was no appreciable inhibition of organic binding by iodide alone.

Summary. Rats fed sulfadiazine (SD) or propylthiouracil (PTU) for 14 days developed thyroid hypertrophy. Addition of 1 mg KI daily did not affect the SD-induced goiter but greatly reduced that due to PTU. Neither SD nor PTU alone, in the doses employed, blocked organic binding of iodine completely. Iodide added to SD completely inhibited organic binding of iodine. Iodide added to PTU did not change the degree of inhibition of thyroidal biosynthesis due to PTU alone but increased the absolute amount of thyroxine and triiodothyronine formed.

1. MacKenzie, C. G., MacKenzie, J. B., *Endocrinology*, 1943, v32, 185.
2. MacKenzie, C. G., *ibid.*, 1947, v40, 137.
3. Richards, J. B., Ingbar, S. H., *ibid.*, 1959, v65, 198.
4. Slingerland, D. W., Graham, D. E., Josephs, R. E., Mulvey, P. F., Jr., Trakas, A. P., Yamazaki, E., *ibid.*, 1959, v65, 178.
5. Iino, S., Yamada, T., Greer, M. A., *ibid.*, 1961, v68, 582.
6. Wolff, J., Chaikoff, I. L., *J. Biol. Chem.*, 1948, v174, 555.
7. Galton, V. A., Pitt-Rivers, R., *Endocrinology*, 1959, v64, 835.

Received October 9, 1961. P.S.E.B.M., 1962, v109.

Sterol Ester Formation by Rat Liver Homogenates.* (27143)

LEON SWELL, M. D. LAW AND C. R. TREADWELL
Veterans Administration Center, Martinsburg, W. Va., and Dept. of Biochemistry, School of Medicine, George Washington University, Washington, D. C.

It has been variously suggested that cholesterol esters are synthesized in the liver, intestine, or in other tissues in which they occur(1-5). Cholesterol esterase systems which catalyze the synthesis and hydrolysis of long chain cholesterol esters have been shown to occur in pancreas and intestinal mucosa(6,7). It has also been shown that bile salts are a necessary co-factor for enzymatic activity of those systems(6). Several studies(8-10) have presented evidence for the existence of a hydrolytic cholesterol esterase in rat and chicken liver which catalyzes the hydrolysis of short chain fatty acid esters of cholesterol. The physiological significance of this type of hydrolytic system is uncertain since the major proportion of the cholesterol esters in liver contains long chain fatty acids.

Mukherjee and co-workers(11) reported the presence of a cholesterol esterifying system in rat liver which synthesized cholesterol palmitate from added cholesterol-4-C^{14} and palmityl CoA. A 2-step system was suggested involving first, a reaction between palmitic acid and coenzyme A and secondly, a reaction between cholesterol and palmityl CoA. Fredrickson(12) has described a system containing mouse liver mitochondria plus a heat-stable soluble fraction which esterified considerable quantities of added cholesterol-4-C^{14} during incubation. In this system a considerable part of the total material esterified appeared to be intermediate products in conversion of cholesterol to bile acids. As a part of our studies on metabolism of cholesterol esters, we have been investigating the formation of sterol esters in rat liver homogenates. As an initial approach, the formation of sterol esters from acetate-1-C^{14}, mevalonic acid-2-C^{14} and cholesterol-4-C^{14} was determined. Any differences between endogenously formed cholesterol and added cholesterol would be expected to be apparent in such studies.

Methods and materials. Non-fasted male rats weighing 100-125 g were sacrificed by decapitation, their livers removed and chilled on cracked ice. The livers were homogenized in a buffer medium containing 0.1 M phosphate buffer pH 7.2, 0.03 M nicotinamide,

* This work was supported in part by U. S. Public Health Service Grant.

TABLE I. Sterol and Sterol Ester Synthesis by Rat Liver Homogenates from Acetate-1-C^{14}.

