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Effect of Ferric Chloride on Mobilization of Accumulated Liver Cholesterol, in Mice.* (23347)

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Siperstein, Nichols, and Chaikoff(1) successfully prevented hypercholesterolemia in cockerels by addition of ferric chloride to a diet rich in bile concentrate and cholesterol. They based their work on two well-known facts: 1. Cholesterol absorption in the intestinal tract of the rat is hindered if bile is not present(2); and 2. Ferric chloride precipitates bile salts, *in vitro*. In our previous experiment(3), addition of varying amounts of ferric chloride to cholesterol-rich diets did not prevent the accumulation of exogenous cholesterol in mouse liver. Friedman, Byers, and Gunning(4) showed that in rats excess organ cholesterol is converted to bile acids prior to excretion. It has been shown by Hummel(5) that the mobilization of excess liver cholesterol in mice is prevented by dietary bile acids. It would follow from these observations that any substance which would remove bile acids from the intestinal tract, should increase the rate of cholesterol mobilization. This experiment attempts to answer 2 questions: Does the addition of ferric ions to the diet increase rate of liver cholesterol mobilization in mice with elevated liver cholesterol? Do ferric ions reverse effects of dietary cholic acid?

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Methods. Approximately 200 albino female Webster strain mice, weighing 15-20 g, were placed on a cholesterol-free diet (C.F.D.) for 2 weeks. The animals were then divided into 2 groups: Group A with 40 mice, and Group B with 160 mice. Group A was continued on the basic C.F.D. while Group B diet was supplemented with 1% cholesterol and 0.5% cholic acid. After a 3-week period on this regime, 7 Group A and 10 Group B animals were sacrificed. As shown in Table I, the supplemented diet resulted in a significant build-up of liver cholesterol in the Group B animals, but in no change in either total lipids or phospholipids. After this build-up period, the remaining Group B animals were divided into 4 groups of approximately 40 mice each and placed on the following diets: Group C received the C.F.D.; Group D received the C.F.D. plus 0.5% cholic acid; Group E, C.F.D. plus 1% ferric ions; and Group F, C.F.D. plus 0.5% cholic acid and 1% ferric ions. At weekly intervals about one-fourth of the mice from each group were sacrificed by decapitation. The livers were immediately excised, drained of excess blood, weighed, and quick frozen. The livers were dried over phosphorous pentoxide, weighed, and extracted with a 1:1 mixture of methanol and chloroform. Aliquots of the extracts were

TABLE I. Effect of Dietary Cholic Acid and Cholesterol on Liver Lipid Pattern in Mice in 3 Weeks.

Group	Phospho-lipid	Total lipid	Total cholesterol
	%		
A. Normals	7.74±1.2	36.30±6.42	1.76±.32
B. Developed fatty livers	6.60± .55	35.09±3.11	7.69±.89

± numbers are stand. dev.

used for gravimetric determination of total lipids. Suitable aliquots were used for determination of cholesterol by the Schoenheimer-Sperry method, as modified by Sperry and Webb(6), and for lipid phosphorous according to the method of Fiske and SubbaRow (7). The phosphorous values were multiplied by 25 to obtain phospholipid levels.

Results. Table II summarizes the weekly regression period analyses for phospholipid, total lipid, and cholesterol in each group. Values for total lipids show slight variations; however, these are not statistically significant. While in all groups the phospholipid values varied during the regression period, none of these values were significantly different from those for Group A.

Total cholesterol values are presented in the last column of Table II. It can be seen that the control group (C) regressed to normal (Group A) levels within the 4-week period. A comparison of Groups C and D shows that dietary cholic acid prevented regression of liver cholesterol, and that in fact it caused an increase in cholesterol levels during that period. Dietary ferric ions did not alter the rate of cholesterol regression (Groups C and E). A comparison of Groups D and F indicates that the simultaneous feeding of ferric ions and cholic acid did not reverse the effect of cholic acid alone as observed in Group D. Such a reversal was expected because of the large excess of ferric ions. From the data presented here, it can be concluded that ferric ions do not prevent cholic acid absorption in mice.

Summary. 1. Dietary ferric ions do not increase rate of liver cholesterol mobilization in mice. 2. Mobilization of elevated liver cholesterol is prevented by addition of dietary cholic acid. 3. Evidence is presented which suggests that cholic acid absorption is not

TABLE II. Effect of Ferric Ions and Cholic Acid on Lipid Pattern in Cholesterol Type Fatty Liver.

