sult of hypertrophy of the adrenals with resultant decrease in ability of tissue to retain Vit. B_{12}. Further investigation is necessary to ascertain whether "Depinar" would be retained to clinical advantage of these patients.

Conclusions. 1. When rats were administered "Depinar" and soluble Vit. B_{12} both tagged with Co^{60}, the group receiving the tannate derivative of Vit. B_{12} had elevated radioactivity in liver and markedly elevated radioactivity in both kidney and intestines, assumed to be due to B_{12} Co^{60}-tannate complex. 2. This material was likewise excreted much more slowly in urine of both rats and man than aqueous Vit. B_{12}. 3. Serum Vit. B_{12} activity of normal human subjects administered "Depinar" was significantly greater and sustained a much longer time than those receiving soluble Vit. B_{12}. 4. Whether Vit. B_{12} administered as tannate derivative is of any metabolic benefit over the soluble form to patients requiring supplements of this vitamin is yet to be investigated.

1. Davis, R. L., Chow, B. F., PROC. SOC. EXP. BIOL. AND MED., 1951, v77, 218.

2. Skeggs, H. R., Huff, J. W., Wright, L. D., Bosshardt, D. K., *J. Biol. Chem.,* 1958, v176, 1459.

3. Davis, R. L., Chow, B. F., *Science,* 1952, v115, 2987.

4. Harte, Robert A., Chow, B. F., Barrows, Lois, *J. Nutrition,* 1953, v49, 669.

5. Skeggs, H. R., Nepple, H. M., Valentik, K. O., Huff, J. W., Wright, L. D., *J. Biol. Chem.,* 1950, v184, 211.

6. Chow, Bacon F., Lang, C. A., Davis, R. L., Conley, C. L., Ellicott, C. E., *Bull. Johns Hopkins Hosp.,* 1950, v87, 156.

7. Becker, B., Lang, C. A., Chow, B. F., *Am. J. Clin. Nutrition,* 1953, v1, 417.

Received February 18, 1959. P.S.E.B.M., 1959, v101.

Build-Up and Regression of Inhibitory Effects of Cholic Acid on *in vivo* Liver Cholesterol Synthesis.* (24887)

WILLIAM T. BEHER AND GIZELLA D. BAKER

Edsel B. Ford Inst. for Medical Research, Henry Ford Hospital, Detroit, Mich.

That certain bile acids inhibit *in vivo* both synthesis and degradation of rat liver cholesterol has been demonstrated(1,2). Whenever dietary cholic acid inhibited liver cholesterol synthesis, there was a small but very significant increase in liver cholesterol level. It is well-known that increases in liver cholesterol levels are attended by decreases in liver cholesterol synthesis rates(3,4,5). Therefore, does cholic acid directly inhibit rate of liver cholesterol synthesis or does it initiate a change in level of liver cholesterol which in turn brings about the inhibition? The present studies were undertaken to clarify the mechanism of action of cholic acid by determining the order of changes in bile acid, cholesterol and phospholipid concentrations, in relation to changes in *in vivo* liver cholesterol synthesis rates. These studies have been made during early build-up and regression phases of effects of cholic acid.

Methods. Exp. A. Thirty-two Sprague-Dawley female albino rats weighing 250-280 g, were maintained *ad lib.* on basal diet consisting of 97% Rockland rat ration and 3% corn oil for 2-wk observation. The animals were then divided into 2 groups. Controls continued on basal diet *ad lib.*, while treated group received basal diet supplemented with 0.5% cholic acid. During experimental period, controls and treated animals consumed 17.8 $\pm$ 3.10 and 17.4 $\pm$ 1.86 g/day respectively. After dietary periods of 12 hours, 1, 2, and 3 days, 4 control and 4 treated rats received intraperitoneal injections of 50 μc of acetate-1-C^{14}. Six hours later the rats were sacrificed and samples of blood and liver removed for analysis. *Exp. B.* Thirty rats similar to those used in Exp. A were maintained on same basal diet. The animals were

* This investigation supported in part by research grant from Michigan Heart Assn.

