The Effects of Experimental Hepatobiliary Disease on Certain Aspects of Tocopherol Metabolism in the Rat

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Research Article

THE EFFECTS OF EXPERIMENTAL HEPATOBILIARY DISEASE ON CERTAIN ASPECTS OF TOCOPHEROL METABOLISM IN THE RAT [1]

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In a previous study (1) it was found that the plasma tocopherol level in patients with hepatocellular disease tended to be low and relatively unresponsive to an oral test dose of tocopheryl acetate. However, repeated doses raised the concentration in a normal manner, while the fraction recovered in the feces was even smaller than in normal subjects (2, 3), suggesting that intestinal absorption was not impaired. One possible explanation for these findings was that the tissue stores of tocopherol were depleted as the consequence of an antecedent dietary deficiency, and that the low concentrations in the feces and the relatively flat tolerance curves were indicative of greater absorption and more rapid transfer of tocopherol from the intestinal tract to the tissues. Consistent with this interpretation was the observation that the plasma levels in liver disease did not differ significantly from those in randomly selected patients with a variety of other disorders in which malnutrition could have been a factor. Since there was no loss of tocopherol during its incubation with feces, aspirated jejunal contents or pure cultures of enteric bacteria (3), it appeared unlikely that the abnormalities noted in patients with hepatocellular damage were due to increased tocopherol degradation in the intestinal tract. However, the report of Rosenkrantz, Milhorat, and Farber (4) that a significant fraction of tocopherol is excreted as tocopherylquinone, an oxidative product which is not detectable in conventional analyses based on the Emmerie-Engel reaction, necessitated further investigation of this possibility. Accordingly, simultaneous determinations of fecal tocopherol and tocopherylquinone were carried out on three-day stool collections before and after a one Gm. oral

test dose of tocopheryl acetate, using a method described by these authors (5). It was found that neither normal nor cirrhotic subjects excreted a significant amount of tocopherylquinone (6), an observation consistent with the impression that the low levels of fecal tocopherol in liver disease were not due to increased degradation in the intestinal tract.

As in the case of hepatocellular disease, patients with partial occlusion of the common bile duct exhibited low levels of plasma tocopherol and relatively flat tolerance curves, and excreted only a small fraction of ingested tocopherol, but, in contrast, showed no increase in plasma concentration when repeated large doses of tocopherol were administered (3). It was difficult to reconcile these findings with either an absorptive defect or a deficiency state leading to greater absorption and more rapid transfer of tocopherol from the intestinal tract to depleted tissue depots. Since Popper, Dubin, Steigmann, and Hesser (7) had reported high levels of plasma tocopherol in both obstructive and hepatocellular jaundice, the possibility was considered that the low output of tocopherol in the feces in these conditions was due to interference with its re-excretion in the bile. This was investigated by measuring the output of tocopherol in T-tube bile from patients with biliary fistulae, and in aspirated duodenal contents of cirrhotic and normal subjects with an occlusive balloon in the jejunum (3). It was found that the amount recovered constituted only a small fraction of the total fecal tocopherol, and did not increase during the absorption of tocopherol from the intestine. Moreover, the excretion in normal and cirrhotic subjects did not differ, so that biliary retention did not appear to be a significant factor in the reduced fecal excretion of tocopherol in liver disease, a conclusion supported by the accompanying low levels of tocopherol found in the

plasma. In contrast to the experience of Popper and his associates (7), no high levels were encountered in either hepatocellular or obstructive jaundice, and there was no correlation between the plasma concentration of tocopherol and that of either bilirubin or cholesterol (3).

The results of these studies on the effects of hepatobiliary disease on the plasma tocopherol level and on the absorption and storage of tocopherol were considered inconclusive, and in some ways contradictory. No doubt some of the factors that could not be controlled or evaluated accurately, such as the nutritional status, the degree of bile stasis, and the severity of the hepatocellular injury, affected the results. It was considered worth while, therefore, to reinvestigate the problem in animals under more uniform conditions. The following is a report of such studies in rats subjected to ligation of the bile duct, bile fistula and acute and chronic carbon tetrachloride-induced hepatic injury.

METHODS

Animals. Male Sprague-Dawley rats, raised on an *ad libitum* diet of Purina Laboratory Chow containing 0.035 mg. of tocopherol per Gm., were subjected to the following procedures when they attained a weight of 200 to 300 Gm.:

1) Ligation of the bile duct. Under Nembutal® anesthesia, the bile duct was doubly ligated and transected as close to the hilum of the liver as possible. Special care was taken to avoid injury to the pancreas. Post-operatively, each animal received 10 ml. of saline subcutaneously to insure adequate hydration.