Additions*	cpm recovered as sterol digitonide			
	Free		Ester	
	Exp. 5	Exp. 6	Exp. 5	Exp. 6
None	30	240	<5	20
DPN	3970	3905	540	505
TPN	8435	7885	865	1040
DPN + TPN	9400	6530	1565	820
ATP	365	425	40	65
DPN + oleic acid		20		<5
TPN + oleic acid		10		<5
ATP + CoA + oleic acid		20		<5
DPN + TPN + ATP + CoA	260	1150	30	265
DPN + TPN + ATP + CoA + oleic acid		<5		<5

* Complete system had 5 μm ATP, 5 μm DPN, 5 μm TPN, 1 mg CoA, 7.0 mg oleic acid (added in .1 cc acetone), 10 μm acetate-1-C^{14} (1.5×10^6 cpm), 1 ml liver homogenate; made to vol of 2.5 ml with phosphate buffer medium; incubation time 2 hr; duplicate incubation flasks.

0.004 M magnesium chloride, 0.01 M glutathione as described by Frantz and Bucher (13). The homogenate was centrifuged for 10 mintues at 500 $\times$ g to eliminate tissue fragment cells and nuclei. One ml of this homogenate was added to each incubation flask and the radioactive substrates and cofactors added as indicated in the Tables. Incubation was carried out in an atmosphere of 95% O_2 – 5% CO_2 for 2 hours in a Dubnoff Incu-Shaker. The contents of the flasks were then poured into 10 volumes of 1:1 acetone-alcohol and lipid extracts prepared as previously described(14). Free and esterified sterol fractions were isolated as the digitonide(14). The sterol digitonides were cleaved with pyridine and the sterols reprecipitated with digitonin. The purified sterol digitonides were dissolved in 2:1 chloroform-methanol and plated directly on concentric copper planchettes and C^{14}-activity determined in a gas flow counter. There was considerable variation in activity in the different homogenates, as has been reported by Bucher *et al.*(15), but duplicate incubations of the same preparation agreed within $\pm$ 5%.

Results. Acetate-1-C^{14} (Table I). Several typical experiments with acetate-1-C^{14} are shown in Table I. Without the addition of co-factors, there was incorporation of acetate-1-C^{14} (particularly in Exp. 6) into free and esterified sterol. Addition of DPN, TPN, or DPN and TPN greatly stimulated incorporation of acetate-1-C^{14} into both free and esterified sterol. The proportion of total C^{14}-sterol present as esterified C^{14}-sterol in those systems was 6-12%. Addition of oleic acid to several of the homogenate systems greatly inhibited formation of both free and esterified sterol from acetate-1-C^{14}.

Mevalonic acid-2-C^{14} (Table II). Demonstrable synthesis of both free and esterified sterol occurred from mevalonic acid-2-C^{14} without addition of cofactors. As with acetate-1-C^{14}, the greatest stimulation of sterol synthesis occurred when DPN and TPN were added. Other cofactors such as ATP, DPN, TPN and CoA in several combinations also stimulated sterol synthesis, but not as well as DPN and TPN. Addition of oleic acid to the homogenate systems markedly inhibited sterol synthesis.

Cholesterol-4-C^{14} (Table III). Cholesterol-4-C^{14} was added as a tracer to the homogenate system to label the reactions occurring without influencing chemical equilibria. Esterification of cholesterol-4-C^{14} occurred with no additions to the homogenate. Addition of DPN, TPN, ATP and CoA, singly and in combinations, stimulated conversion of cholesterol-4-C^{14} to C^{14}-sterol ester more than that of the system with no ad-

TABLE II. Sterol and Sterol Ester Synthesis by Rat Liver Homogenates from Mevalonic Acid-2-C^{14}.

Additions*	cpm recovered as sterol digitonide	
	Free	Ester
None	1053	73
DPN	11750	1018
TPN	19825	1455
DPN + TPN	19355	1075
ATP	750	70
DPN + oleic acid	150	<5
TPN + oleic acid	250	43
ATP + CoA	330	63
ATP + CoA + oleic acid	<5	<5
DPN + TPN + ATP + CoA	4715	100
Idem + oleic acid	120	20

* See Table I for amounts of cofactors added; mevalonic acid-2-C^{14} (1.54×10^5 cpm) was added to each flask.