Group	Time, wk	Phospholipid	Total lipid	Total cholesterol
		%		
A. Normals	1	5.21 ± .75	39.85 ± 5.60	2.29 ± .41
	2	2.86 ± .67	36.01 ± 4.28	1.85 ± .31
	3	4.03 ± 1.06	33.17 ± 2.97	2.07 ± .25
	4	4.52 ± .68	36.99 ± 3.01	2.80 ± .93†
C. R*—Controls	1	4.80 ± .70	44.30 ± 4.84	4.93 ± .85
	2	2.66 ± .77	29.44 ± 2.62	3.65 ± .60
	3	4.93 ± .78	40.24 ± 3.92	2.93 ± .96
	4	3.39 ± .96	37.75 ± 7.36	3.15 ± 1.51†
D. R—Cholic acid	1	4.85 ± .88	33.97 ± 3.78	4.81 ± .72
	2	3.63 ± .48	33.91 ± 4.16	5.19 ± 1.04
	3	5.86 ± 1.24	34.36 ± 4.08	6.54 ± 1.33
	4	3.10 ± .55	34.08 ± 3.64	6.30 ± 1.42†
E. R—Ferric ions	1	4.95 ± .78	34.01 ± 2.90	4.17 ± 1.19
	2	2.85 ± .55	31.73 ± 4.52	3.02 ± 1.00
	3	4.22 ± .72	37.14 ± 4.16	3.40 ± 1.01
	4	3.85 ± .82	40.50 ± 4.77	3.57 ± .47†
F. R—Cholic acid and ferric ions	1	3.62 ± .67	32.45 ± 3.40	7.64 ± 1.22
	2	3.66 ± .45	35.50 ± 3.46	5.52 ± .97
	3	4.63 ± .67	30.13 ± 4.60	4.56 ± 1.66
	4	4.29 ± .81	31.75 ± 1.45	4.86 ± 1.89†

* R = Regression.

† Groups C and A, $P = .65$. Groups C and D, $P < .01$. Groups C and E, $P = .55$. Groups D and F, $P = .13$.

± numbers are stand. dev.

prevented by dietary ferric ions.

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Association of Thyrotropin with γ-Globulin in Myxoedema.* (23348)

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In human serum, thyrotropin has been stated to be associated with β-globulin(1,2). However, Postel(3) demonstrated that when bovine pituitary thyrotropin was added to human serum, and this solution fractionated by electrophoresis in a starch block, thyrotropic activity was present only in the gamma globulin fraction. Using a highly sensitive bioassay for thyrotropin(4), endogenous thyrotropic activity has now been found only in association with gamma globulin.

Methods. The bioassay procedure was

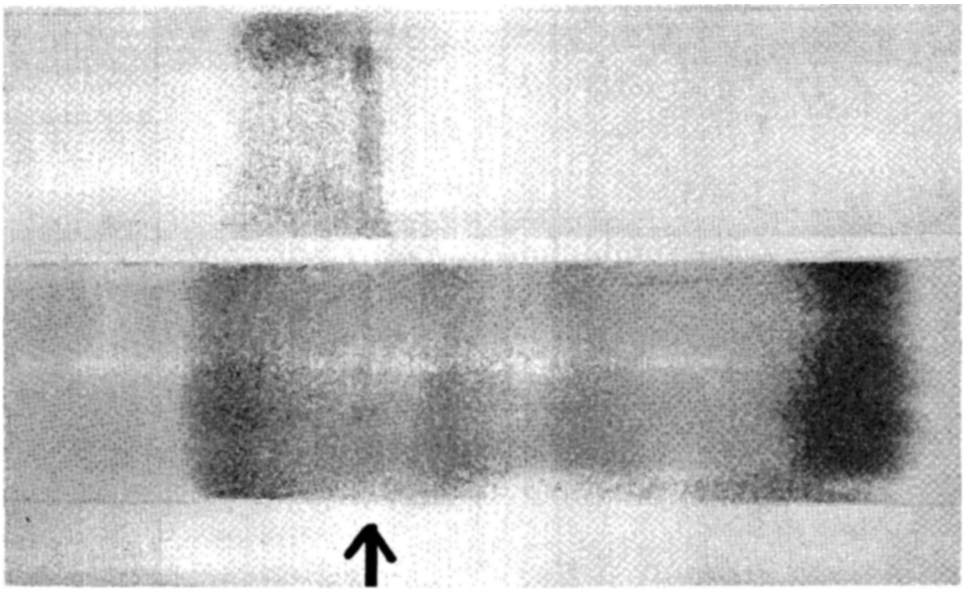

FIG. 1. Identification of a fraction of serum obtained by electrophoresis on a starch block. The gamma globulin fraction (top) and whole serum (bottom) simultaneously electrophoresed on paper at pH 8.6.

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