2) Bile fistula. Under Nembutal® anesthesia, a polyethylene catheter with a bore of 0.58 mm. was introduced into the bile duct and tied in place approximately 1 cm. from the hilum of the liver and proximal to a double ligature occluding the distal segment of the duct. The free end of the catheter was then threaded subcutaneously through a hollow needle to the nape of the neck where it was allowed to drain into a rubber finger cot lying on the back and held in place by a protective plastic shield sutured to the skin, as described by Hoffbauer, Watson, and Schwartz (8). The open end of the finger cot was sealed with a cork through which the catheter entered, while the tip emptied into a length of rubber tubing containing a removable glass plug to permit daily drainage. The water-tight sac thus created was sufficiently large to accommodate a 24 hour bile collection without tension, so that no hyperbilirubinemia ensued. Post-operatively, 10 ml. of saline was administered subcutaneously. Thereafter, normal saline was substituted for the drinking water to compensate for electrolyte losses in the bile. Animals maintained on this regimen tolerated biliary drainage

for the seven day experimental period without apparent ill effects.

3) Acute hepatic injury. Three subcutaneous injections of carbon tetrachloride in doses of 0.15 ml., 0.10 ml., and 0.05 ml. per 100 Gm. of body weight were given at two day intervals to insure the presence of acute hepatic necrosis throughout the experimental period. This was confirmed histologically. A number of animals died, but the survivors used in subsequent studies appeared to be in a good state of health.

4) Chronic hepatic injury and cirrhosis. Carbon tetrachloride was injected subcutaneously at the rate of 0.05 ml. per 100 Gm. of body weight twice weekly for five months. Histological examination at this time revealed the presence of a finely granular cirrhosis. The mortality rate in this group was high, but the surviving animals used in subsequent tocopherol studies appeared healthy, had no signs of jaundice or ascites, and exhibited a reasonably rapid growth rate, attaining an average weight of 438 Gm., representing a gain of approximately 180 Gm.

Tocopherol studies. The acute effects of these procedures on the serum tocopherol concentration were measured daily for a period of one week in samples of blood obtained from a tail vein.

The method of evaluating changes in tocopherol absorption, transport and storage was based on a study of the serum tocopherol responses to test doses of tocopheryl acetate administered either (a) orally in the form of a suspension in dilute alcohol, (b) orally in the form of a stable emulsion, or (c) intramuscularly in the form of a stable emulsion. Serum tocopherol concentration was estimated at 0, 2, 4, 6, 8, 10, 12, 24, 48, 72 and 96 hours, each of the three tolerance tests being carried out in an individual group of six animals. The curves obtained were then analyzed statistically in terms of maximum and mean increase in concentration over the control level, and were compared with each other in each of the four experimental groups, and with those obtained in control animals.

The oral suspension was prepared by vigorously mixing a 10 per cent solution of *dl*-alpha-tocopheryl acetate [3] in 95 per cent ethyl alcohol (w/v) with 40 times its volume of water in a small test tube with the aid of a fine needle and syringe, thus yielding a final alcohol concentration of approximately 2.3 per cent. The required amount was then promptly drawn into a syringe and instilled into the stomach through a metal cannula passed down the esophagus.

The oral emulsion,[4] which was a 3.3 per cent (w/v) stable dispersion of *d*-alpha-tocopheryl acetate in a 10 per cent aqueous solution of Tween 80, was administered in the same fashion. It has been shown that stabilization in the Tweens enhances the absorption of tocopheryl ace-

[3] Obtained from Hoffman-LaRoche, Inc., Nutley, N. J., through the courtesy of Dr. Robert J. Floody.

[4] Obtained from the United States Vitamin Corporation, New York, N. Y., through the courtesy of Dr. Louis Freedman.

tate in normal individuals (9), and, at least in the case of vitamin A, is capable of abolishing the absorptive defect in patients with steatorrhea (10). For that reason it was hoped that a comparison of the tolerance curves obtained with the emulsion and suspension might reveal any impairment of intestinal absorption related to disturbances in bile flow.

The intramuscular preparation [4] was a stabilized 2.5 per cent emulsion of dl-alpha-tocopheryl acetate in water containing 5 per cent Tween 20, 2 per cent procaine HCl and 0.5 per cent chlorbutanol (all values expressed as w/v). Since preparations of this type are known to be absorbed rapidly from muscle (11), study of the serum concentrations following an injection appeared to provide an indirect method for measuring changes in the rate of tocopherol utilization and/or transfer to the tissues.

The tolerance tests were carried out (a) 72 hours after ligation or external drainage of the bile duct to avoid any immediate post-operative effects, and to rid the intestinal tract of previously secreted bile, (b) 48 hours after the initial injection of carbon tetrachloride to coincide with the point of maximum hepatic necrosis; the second injection was given at the same time as the tocopheryl acetate, while the third was administered 48 hours later to perpetuate the hepatic injury throughout the course of the 96 hour observation period, and (c) two weeks after the cessation of a five month series of carbon tetrachloride injections to avoid the complicating effects of acute hepatic injury related to the most recent injections.

In the animals with a fistula the bile was collected daily and analyzed for its tocopherol content.

Other studies. Following completion of the tolerance tests, the animals were sacrificed and examined for evidence of recanalization or rupture of the bile duct in those with ligated ducts, and for evidence of biliary obstruction or leakage in those with a biliary fistula. If any of these complications were found, the experimental results were discarded.

