	1.03	1.12	1.39	1.38	1.42	1.43	1.35	1.21	1.11	0.96	0.67*	
±s.d.	0.23	0.27	0.56	0.45	0.49	0.55	0.56	0.36	0.34	0.09	0.51	

* Difference between means statistically significant ($t = 2.82$, $P < 0.01$).

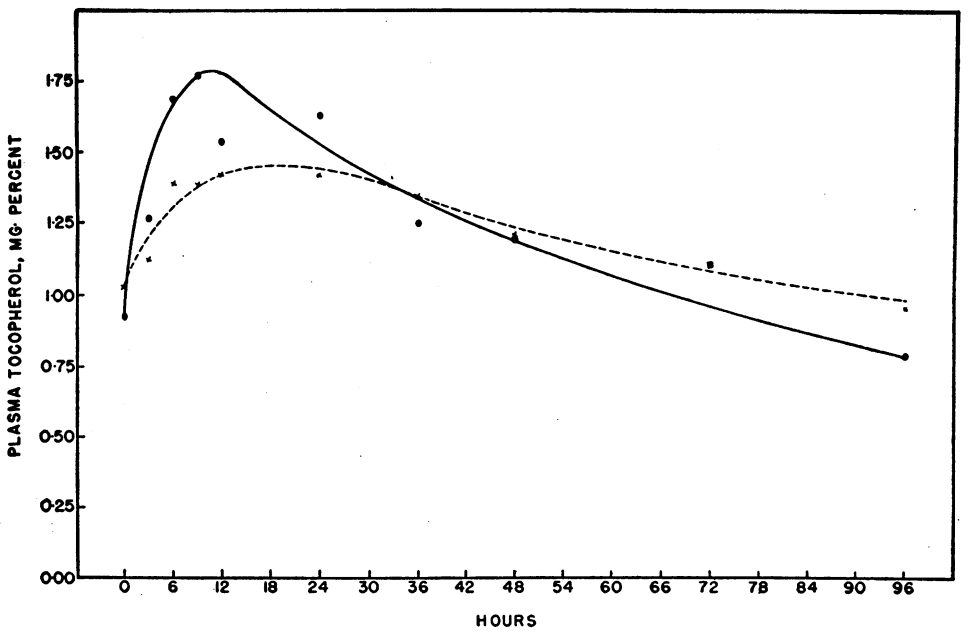

FIG. 1. PLASMA TOCOPHEROL CURVES FOLLOWING THE INGESTION OF 500 MG. OF DL-ALPHA-TOCOPHERYL ACETATE

Solid line = mean for hospitalized control subjects.
Broken line = mean for liver disease subjects.

studied there was a small but significant increase in plasma tocopherol following 1,500 mg. of dl-alpha-tocopheryl acetate *orally*. In the present series the one subject with obstructive jaundice (subject 71, Table V) showed a similar small but significant increase following the administration of only 500 mg. of the same material.

In an attempt to elucidate the difference between the tocopherol tolerance curves observed in the control and liver disease groups, tolerance tests were carried out in six liver disease subjects with *orally* administered d-alpha-tocopheryl di-sodium phosphate (Table VI). It seemed possible that the relatively flat curves in liver disease might be due to a faulty absorption of dl-alpha-tocopheryl acetate related to its fat-solubility. If this were so, the *oral* administration of a water-soluble ester of tocopherol, such as the phosphate, might be ex-

TABLE VI

The plasma tocopherol level following the oral administration of 500 mg. d-alpha-tocopheryl di-sodium phosphate to subjects with liver disease

Subject	Hours										Maximum rise	
	0	3	6	9	12	24	36	48	72	96		
	mg. per cent	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*hours*
48	0.29	0.47	0.68	0.55	0.70	0.77	1.02	0.77	0.99	0.75	0.73	36
51	0.60	0.79	0.77	0.96	1.04	0.73	0.90	0.96	0.80	0.91	0.44	12
54	0.67	0.80	0.71	0.72	0.55	0.50	0.80	1.34	—	1.88	1.21	96
56	0.70	0.71	—	0.72	0.69	0.94	1.04	1.35	0.97	1.00	0.65	48
64	0.95	1.06	0.97	0.79	1.22	1.13	0.82	0.59	0.86	1.18	0.27	12
74	0.40	—	1.60	1.40	1.45	1.35	—	1.42	—	—	0.20	6
Mean	0.77	0.77	0.95	0.86	0.94	0.90	0.92	1.06	0.91	1.14	0.58*	
±s.d.	0.34	0.19	0.34	0.27	0.32	0.28	0.10	0.37	0.08	0.39	0.34	