TABLE I. Effect of Dietary Cholic Acid on Serum and Liver Total Cholesterol, and on Serum Bile Acid, as a Function of Time.

	Serum total cholesterol		Liver total cholesterol			Serum bile acid		
	Control	Cholic acid*	Control	Cholic acid*		Control	Cholic acid*	
Exp. period	mg %		mg/g†		P	mg %		P
12 hr	99.0 ± 8.6	98.5 ± 9.7	2.47 ± .08	2.62 ± .33	.43	3.80 ± .36	3.90 ± .06	.66
1 day	80.3 ± 9.1	88.2 ±16.6	2.63 ± .08	3.05 ± .13	<.01	3.79 ± .68	3.64 ± .70	.78
2 days	98.3 ± 7.4	83.0 ± 5.3	2.38 ± .11	3.13 ± .48	.025	3.74 ± .55	7.20 ± .34	<.01
3 "	89.9 ±12.0	98.3 ± .6	2.44 ± .12	3.13 ± .44	.021	4.06 ± .45	7.70 ±1.49	.02

* .5% cholic acid in diet. † Wet wt.

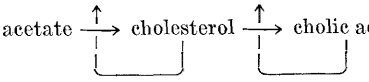
P indicates probability that observed difference between the 2 means is due to chance.

divided into 2 groups. Controls were maintained on basal diet. Treated rats received basal diet supplemented with 0.5% cholic acid for 14 days, after which they were placed on unsupplemented basal diet for the regression study. After regression intervals of 0, 1, 3, 7 and 14 days, 3 control and 3 treated rats were injected intraperitoneally with 50 μc of acetate-1-C^{14} in saline. Six hours following injections, the animals were sacrificed and blood and liver samples removed for assay. In Exp. A and B, serum and liver total cholesterol, serum bile acid, and serum and liver cholesterol-x-C^{14} were determined as previously described (2). In addition, in Exp. A, samples of liver were weighed and dried over P_2O_5. Total lipids were extracted with 1:1 mixture of methanol and chloroform, and determined gravimetrically. Aliquots of extracts were used for total cholesterol(2) and phospholipid(6) determinations.

Results. Exp. A. Relationship of changes in liver composition to decrease liver cholesterol synthesis effected by cholic acid, are presented in Tables I, II, and III. Serum bile acid levels became elevated by second day of experiment (Table I). Previous results(2) show that this level remains constant for an additional 12 days of cholic-acid feeding. Liver total cholesterol (Tables I and II) was significantly elevated in one day and reached a constant level in 2. Liver moisture content and total lipid (Table II) did not change during experiment. On the other hand, total phospholipid decreased significantly during the 3-day build-up period. Incorporation of acetate-1-C^{14} into liver cholesterol (Table III) started to drop during first day of cholic acid treatment. Synthesis rate continued to decrease through 7th day(2), and remained at that level as long as cholic acid feeding was continued. There were no changes in serum total cholesterol concentrations; however, specific activity of cholesterol-x-C^{14} in serum followed the same pattern as in liver.

Exp. B. Tables IV and V give results on regression of dietary cholic acid effects. Serum bile acids (Table IV) remained elevated through third day of regression, while liver cholesterol levels returned to near normal within 3 days. Serum cholesterol levels remained constant throughout experiment. Incorporation of acetate-1-C^{14} into total liver cholesterol increased exponentially during 14-day regression period. Increasing level of serum cholesterol-x-C^{14} paralleled that in liver.

Discussion. In a previous study(2), it was proposed that inhibition of cholesterol synthesis by cholic acid is probably due to a feed-back reaction:



(7)

This mechanism was suggested because observed increases in liver total cholesterol levels caused by dietary cholic acid were accompanied by simultaneous decreases in liver cholesterol synthesis rates. The possibility remained that changes observed in liver total cholesterol concentration were misleading due to decreases in hepatic moisture content. However, since results presented in Table II show that there were *no* significant changes in moisture content, the increases in liver cholesterol are real. This is further confirmed by results obtained from determination of liver total cholesterol on a dry-weight basis.