Serum bilirubin determinations were carried out serially in each of the experimental groups to investigate the possible relationship between the serum tocopherol level and bile retention. The same samples were analyzed for total cholesterol, since previous investigators (12) had noted a direct relationship between the concentration of tocopherol and cholesterol in a number of conditions. It should be pointed out that these studies were not carried out in the animals undergoing the tocopherol tolerance tests. Unfortunately, the amount of blood required was too large to permit simultaneous serial analyses of tocopherol, bilirubin and cholesterol in the same animals.

Chemical methods. Serum tocopherol determinations were carried out in triplicate by the micromethod of Quaife, Schrimshaw, and Lowry (13). The three values checked closely, as evidenced by the fact that the standard deviation of the means for all individual samples analyzed was only ± 0.04 mg. per cent.[5] Moreover, parallel analyses of 15 pairs of serum revealed that the results were

[5] $\sqrt{\dfrac{\text{Sum of (deviations from the mean of each sample)}^2}{\text{Sum of (n-1 for each sample)}}}$.

in close agreement with those obtained by the macromethod of Quaife and Biehler (14).

Estimations of serum bilirubin and cholesterol were carried out by microscale modifications of the Malloy-Evelyn (15) and Saifer-Kammerer (16) methods, respectively.

RESULTS

Control studies

The serum tocopherol concentration in rats of identical age and sex and raised on a uniform diet varied over a wide range (Table I). However, the hourly and daily fluctuations in individual animals were relatively small (Table II), suggesting that the variations within the group were not due to postprandial changes in serum concentration related to *ad libitum* feeding.

Following the oral administration of dl-alpha-tocopheryl acetate in the form of an unstable suspension in 2.3 per cent ethanol, the serum tocopherol level rose significantly, reaching a peak in 8 to 12 hours, and returned to the initial concentration, or slightly above, in 24 to 48 hours. The magnitude of the response varied with the amount of tocopherol administered, approaching a ceiling at a dose of 10 mg. of free tocopherol per 100 Gm. of body weight. Presumably, absorption was maximal at this level, so that the 10 mg. dose was employed in all subsequent experiments. In comparing the effects of different preparations and routes of administration, the curve obtained with the oral suspension of tocopheryl acetate was adopted as a standard of reference. To facilitate their comparison, the curves were analyzed statisti-

TABLE I

Serum tocopherol level in rats under varying experimental conditions

Experimental group	No. of animals	Mean ± S.D.*	Range
		mg. %	*mg. %*
Controls	64	0.39 ± 0.23	0.03–1.05
Ligation of bile duct			
72 hours	27	0.61 ± 0.45†	0.00–1.38
7–11 days	24	0.42 ± 0.18	0.00–0.65
Bile fistula—72 hours	18	0.90 ± 0.47†	0.11–1.72
Carbon tetrachloride			
48 hours	24	0.78 ± 0.48†	0.09–1.93
5 months	22	0.46 ± 0.23	0.09–0.98

* S.D. refers to standard deviation.
† Statistically significant differences: ligation of bile duct (72 hours) > controls (p < 0.02); bile fistula > controls (p < 0.01); carbon tetrachloride (48 hours) > controls (p < 0.01).

TABLE II

Fluctuations in serum tocopherol concentration of rats maintained on an ad libitum stock diet

Rat no.	Serial serum tocopherol determinations						Mean
	1	2	3	4	5	6	
			Hourly intervals				
			mg. %				*mg. %*
1	0.54	0.55	0.48	0.50	0.30	0.35	0.45
2	0.55	0.51	0.58	0.44	0.43	0.45	0.49
3	0.29	0.32	0.45	0.32	0.35	0.33	0.34
4	0.48	0.31	0.47	0.31	0.31	0.34	0.37
5	0.32	0.29	0.30	0.16	0.23	0.22	0.25
6	0.28	0.28	0.27	0.26	0.25	0.19	0.26

Standard deviation of the observations in the group* = ±0.07 mg. per cent

			Daily intervals				
7	1.05	0.68	0.68	0.81	0.99	0.86	0.85
8	0.66	0.68	0.75	0.73	1.04	0.77	0.77
9	0.85	0.78	0.69	0.74	0.98	0.86	0.82
10	0.88	0.44	0.56	0.74	0.81	0.76	0.70
11	0.59	0.66	0.65	0.68	1.14	1.02	0.79
12	0.84	0.83	0.86	0.94	0.95	0.88	0.88
13	0.32	0.28	0.18				0.26
14	0.29	0.48	0.40				0.39
15	0.54	0.58	0.45				0.52
16	0.30	0.29	0.44				0.34
17	0.36	0.31	0.47				0.38
18	0.33	0.29	0.53				0.38
19	0.52	0.57	0.59				0.56