* Difference between means for maximum rise in plasma tocopherol following *oral* administration of dl-alpha-tocopheryl acetate and phosphate in liver disease not statistically significant ($t = 0.45$, $P = >0.90$).

pected to yield normal plasma tocopherol curves in subjects with liver disease. Such was not the case, for the maximum rise in plasma tocopherol following the phosphate (0.58 ± 0.34 mg. per cent) did not differ significantly from that observed following the *oral* administration of acetate (0.67 ± 0.51 mg. per cent). All six of the subjects receiving the phosphate but only 15 of the 19 receiving the acetate exhibited a rise in plasma tocopherol. However, this difference proved to have no statistical significance (chi-square = 1.50, P = > 0.20). The results of this experiment cannot be regarded as conclusive, since the phosphate ester administered contained only 85 per cent as much tocopherol as the acetate ester, taking differences in molecular weight into account. Unfortunately, due to the limited supply of material available, it was not possible to repeat these experiments with larger amounts, nor to study its effects in normal and hospitalized control subjects.

The results of the *oral* tocopherol tolerance tests suggested that there was a defect in absorption or, possibly, an increased destruction of tocopherol esters in the intestinal tract of subjects with liver disease. To exclude other possible explanations for the relatively flat plasma tocopherol curves observed in liver disease, the effects of parenterally administered tocopherol were studied in the hope that differences in the curves due to alterations in intestinal absorption or destruction could be eliminated. Unfortunately, the results of these experiments were equivocal, largely because the two forms of tocopherol available for parenteral use appeared to be poorly absorbed from the muscles.

Following the *intramuscular* injection of 500

TABLE VII

The plasma tocopherol level following the intramuscular injection of 500 mg. dl-alpha-tocopherol in sesame oil

Subject	Hours						Maximum rise	
	0	3	6	9	12	24		
Controls								
	mg. per cent	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*hours*
16	0.82	0.94	0.98	1.11	1.08	1.14	0.32	24
22	0.94	0.89	1.21	1.42	1.30	1.40	0.48	9
75	0.94	1.58	2.10	1.02	0.93	1.02	1.16	6
76	0.96	1.05	1.13	1.18	1.13	0.93	0.22	9
77	0.97	1.16	1.88	1.56	1.30	1.12	0.91	6
78	1.18	1.43	1.62	1.53	1.27	1.33	0.44	6
35	1.20	1.50	1.75	2.72	1.84	1.62	1.52	9
79	1.47	1.74	1.88	1.19	1.28	1.45	0.41	6
40	1.48	1.61	1.27	1.29	1.50	1.35	0.13	3
80	1.64	2.02	1.56	1.30	1.28	1.47	0.38	3
Mean	1.16	1.39	1.54	1.43	1.29	1.28	0.60*	
s.d.±	0.27	0.36	0.35	0.47	0.24	0.23	0.42	
Liver disease								
55	0.69	0.72	0.77	0.81	0.80	0.78	0.12	9
54	0.72	0.78	0.82	0.84	0.72	0.94	0.22	24
58	0.78	0.86	1.34	1.05	1.50	1.05	0.72	12
60	0.84	0.63	0.86	0.75	0.56	1.01	0.17	24
61	0.84	0.92	0.98	1.10	1.08	1.12	0.28	24
59	0.88	—	0.99	1.01	1.20	1.02	0.32	12
70	1.16	1.07	0.94	1.17	1.20	—	0.04	12
62	1.60	0.62	—	1.49	1.03	2.10	0.50	24
48	1.70	0.68	1.07	1.37	1.36	0.96	0.00	—
Mean	1.02	0.79	0.97	1.07	1.05	1.12	0.26*	
s.d.±	0.36	0.15	0.17	0.24	0.29	0.38	0.22	

* Difference between means of borderline significance (t = 2.04, P = <0.10, >0.05). Rise following *intramuscular* dl-alpha-tocopherol in oil significantly smaller than that following *oral* dl-alpha-tocopheryl acetate, both in the hospitalized control and liver disease groups (controls: t = 2.36, P < 0.05; liver disease group: t = 2.25, P = <0.05).