Another possible explanation for the increase of liver cholesterol and subsequent

TABLE II. Effect of Dietary Cholic Acid on Rat Liver Moisture Content, Total Lipid, Phospholipid, and Total Cholesterol.

Exp. period	Moisture content		Total lipid		Total phospholipid		Total cholesterol		P
	Control	Cholic acid*	Control	Cholic acid*	Control	Cholic acid*	Control	Cholic acid*	
	%		mg/g†						
12 hr	68.7 ± .52	68.3 ± .27	279 ± 14	310 ± 17	63.4 ± 3.9	67.9 ± 7.8	7.59 ± .26	8.41 ± .37	.04
1 day	69.6 ± 1.92	68.4 ± 1.20	315 ± 15	331 ± 20	62.2 ± 3.9	54.5 ± 6.9	7.60 ± .39	8.31 ± .42	.05
2 days	70.0 ± 2.0	69.4 ± .63	326 ± 41	303 ± 25	55.2 ± 7.0	49.0 ± 4.5	7.60 ± .50	9.21 ± .98	.07
3 "	69.3 ± .60	70.1 ± .60	313 ± 19	297 ± 23	60.5 ± 3.1	48.2 ± 7.1	7.92 ± .16	9.30 ± .34	<.01

* .5% cholic acid in diet.
† Dry wt.
P indicates probability that observed difference between the 2 means is due to chance.

TABLE III. Incorporation of Acetate-1-C^{14} into Liver and Serum Total Cholesterol at Different Time Intervals.

Exp. period	Liver total cholesterol		Serum total cholesterol	
	Control	Cholic acid*	Control	Cholic acid*
	counts/min./mg			
12 hr	3,501	2,943	2,916	2,521
1 day	3,195	2,208	2,983	1,876
2 days	2,875	1,595	2,333	1,378
3 "	3,296	1,368	2,717	1,171

* .5% cholic acid in diet.

slowing of turnover rate has been suggested by Friedman and Byers(8,9). They showed that increased plasma cholesterol by infusion of large amounts of cholic acid is preceded by increased plasma phospholipid levels. However, this mechanism can not be applied to our experiments on liver, in which liver phospholipid decreased to some extent while liver cholesterol increased (Table II).

During feeding of cholic acid (Tables I and III), increase of liver cholesterol concentration was paralleled by simultaneous decrease in liver cholesterol-x-C^{14} activity. Bile acid levels did not rise until after first day of treatment; this was to be expected because serum rather than liver bile acids were being measured.

In the regression study (Tables IV and V), levels of liver cholesterol and serum bile acids returned to control levels by the 7th day. Although liver cholesterol levels receded somewhat more rapidly (2nd - 3rd day) than serum bile acid levels (3rd - 7th day), this is probably due to metabolic changes in cholic acid (10,11) which alter its effectiveness in elevating liver cholesterol. The method employed in determining serum bile acids was non-specific, and would measure bile acid conjugates as well as free bile acids.

Along with regressions of serum bile acid and liver cholesterol, there was an exponential increase in liver cholesterol-x-C^{14} activity. Although the most rapid increase in activity corresponded to first 4 to 7 days of study, activities were still slightly low even at the end of 14 days. These findings are similar to those of Taylor *et al.*(5) who found that feeding cholesterol to rats for 7 days decreased the rate of synthesis in liver slices, and

TABLE IV. Regression of Effects of Dietary Cholic Acid on Cholesterol Metabolism in Rat Serum and Liver.