Standard deviation of the observations in the group* = ±0.13 mg. per cent

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

cally in terms of the maximum and mean increases in serum tocopherol concentration over 24 and 96 hour periods. The latter were calculated from the areas under the curves. Since the results ob-
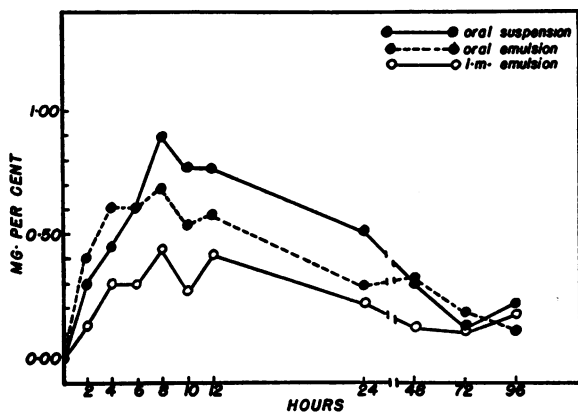

FIG. 1. INCREASE IN SERUM TOCOPHEROL IN RESPONSE TO THE ADMINISTRATION OF TOCOPHERYL ACETATE TO CONTROL RATS

Values shown represent the means for groups of six animals each.

tained by all methods of analysis were in close agreement, only those pertaining to the maximum increase in concentration are presented.

As might be expected in control animals with an intact digestive mechanism, homogenization of the tocopheryl acetate to produce a stable dispersion of microscopic particles did not enhance its rate of absorption from the intestinal tract (Figure 1, Table III). However, it did permit its absorption from muscle, although the rate of absorption, judging from its effect on serum tocopherol, was significantly lower than that in the intestinal tract (Figure 1, Table III).

Biliary obstruction

Following ligation of the bile duct, the serum tocopherol concentration rose transiently, reaching a peak in 72 hours, and returned to the control level by the fifth day (Figure 2). This was not a constant finding, but in the 27 experiments carried out the mean of the concentrations found at 72 hours was significantly higher than that of the

controls, while the mean of the values found between the fifth and eleventh days was within normal limits (Table I). The transient character of the rise in the face of persistent complete biliary obstruction, as evidenced by the progressive increase in serum bilirubin (Figure 2), made it unlikely that it was due to retention of tocopherol normally excreted in the bile. The possibility that it was related to the hypercholesteremia that accompanied it was suggested by the striking similarity between the concentration curves of the two lipids (Figure 2). According to Byers, Friedman, Biggs, and Gunning (17), the hypercholesteremia that follows ligation of the bile duct in the rat is due primarily to an accumulation of cholate in the blood, leading to an increase in the cholesterol-binding capacity of the serum proteins and a consequent retention of cholesterol. Unfortunately, the period of observation in the experiments carried out by these investigators was limited to 72 hours, so that no data are available on the serum cholate level for the subsequent period during

which the serum cholesterol was found to fall both in the present study and that of Chanutin and Ludewig (18). Although the serum cholate concentration tends to remain elevated following bile duct ligation in most animals (19, 20), it is conceivable that the rat is an exception, and that the rise and fall in the concentrations of both cholesterol and tocopherol were due to parallel fluctuations in the cholate level. Since the rat is incapable of synthesizing tocopherol, any increment in the serum must have been derived from either the diet or the tissues. Accordingly, the possibility had to be considered that the fluctuations in serum tocopherol were due to alterations in intestinal absorption, or to changes in the output or uptake of tocopherol by the tissues, and particularly by the liver, which contains one of the largest and most readily mobilized depots of tocopherol (21).

As is evident from Figure 3 and Table III, orally administered tocopheryl acetate in suspension raised the serum tocopherol concentration only slightly. In emulsified form it had a somewhat

TABLE III

Maximum increase in serum tocopherol concentration following the administration of tocopheryl acetate *

| | | Statistically significant differences† | | | | | |
| | | Comparison with control animals | | | Comparison with animals in the same experimental group | | |
Experimental group‡	Mean ± S.D.	Oral suspension	Oral emulsion	I.M. emulsion	Oral suspension	Oral emulsion	I.M. emulsion
	mg. %						
Controls							
Oral suspension	0.98 ± 0.34						
Oral emulsion	0.76 ± 0.13						
I.M. emulsion	0.51 ± 0.16				$p < 0.02$	$p < 0.02$	
Ligated bile duct							
Oral suspension	0.12 ± 0.05	$p < 0.01$					
Oral emulsion	0.32 ± 0.17	$p < 0.01$	$p < 0.01$				
I.M. emulsion§	4.24 ± 1.20	$p < 0.01$		$p < 0.01$	$p < 0.01$	$p < 0.01$	
Bile fistula							
Oral suspension	0.24 ± 0.21	$p < 0.01$					
Oral emulsion	0.76 ± 0.90						
I.M. emulsion§	1.10 ± 0.38			$p < 0.01$	$p < 0.01$		
CCl₄ (acute)							
Oral suspension	0.50 ± 0.44						
Oral emulsion	1.51 ± 0.73		$p < 0.05$			$p < 0.02$	
I.M. emulsion	1.03 ± 0.21			$p < 0.01$		$p < 0.05$	
CCl₄ (chronic)							
Oral suspension	0.61 ± 0.14						
Oral emulsion	1.18 ± 0.63						
I.M. emulsion	0.53 ± 0.33					$p < 0.05$	