TABLE VIII

The plasma tocopherol level following the intramuscular injection of 500 mg. dl-alpha-tocopherol acetate in an aqueous emulsion stabilized with a mixture of Tweens

Subject	Hours						Maximum rise	
	0	3	6	9	12	24		
Controls								
	mg. per cent	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*hours*
18	0.84	1.02	0.94	0.82	0.94	1.00	0.18	3
20	0.94	0.92	0.87	1.06	1.10	1.00	0.16	12
21	0.94	1.02	1.10	1.08	1.00	1.08	0.16	6
23	0.97	0.92	0.84	0.97	1.00	0.90	0.03	12
32	1.12	1.04	1.21	1.20	1.00	0.88	0.09	6
Mean	0.96	0.98	0.99	1.03	1.01	0.97	0.12*	
±s.d.	0.29	0.17	0.14	0.13	0.05	0.07	0.06	
Liver disease								
53	0.62	0.84	0.94	1.00	0.81	0.84	0.38	9
59	0.79	0.92	0.98	1.01	1.14	—	0.35	12
Mean	0.71	0.88	0.96	1.01	0.98	0.84	0.37†	
±s.d.	0.08	0.04	0.02	0.02	0.13	—	0.01	

* Significantly smaller than rise following *intramuscular* dl-alpha-tocopherol in oil ($t = 2.33$, $P = <0.05$) and that following *oral* dl-alpha-tocopherol acetate in hospitalized controls ($t = 2.95$, $P = <0.02$).

† Too few values to warrant statistical analysis.

mg. of dl-alpha-tocopherol in sesame oil, the increases in plasma tocopherol were significantly smaller than those following the *oral* administration of dl-alpha-tocopheryl acetate, both in the hospitalized control and liver disease groups (Table VII). This difference was all the more significant in that the *intramuscular* injection contained almost 9 per cent more tocopherol than the *oral* dose. These findings suggest a defect in the absorption of *intramuscularly* administered dl-alpha-tocopherol similar to that observed in the rabbit (35) and in the rat (36). The mean rise in plasma tocopherol concentration was lower in the liver disease group than in the controls, although the difference was of only borderline significance statistically ($t = 2.04$, $P = < 0.10$, > 0.05). These observations suggest that the lower tocopherol tolerance curves in liver disease may reflect alterations in storage or utilization, rather than faulty absorption of tocopherol from the intestinal tract. However, the results of this experiment are not conclusive, since the difference between the control and liver disease groups may have been related to irregular absorption of tocopherol from muscle. The extremely variable responses in both groups lends support to this possibility.

To eliminate the effects of faulty absorption from muscle, a readily absorbed, water-soluble, preparation of tocopherol suitable for parenteral injection was sought. Recent reports (35, 37, 38) suggested that the di-sodium salt of d-alpha-tocopheryl phosphate might meet these requirements. Preliminary experiments were carried out in rats with an *intramuscular* injection of 50 mg. in 0.5 cc. of water.[6] Extensive necrosis of muscle resulted at each of the injection sites. A similar injection into one human subject evoked both a severe local reaction and constitutional symptoms. Intravenous injections also produced severe local and constitutional reactions.[7] These side effects of injected d-alpha-tocopheryl di-sodium phosphate precluded its use for experimental purposes.

Although the solubility of dl-alpha-tocopheryl acetate in aqueous media is limited and its absorption from muscle very poor (39), it seemed possible that a finely dispersed aqueous emulsion might be readily absorbed from the muscles. This was suggested by the effectiveness of emulsifying

[6] These experiments were carried out in the Pharmacology Laboratory of Hoffmann-LaRoche, Inc. through the courtesy of Dr. R. J. Floody.