Exp. period (days)	Serum total cholesterol		Liver total cholesterol			Serum bile acid		
	Control	Cholic acid*	Control	Cholic acid*	P	Control	Cholic acid*	P
	mg %		mg/g†			mg %		
0	81.0 ± 4.0	91.1 ± 5.0	2.53 ± .25	3.56 ± .50	.02	3.07 ± .64	6.97 ±1.91	.03
1	92.8 ±11.5	89.1 ± 6.8	2.42 ± .25	2.71 ± .15	.18	3.49 ± .20	4.73 ± .97	.19
3	87.6 ± 7.8	89.0 ±19.7	2.44 ± .20	2.34 ± .13	.51	2.58 ± .17	3.79 ± .55	.02
7	85.9 ± 3.9	88.8 ±13.8	2.34 ± .14	2.35 ± .84	.35	2.79 ± .14	2.74 ± .10	.75
14	95.2 ± 1.5	90.1 ± 4.0	2.43 ± .11	2.43 ± .74	1.00	2.82 ± .39	2.75 ± .65	.90

* .5% cholic acid in diet. † Wet wt.

P indicates probability that observed difference between the 2 means is due to chance.

that recovery to near normal but slightly low rates of synthesis took from 6 to 8 days. Unfortunately these authors made no attempt to correlate decreasing liver cholesterol levels with increasing synthesis rates. If the evidence is considered as a whole, there are indications that the proposed feed-back reaction is operating with cholic acid. Whether other bile acids would act in the same way is open to question and is being investigated.

Summary. A study of sequence of events during initiation and regression of inhibition of cholesterol synthesis by dietary cholic acid was made in rats. In the initiation study, increases of liver cholesterol and serum bile acid levels paralleled decreases of liver cholesterol-x-C^{14} activity. There was a decrease in liver phospholipid during the same time interval. In the regression study, serum bile acid and liver cholesterol returned to control levels more rapidly than the rate of liver cholesterol synthesis. The results suggest that dietary cholic acid initially elevates liver cholesterol, which in turn leads to the inhibition of acetate-1-C^{14} incorporation into liver cholesterol.

TABLE V. Regression of Effects of Dietary Cholic Acid on Incorporation of Acetate-1-C^{14} into Liver and Serum Total Cholesterol.

Exp. period (days)	Liver total cholesterol		Serum total cholesterol	
	Control	Cholic acid*	Control	Cholic acid*
	counts/min./mg			
0	2,361	894	2,366	854
1	3,154	1,135	2,800	1,009
3	2,895	1,659	2,549	1,395
7	3,148	2,126	2,823	1,808
14	3,525	2,715	2,721	2,126

* .5% cholic acid in diet.

1. Beher, W. T., Baker, G. D., PROC. SOC. EXP. BIOL. AND MED., 1958, v98, 892.

2. Beher, W. T., Baker, G. D., Anthony, W. L., *ibid.*, 1959, v100, 3.

3. Tomkins, G. M., Sheppard, H., Chaikoff, I. L., *J. Biol. Chem.*, 1953, v201, 137.

4. Frantz, I. D., Schneider, H. S., Hinkelman, B. T., *ibid.*, 1954, v206, 465.

5. Taylor, C. B., Nelson, L. G., Stumpe, M., Cox, G. E., Tamura, R., *Fed. Proc.*, 1956, v15, 534.

6. Fiske, C. H., SubbaRow, Y., *J. Biol. Chem.*, 1925, v66, 375.

7. Gould, R. G., Cook, R. P., in *Cholesterol Chemistry, Biochemistry and Pathology,* edited by R. P. Cook, Academic Press, N. Y., 1958. p273.

8. Friedman, M., Byers, S. O., *Am. J. Physiol.*, 1957, v188, 337.

9. ———, *ibid.*, 1957, v191, 551.

10. Bremer, J., *Acta Chem. Scand.*, 1956, v10, 56.

11. Norman, A., Sjövall, J., *J. Biol. Chem.*, 1958, v233, 872.

Received February 26, 1959. P.S.E.B.M., 1959, v101.