* Dose used equivalent to 10 mg. free tocopherol per 100 Gm. body weight.
† Determined by Students' "t" test.
‡ Six animals per group, except: CCl₄ (acute), oral emulsion, seven; CCl₄ (chronic), oral suspension, four.
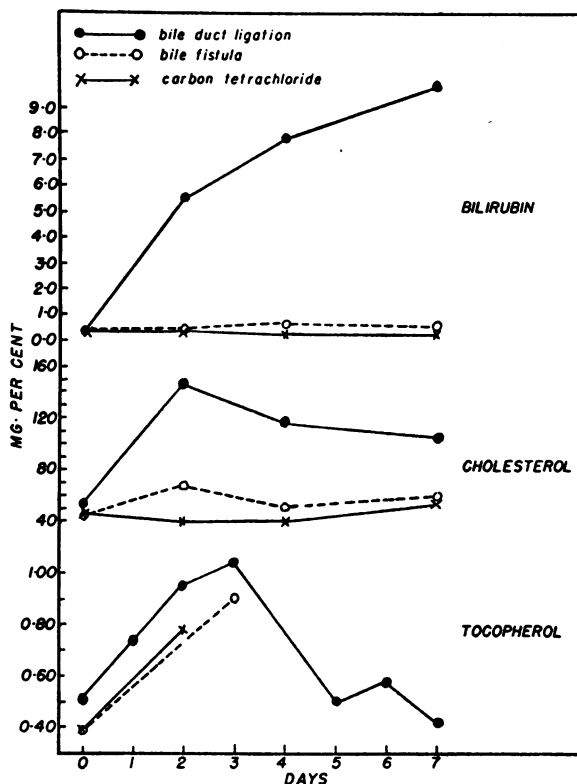§ Difference statistically significant, p < 0.01.

FIG. 2.   CHANGES IN SERUM BILIRUBIN, CHOLESTEROL,
AND TOCOPHEROL FOLLOWING LIGATION OF THE BILE DUCT,
EXTERNAL DRAINAGE OF THE BILE DUCT, OR THE ADMINIS-
TRATION OF CARBON TETRACHLORIDE

Values shown represent the means for groups of six
animals each, except in the case of the serum tocopherol
levels in animals with a bile fistula or acute carbon tetra-
chloride intoxication, which were derived from the data
in Table I.

greater effect, but the difference was not statisti-
cally significant.   Moreover, in neither case was the
response equal to that in the controls, suggesting
that tocopherol absorption from the intestinal tract
was impaired, presumably due to the absence of
bile.   It is highly improbable, therefore, that the
increment in serum tocopherol following ligation
of the bile duct was of dietary origin, particularly
since the diet provided only 0.14 to 0.18 mg. per
100 Gm. of body weight.

In contrast, the intramuscular administration of
tocopheryl acetate produced a striking increase in
serum tocopherol that greatly exceeded the re-
sponse in any of the controls (Figure 3, Table
III).   Since the level remained elevated for a
longer period than the hypercholesteremia, it is
unlikely that a high blood cholate concentration

was a major factor in the retention of tocopherol
in the serum.

To investigate further the possibility that liga-
tion of the bile duct interrupted a normal pathway
of tocopherol excretion, or reduced the capacity of
the liver to store tocopherol, similar studies were
carried out in animals with bile fistula and with
carbon tetrachloride-induced acute hepatic injury.

Bile fistula

The serum tocopherol level rose transiently fol-
lowing the creation of a bile fistula, reaching a
peak in 72 hours (Table I).   Since there was no
accompanying hypercholesteremia (Figure 2), it
is unlikely that this effect was due to an increase
in blood cholate.

Orally administered tocopheryl acetate in sus-
pension raised the serum concentration less than
in the controls (Figure 4, Table III), suggesting
that intestinal absorption was impaired.   How-
ever, the defect appeared to be less severe than in
animals with biliary obstruction since homogeniza-
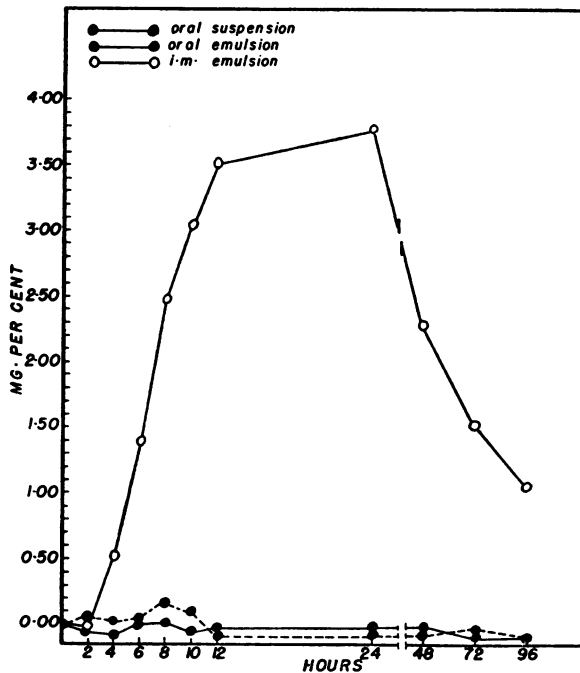tion of the tocopherol in Tween 80, which was in-

FIG. 3.   INCREASE IN SERUM TOCOPHEROL IN RESPONSE
TO THE ADMINISTRATION OF TOCOPHERYL ACETATE 72
HOURS FOLLOWING LIGATION OF THE BILE DUCT

Values shown represent the means for groups of six ani-
mals each.