[7] Personal communication from Dr. R. J. Floody.

agents, like the Tweens, in promoting the absorption of vitamin A in liver disease (40) and in steatorrhea (41), and by the report of Quaife (36) that Tween 80 promoted the absorption of *intramuscularly* injected alpha-tocopherol in vitamin E-deficient rats. Accordingly a finely dispersed emulsion of dl-alpha-tocopheryl acetate, containing particles 93 per cent of which were less than 14 microns in diameter, was prepared, using a mixture of Tweens as stabilizing agents. However, when the emulsion was injected *intramuscularly* into five control subjects not one showed a significant rise in plasma tocopherol, and the rises in the two liver disease subjects tested were exceedingly small (Table VIII), indicating that it was absorbed from muscle even more slowly than an oily solution of dl-alpha-tocopherol.

The experiments employing *intramuscular* injections of tocopherol differed from those in which dl-alpha-tocopheryl acetate was administered *orally* in that the plasma tocopherol level was followed for only 24 hours in the former and 48 to 96 hours in the latter. However, it is evident from Table V that the peak was attained within a period of 24 hours in all but three of the 40 subjects tested following *orally* administered tocopherol, so that the small peaks following *intramuscular* injections can be interpreted as indicating a delay in absorption. Indeed experiments in vitamin E-deficient animals suggest that alpha-tocopherol in oil is not at all absorbed from muscle (36), or only very slowly (35).

An attempt was made to analyze the factors which determined the height of the plasma tocopherol rise following the *oral* administration of tocopherol esters. No significant correlation could be demonstrated between the peak response and the initial plasma tocopherol level, the serum cholesterol concentration, or the functional status of the liver, judged by the results of liver function tests (Table II).

DISCUSSION

The plasma tocopherol level probably reflects the interplay of many factors, including the dietary intake, the absorptive capacity of the intestinal tract, the destruction of tocopherol in the intestinal tract or tissues, the state of the tissue stores, the efficiency of utilization, and, possibly, the rate of tocopherol excretion. There is evidence to show that the concentration of tocopherol in both plasma (32, 42) and tissue (43) is affected by the dietary intake. Darby's studies in sprue indicate that changes in the absorptive capacity of the intestine are reflected in the level of plasma tocopherol (18). Dietary excesses of unsaturated fat lead to the destruction of tocopherol in the intestine and to the development of vitamin E deficiency (13, 44). Little is known about the relative efficiency of tocopherol utilization under various conditions, but it is known that the tocopherol requirement can be influenced by such factors as the dietary content of protein (10, 11) and of vitamin A (15). As far as is known, tocopherol is not excreted in the urine (32), except under conditions of prolonged excessive intake (45). Similarly, there is little or no fecal excretion of tocopherol in experimental animals under conditions of normal, or low vitamin E intake, but very considerable fecal losses can occur under conditions of prolonged, excessive tocopherol intake (45). Whether such losses represent an unabsorbed fraction of ingested tocopherol, or a true excretion product is not known. The demonstration of significant concentrations of tocopherol in bile (19), suggests that there may be an enterohepatic circulation of tocopherol. Unfortunately, excretion studies have not been carried out following hepatic damage, so that important data necessary for the interpretation of the plasma tocopherol findings in the present experiments, are lacking.

The low plasma tocopherol levels observed in some patients with liver disease probably reflect low tissue stores (19). Whether this decrease is due to alterations in absorption, utilization or excretion directly related to liver damage, or to some other factor, such as malnutrition, is not known. The almost identical frequency distribution of plasma tocopherol levels in liver disease and randomly selected, convalescent, hospital patients, and the lack of any correlation between the tocopherol level and the functional status of the liver are points against a direct, causal relationship between liver damage and low plasma tocopherol concentrations. The relatively low plasma tocopherol curves after oral administration of tocopherol suggest impaired intestinal absorption in some cases of liver disease. However, the lack of any correlation between the initial plasma tocopherol level and the response to orally administered to-

copherol, would seem to indicate that the low plasma concentrations in some cases of liver disease are not due to defective tocopherol absorption.