TABLE IV

*Biliary excretion of tocopherol before and after the administration of tocopheryl acetate **

Tocopheryl acetate preparation	Days before or after tocopheryl acetate administration						
	−3	−2	−1	+1	+2	+3	+4
Tocopherol concentration in bile, mg. % †							
Oral suspension	0.43	0.26	0.25	0.14	0.16	0.20	0.19
Oral emulsion	0.47	0.28	0.23	0.26	0.26	0.24	0.33
I.M. emulsion	0.37	0.50	0.46	0.42	0.41	0.30	0.28
Amount of tocopherol excreted, mg.†							
Oral suspension	0.05	0.03	0.03	0.02	0.02	0.03	0.02
Oral emulsion	0.06	0.03	0.03	0.03	0.03	0.03	0.04
I.M. emulsion	0.03	0.05	0.05	0.05	0.05	0.03	0.03

* Dose used equivalent to 10 mg. free tocopherol per 100 Gm. body weight.
† Mean values for groups of six animals each.

effective in the latter, permitted normal absorption in animals with a bile fistula, as evidenced by a serum response that was equal to that of the controls (Table III). Although ligation and transection of the bile duct were carried out as close to the porta hepatis as possible to avoid injury or obstruction to the pancreatic ducts, which enter the bile duct along most of its length, it is conceivable that the inflammatory reaction and local pressure effects associated with elongation and distension of the proximal occluded segment of the bile duct interfered with pancreatic function, thus compounding the absorptive defect due to the absence of bile.

Emulsified tocopheryl acetate produced a greater rise in serum tocopherol concentration when injected intramuscularly than when administered orally (Figure 4, Table III). This difference could not have been due solely to the more rapid absorption of tocopherol from muscle, since the increase in serum concentration was even larger than that observed following a similar injection in normal animals (Table III). Inasmuch as hypercholesteremia and bile retention could be excluded as factors, a decrease in the uptake of tocopherol by the liver appeared to be an attractive possibility worthy of further investigation.

It is noteworthy that the increase in serum tocopherol following an intramuscular injection of tocopheryl acetate was significantly greater in animals with obstruction of the bile duct than in those with a biliary fistula (Table III). Hypercholesteremia and/or cholate retention could have been responsible for this difference, although the possibility that the mechanism for the uptake of

tocopherol by the liver was more severely impaired under conditions of biliary obstruction could not be excluded. As is evident from the data in Table IV, retention in the serum of tocopherol normally excreted in the bile could not have been a significant factor. Thus, it will be noted that the amount of tocopherol excreted in the bile constituted only a negligible fraction of the intake, and did not increase significantly when large doses of tocopherol were administered either orally or intramuscularly.

Carbon tetrachloride-induced acute hepatic injury

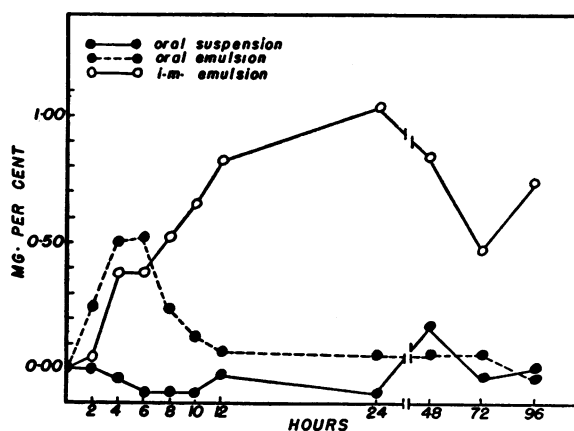At the height of the hepatic injury 48 hours after the administration of carbon tetrachloride,

FIG. 4. INCREASE IN SERUM TOCOPHEROL IN RESPONSE TO THE ADMINISTRATION OF TOCOPHERYL ACETATE 72 HOURS FOLLOWING THE CREATION OF AN EXTERNAL BILE FISTULA

Values shown represent the means for groups of six animals each.
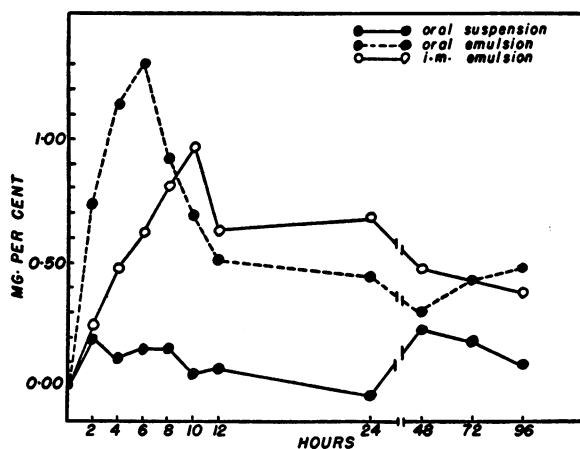
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FIG. 5. INCREASE IN SERUM TOCOPHEROL IN RESPONSE TO THE ADMINISTRATION OF TOCOPHERYL ACETATE 48 HOURS FOLLOWING CARBON TETRACHLORIDE INTOXICATION