No correlation could be demonstrated between the plasma response to orally administered tocopherol and the state of hepatic function which suggests that other associated factors may be responsible for the flat tocopherol tolerance curves in liver disease.

The antecedent diet may have played a role in producing the low plasma tocopherol levels observed in both groups of hospitalized subjects. The average American diet contains less (46) than the estimated normal daily tocopherol requirement of man (32, 47), so that low plasma levels, presumably indicative of decreased tissue stores, may occur in hospital patients, some of whom subsist on less-than-average diets for long periods. It may be of significance in this connection that normal plasma tocopherol levels have been reported in acute liver disease, such as infectious hepatitis (21), and that a fatal case of acute hepatitis in the present series (subject 68, Table I) had a high normal value.

It is obvious that no definite conclusions regarding the alterations in vitamin E metabolism in liver disease are warranted at the present time. Further study of plasma tocopherol curves following the parenteral administration of readily absorbed tocopherol esters, and studies of fecal tocopherol excretion are needed to determine the role of altered intestinal absorption in liver disease. More observations are also needed on the relation of plasma to tissue concentration of tocopherol and of the effects of continued tocopherol administration, to determine the significance of the plasma tocopherol concentration as an index of tissue tocopherol saturation and utilization. Although clinical evidence of vitamin E deficiency in man is still lacking, the findings of Pappenheimer and Victor (20) are sufficiently suggestive to encourage further investigation of the physiological significance of low plasma tocopherol levels.

SUMMARY

1. The plasma tocopherol level was determined in three groups of subjects: *a*) young healthy adults, *b*) hospitalized convalescent controls with no evidence of liver damage, and *c*) patients with liver disease.

2. The mean plasma tocopherol concentration was significantly lower, and the range and distribution of values were significantly wider in the liver disease group than in young healthy adults, but there was no significant difference between liver disease subjects and hospitalized controls. Moreover, the plasma tocopherol level could not be correlated with the degree of hepatic dysfunction, the type of liver damage, the presence of jaundice, the serum cholesterol concentration, or age.

3. Plasma tocopherol curves were obtained in liver disease subjects and hospitalized controls following single test doses of various preparations of alpha-tocopherol, given either orally or intramuscularly.

4. The rise in plasma tocopherol concentration following the *oral* administration of dl-alpha-tocopheryl acetate was significantly smaller in liver disease subjects than in hospitalized controls. The responses to *orally* administered water-soluble disodium phosphate ester of d-alpha-tocopherol did not differ from those of the fat-soluble acetate in subjects with liver disease. However, the results of the latter experiment were inconclusive, since the phosphate ester contained only 85 per cent as much tocopherol, taking differences in molecular weight into account. The increase in plasma tocopherol following administration of either ester bore no significant relationship to the initial fasting plasma tocopherol level or to the degree of hepatic dysfunction.

5. The increases in plasma tocopherol following the intramuscular injection of dl-alpha-tocopherol, either in the form of an oil solution, or as a stabilized emulsion of the acetate, were significantly smaller than those following the *oral* administration of tocopherol esters, both in liver disease and hospitalized control subjects. The peak response after dl-alpha-tocopherol in sesame oil was lower in liver disease subjects than in hospitalized controls, but the difference was of only borderline significance statistically. The emulsion of alpha-tocopherol acetate was absorbed even more slowly than alpha-tocopherol in oil.

6. The results do not warrant definite conclusions regarding alterations in tocopherol metabolism in liver disease. The implications of these observations have been discussed, and the neces-

sity for further investigation along other lines has been stressed.