Values shown represent the means for groups of six animals each, except in the case of those given the oral emulsion, of which there were seven.

the serum tocopherol concentration was significantly higher than that in the controls (Table I). Since there was no accompanying change in the serum bilirubin or cholesterol (Figure 2), it is unlikely that the increase in serum tocopherol was a retention phenomenon related to the suppression of bile secretion or the accumulation of blood cholate.

Tocopheryl acetate raised the serum tocopherol to a significantly higher level when it was administered as an emulsion in Tween 80 than when it was administered as a suspension in dilute alcohol (Figure 5, Table III), suggesting that intestinal absorption might be impaired. However, this appears unlikely since the response to the suspension was not significantly smaller than that in the controls (Table III). Moreover, it is evident that some other factor must have been involved to account for the fact that the increase in serum tocopherol following the oral or intramuscular administration of emulsified tocopheryl acetate was significantly greater in animals receiving carbon tetrachloride than in the normal controls (Table III). A decrease in the hepatic uptake of tocopherol as a consequence of injury would best explain these findings, and might, in part at least, account for the supranormal responses to intramuscular tocopherol observed in animals with ligation of the bile duct and biliary fistula.

The question arises whether the transient elevations of serum tocopherol observed following ligation of the bile duct, creation of a bile fistula, and the administration of carbon tetrachloride also were due to a decrease in the hepatic uptake of tocopherol entering the serum from the intestinal tract or other tissues. This appears unlikely, since the progressive hepatic damage seen under these conditions might be expected to produce a sustained rather than a transient retention of tocopherol in the serum. The alternative possibility, that hepatic injury resulted in the release of tocopherol from the liver itself, appears more plausible, and is consistent with the observation that the increase in serum tocopherol was independent of bile retention or elevation of the serum cholesterol level. The recent report of Yamamoto, Okuda, and Chow (22) that the administration of carbon tetrachloride increases the release of vitamin B_{12} from the liver and raises its concentration in the serum lends credence to this interpretation.

Carbon tetrachloride-induced chronic hepatic injury and cirrhosis

The mean serum tocopherol concentration in rats given carbon tetrachloride for a period of five months and then allowed to recover for two weeks did not differ significantly from that of the controls (Table I). This suggests that the tissue stores of tocopherol were not depleted and, hence, that chronic hepatic injury and cirrhosis had not affected the absorption of dietary tocopherol, an impression that was confirmed by the results of oral tolerance tests. These revealed that the rise in serum tocopherol following the administration of tocopheryl acetate, in the form of either a suspension or an emulsion, was equal to that in the controls (Figure 6, Table III). Also, judging from the normal serum response to intramuscularly injected tocopheryl acetate (Table III), it is evident that the hepatic uptake of tocopherol was not impaired.

COMMENT

The concentration of tocopherol in the serum depends on the relative rates at which tocopherol enters and leaves the blood. Thus, the level at which equilibrium is established may be influenced by the dietary intake of tocopherol, its rate of ab-

sorption from the intestinal tract, and the capacity of the tissues to store, utilize, and release tocopherol. As far as is known, endogenous synthesis is not a factor, and, as shown previously, the negligible losses of tocopherol in the bile and urine are of little consequence (2). The binding capacity of the serum proteins may affect the amount of tocopherol retained, and may govern the maximum concentration attainable in the serum. However, it is doubtful that a reduction in the binding capacity is ever responsible for low serum concentrations, since even at low levels it is possible to raise the concentration very considerably with an intramuscular injection of tocopherol. Considering the multiplicity of factors involved, it is not surprising that the serum concentration of tocopherol varies over a wide range, even in animals raised under identical conditions.

Since the concentration of tocopherol in the liver is more responsive to changes in the dietary intake than that in any of the other tissues (21), it must be assumed that the liver is capable of storing and mobilizing tocopherol more readily, and, hence, may play a significant role in the regulation of its concentration in the serum. It is reasonable to postulate, therefore, that the transient rise in concentration following experimental hepatocellular injury or surgical manipulation of the bile duct, and the abnormally high levels attained under these conditions following an intramuscular injection of tocopherol, are attributable to alterations in the mechanism for the release and uptake of tocopherol by the liver. In the case of biliary obstruction, an increase in serum binding capacity, possibly related to the retention of cholesterol or cholate, may be a contributory factor.