BIBLIOGRAPHY

1. Brinkhous, K. M., and Warner, E. D., Muscular dystrophy in biliary fistula dogs; possible relationship to vitamin E deficiency. Am. J. Path., 1941, **17**, 81.
2. Greaves, J. D., and Schmidt, C. L. A., Relation of bile to absorption of vitamin E in the rat. Proc. Soc. Exper. Biol. & Med., 1937, **37**, 40.
3. György, P., Relation of dietary factors to liver injury. Tr. Sixth Liver Injury Conference. Josiah Macy, Jr. Found., New York, N. Y., 1947, p. 67.
4. György, P., and Goldblatt, H., Further observations on the production and prevention of dietary hepatic injury in rats. J. Exper. Med., 1949, **89**, 245.
5. Himsworth, H. P., and Lindan, O., Dietetic necrosis of the liver: the influence of alpha-tocopherol. Nature, 1949, **163**, 30.
6. Schwarz, K., Über einen todlichen ernahrungsbedingten Leberschaden und das Vorkommen von Leberschutzstoffen. Ztschr. f. physiol. Chem., 1944, **281**, 101.
7. Schwarz, K., Dietetic hepatic injuries and the mode of action of tocopherol. Ann. New York Acad. Sc., 1949, **52**, 225.
8. Hove, E. L., Interrelation between alpha-tocopherol and protein metabolism. III. The protective effect of vitamin E and certain nitrogenous compounds against CCl_4 poisoning in rats. Arch. Biochem., 1948, **17**, 467.
9. Hove, E. L., Comparison of a fatal tocopherol deficiency disease in rats with the syndrome caused by CCl_4. Ann. New York Acad. Sc., 1949, **52**, 217.
10. Hove, E. L., Interrelation between alpha-tocopherol and protein metabolism: body weight and tooth pigmentation of rats. Proc. Soc. Exper. Biol. & Med., 1946, **63**, 508.
11. Moore, T., The significance of protein in vitamin E deficiency. Ann. New York Acad. Sc., 1949, **52**, 206.
12. Menschik, Z., Munk, M. K., Rogalski, T., Rymaszewski, O., and Szczesniak, T. J., Vitamin E studies on mice with special reference to the distribution and metabolism of lipids. Ann. New York Acad. Sc., 1949, **52**, 94.
13. Mason, K. E., and Filer, L. J., Jr., Interrelationships of dietary fat and tocopherols. J. Am. Oil Chem. Soc., 1947, **24**, 240.
14. Butturini, U., Clinical and experimental studies on vitamin E. Ann. New York Acad. Sc., 1949, **52**, 397.
15. Hickman, K. C. D., Harris, P. L., and Woodside, M. R., Interrelationship of vitamins A and E. Nature, 1942, **150**, 91.
16. Moore, T., The effect of vitamin E deficiency on the vitamin A reserves of the rat. Biochem. J., 1940, **34**, 1321.
17. Steinberg, C. L., A new method of treatment of Dupuytren's contracture, a form of fibrositis. M. Clin. North America, 1946, **30**, 221.
18. Darby, W. J., Cherrington, M. E., and Ruffin, J. M., Plasma tocopherol levels in sprue. Proc. Soc. Exper. Biol. & Med., 1946, **63**, 310.
19. Popper, H., Dubin, A., Steigmann, F., and Hesser, F. P., Plasma tocopherol levels in various pathologic conditions. J. Lab. & Clin. Med., 1949, **34**, 648.
20. Pappenheimer, A. M., and Victor, J., "Ceroid" pigment in human tissues. Am. J. Path., 1946, **22**, 395.
21. Darby, W. J., Ferguson, M. E., Furman, R. H., Lemley, J. M., Ball, C. T., and Meneely, G. R., Plasma tocopherol in health and disease. Ann. New York Acad. Sc., 1949, **52**, 328.
22. Morrione, T. G., Effect of estrogens on the testis in hepatic insufficiency. Arch. Path., 1944, **37**, 39.
23. Rather, L. J., Hepatic cirrhosis and testicular atrophy. Arch. Int. Med., 1947, **80**, 397.
24. Glass, S. J., Edmundson, H. A., and Soll, S. N., Sex hormone changes associated with liver disease. Endocrinology, 1940, **27**, 749.
25. Lloyd, C. W., and Williams, R. H., Endocrine changes associated with Laennec's cirrhosis of the liver. Am. J. Med., 1948, **4**, 315.
26. Klatskin, G., Munson, P. L., and Salter, W. T., Unpublished data.
27. Klinefelter, H. F., Jr., Reifenstein, E. C., Jr., and Albright, F., Syndrome characterized by gynecomastia, aspermatogenesis without A-leydigism and increased excretion of follicle-stimulating hormone. J. Clin. Endocrinol., 1942, **2**, 615.
28. Klatskin, G., and Yesner, R., Factors in the treatment of Laennec's cirrhosis. I. Clinical and histological changes observed during a control period of bed-rest, alcohol withdrawal, and a minimal basic diet. J. Clin. Invest., 1949, **28**, 723.
29. Quaife, M. L., and Harris, P. L., The chemical estimation of tocopherols in blood plasma. J. Biol. Chem., 1944, **156**, 499.
30. Quaife, M. L., and Biehler, R., A simplified hydrogenation technique for the determination of blood plasma tocopherols. J. Biol. Chem., 1945, **159**, 663.
31. Wechsler, I. S., Mayer, G. G., and Sobotka, H., Tocopherol level in serum of normals and patients with amyotrophic lateral sclerosis. Proc. Soc. Exper. Biol. & Med., 1941, **47**, 152.
32. Engel, C., Vitamin E in human nutrition. Ann. New York Acad. Sc., 1949, **52**, 292.
33. Lemley, J. M., Gale, R. G., Furman, R. H., Cherrington, M. E., Darby, W. J., and Meneely, G. R., Plasma tocopherol levels in cardiac patients. Am. Heart J., 1949, **37**, 1029.
34. Steigmann, F., Meyer, K. A., and Popper, H., Se-

vere interference with bile flow in primary hepatitis: its significance in the differential diagnosis of jaundice. Arch. Surg., 1949, **59**, 101.

35. Mackenzie, C. G., and McCollum, E. V., Effect of oral and parenteral administration of vitamin E on creatinuria and symptoms of dystrophic rabbits. Proc. Soc. Exper. Biol. & Med., 1941, **48**, 642.
36. Quaife, M. L., Discussion. Ann. New York Acad. Sc., 1949, **52**, 371.
37. Karrer, P., and Bussmann, G., d,l-alpha-Tocopherol-phosphorsäure-ester. Helvet. chim. acta, 1940, **23**, 1137.
38. Hove, E. L., and Harris, P. L., Relative activity of the tocopherols in curing muscular dystrophy in rabbits. J. Nutrition, 1947, **33**, 95.
39. Eppstein, S. H., and Morgulis, S., Factors influencing the onset and cure of nutritional muscular dystrophy. J. Nutrition, 1942, **23**, 473.
40. Popper, H., Steigmann, F., and Dyniewicz, H. A., Treatment of endogenous hypovitaminemia A in liver disease. Proc. Cent. Soc. Clin. Res., 1947, **20**, 12; *also* J. Lab. & Clin. Med., 1947, **32**, 1403.
41. May, C. D., and Lowe, C. U., The absorption of orally administered emulsified lipid in normal children and in children with steatorrhea. J. Clin. Invest., 1948, **27**, 226.
42. Harris, P. L., Hickman, K. C. D., Jensen, J. L., and Spies, T. D., Survey of the blood plasma levels of vitamin A, carotene, ascorbic acid, and tocopherols of persons in an area of endemic malnutrition. Am. J. Pub. Health, 1946, **36**, 155.
43. Mason, K. E., Distribution of vitamin E in the tissues of the rat. J. Nutrition, 1942, **23**, 71.
44. Mackenzie, C. G., Mackenzie, J. B., and McCollum, E. V., Uncomplicated vitamin E deficiency in the rabbit and its relation to the toxicity of cod liver oil. J. Nutrition, 1941, **21**, 225.
45. Cuthbertson, W. F. J., Ridgeway, R. R., and Drummond, J. C., The fate of tocopherols in the animal body. Biochem. J., 1940, **34**, 34.
46. Quaife, M. L., Swanson, W. J., Dju, M. Y., and Harris, P. L., Vitamin E in foods and tissues. Ann. New York Acad. Sc., 1949, **52**, 300.
47. Harris, P. L., Practical nutritional aspects of vitamin E; introductory remarks. Ann. New York Acad. Sc., 1949, **52**, 240.