It is evident from these studies that, in the rat at least, complete interruption of bile flow into the intestinal tract, as a consequence of either ligation or external drainage of the bile duct, interferes with the absorption of tocopherol, while acute or chronic carbon tetrachloride-induced hepatocellular injury does not. No doubt the absence of bile retention in the latter accounts for this difference. Accordingly, it might be anticipated that other forms of hepatic damage that give rise to jaundice would produce a similar absorptive defect. However, on the basis of studies in man, it would appear that incomplete suppression of bile flow does not affect tocopherol absorption. Thus, as previ-
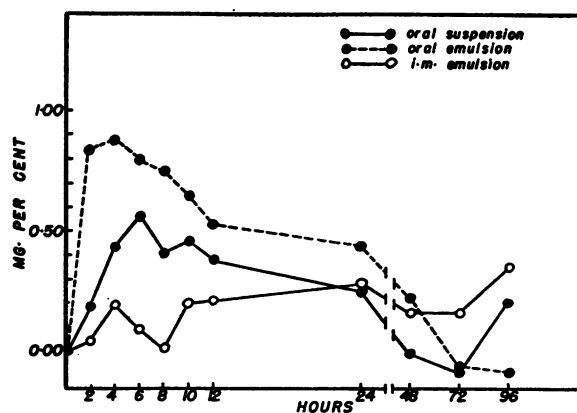

FIG. 6. INCREASE IN SERUM TOCOPHEROL IN RESPONSE TO THE ADMINISTRATION OF TOCOPHERYL ACETATE TO ANIMALS GIVEN BIWEEKLY INJECTIONS OF CARBON TETRACHLORIDE FOR FIVE MONTHS AND THEN ALLOWED TO RECOVER FOR TWO WEEKS

Values shown represent the means for groups of six animals each, except in the case of those given the oral suspension, of which there were only four.

ously reported (2, 3), patients with partial biliary obstruction, acute viral hepatitis, and Laennec's cirrhosis, in whom bile excretion is diminished but not abolished, do not show excessive losses of tocopherol in the feces. Indeed, the fraction of ingested tocopherol excreted is even smaller than in normal subjects. Since there is no evidence of increased tocopherol destruction in the intestinal tract of such individuals, it must be assumed that a larger fraction is absorbed, possibly in response to depletion of the tissue stores resulting from the malnutrition of prolonged illness. The low plasma tocopherol levels and the relatively flat oral tolerance curves observed in such patients are consistent with this interpretation, and suggest that when the depots are depleted the transfer of tocopherol from the intestinal tract to the tissues may be accelerated. The fact that animals maintained in a good nutritional state do not exhibit comparable changes in serum concentration and tocopherol tolerance when subjected to acute or chronic hepatic injury lends support to the concept that hepatocellular disease per se does not alter the serum concentration or intestinal absorption of tocopherol.

It is evident that malnutrition and impairment of absorption due to the absence of bile in the intestinal tract may ultimately lower the serum tocopherol level. However, the retention of bile,

possibly by increasing blood cholesterol or cholate, and hepatocellular injury, by increasing the release and/or decreasing the uptake of tocopherol by the liver, tend to raise the level. Accordingly, the concentration found in any given case of hepato-biliary disease will depend on the interplay of these factors. Although those tending to lower the concentration usually predominate, exceptions are to be expected. No doubt this accounts for the wide range of values reported in liver disease, and for the apparent discrepancy between the findings in different laboratories.

SUMMARY

The effects of biliary obstruction, external drainage of the bile duct, and acute and chronic carbon tetrachloride-induced hepatocellular injury on the serum concentration of tocopherol have been investigated in the rat both before and after the administration of oral and intramuscular test doses of tocopheryl acetate. It has been found that in well nourished rats (a) obstruction or external drainage of the bile duct interferes with the intestinal absorption of tocopherol, while acute hepatic necrosis and postnecrotic cirrhosis do not, (b) the serum tocopherol level tends to rise transiently immediately following acute hepatocellular injury or surgical manipulation of the bile duct, but does not fall below the control level in subsequent short-term periods of observation; similarly, there is no decrease in tocopherol concentration as a consequence of chronic carbon tetrachloride-induced cirrhosis, (c) the increase in serum tocopherol following an intramuscular injection of tocopheryl acetate is significantly greater in animals with biliary obstruction, bile fistula, or acute hepatic necrosis than in normal controls. This phenomenon is not due to the suppression of tocopherol excretion in the bile, nor is it, in the case of bile fistula and acute hepatic necrosis, related to the retention of cholesterol in the serum; however, the latter may be a factor in the case of biliary obstruction, since the rise in serum tocopherol concentration is significantly higher than in either of these other conditions.

These observations suggest that (a) acute hepatic injury, produced either by carbon tetrachloride administration or by surgical manipulation of the bile duct, leads to the release of tocopherol stored in the liver, and reduces the hepatic uptake of tocopherol from the serum, (b) complete interruption of bile flow into the intestinal tract impairs the absorption of tocopherol, (c) hepatocellular injury *per se* does not affect the serum concentration, absorption or tissue uptake of tocopherol, and (d) the factors leading to hypercholesteremia in obstructive jaundice may play a role in the retention of tocopherol in the serum.

The implications of these findings in the interpretation of alterations in tocopherol metabolism observed in human liver disease have been discussed.

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