TABLE III. Esterification of Cholesterol-4-C^{14} by Rat Liver Homogenates.

Additions*	cpm × 10^2 recovered as sterol digitonide			
	Free		Ester	
	Exp. 7	Exp. 8	Exp. 7	Exp. 8
None	1970	1882	99.4	103.3
DPN	1777	1706	207.2	185.8
TPN	1824	1615	165.8	157.7
DPN + TPN	1542	1462	284.0	174.6
ATP	1713	1578	157.0	136.2
ATP + CoA		1692		163.0
ATP + CoA + oleic acid		1624		62.3
DPN + oleic acid	1758	1744	104.9	74.1
DPN + TPN + ATP + CoA + oleic acid	1736	1775	109.5	58.6

* See Table I for amounts of cofactors added; cholesterol-4-C^{14} (2.2×10^5 cpm) was added to each flask.

dition. The greatest stimulation occurred in the systems with DPN and DPN plus TPN. When oleic acid was added to any of the above systems no stimulation of cholesterol-4-C^{14} esterification occurred beyond that of the control and in several cases it actually inhibited esterification.

Discussion. Our results have demonstrated that rat liver homogenates form sterol esters from acetate-1-C^{14}, mevalonic acid-2-C^{14} and cholesterol-4-C^{14}. Addition of cofactors which stimulated the synthesis of free sterol from acetate-1-C^{14} and mevalonic acid-2-C^{14} correspondingly increased the formation of sterol esters; the systems with added DPN and TPN were most active. It is known that these cofactors are involved in synthesis of cholesterol from acetate and that TPNH is required for hydroxylation of the sterol nucleus to form bile acids, but it has not been suggested that they are directly involved in the esterification reaction; presumably, fatty acid acyl CoA or some other fatty acid donor participates in the esterification reaction. It is possible that when free sterol synthesis is stimulated a corresponding increase in the formation of sterol esters and other products (bile acids) must occur to prevent the accumulation of free sterol. Thus far, no functional role for liver cholesterol esters has been demonstrated, but they may be an intermediate in the biosynthetic pathway to bile acids(12).

The present data obtained with cholesterol-4-C^{14} are not in complete agreement with the findings of Mukherjee *et al.*(11) since considerable esterification occurred in the absence of any added cofactors. Also, when ATP, CoA and oleic acid were included in the incubation medium no stimulation of cholesterol esterification occurred. However, there may have been adequate amounts of preformed fatty acid acyl CoA or some other fatty acid donor in the present homogenate system or the differences might be attributed to experimental conditions. Further studies are needed to determine what cellular fraction or fractions are associated with the esterification reaction.

An interesting finding was that added oleic acid markedly inhibited the synthesis of free and esterified sterol from acetate-1-C^{14} and mevalonic acid-2-C^{14}. Fluck and Pritham (16) have also observed an inhibition of sterol synthesis in the presence of added fatty acids; the greatest inhibition occurred with the short chain fatty acids. The inhibition of sterol synthesis may have been due to removal of necessary cofactors by stimulation of other pathways. Also, the failure to observe stimulation, and in some cases inhibition, of esterification of cholesterol-4-C^{14} by oleic acid suggests that free fatty acid may not be directly involved in the esterification reaction.

Summary. The formation of sterol esters from acetate-1-C^{14}, mevalonic acid-2-C^{14} and cholesterol-4-C^{14} by whole rat liver homogenates has been investigated. Labeled sterol esters were formed from the above substrates without addition of cofactors. The greatest synthesis of free and esterified sterols from acetate-1-C^{14} and mevalonic acid-2-C^{14} occurred in systems with DPN and/or TPN. Oleic acid markedly inhibited the synthesis of both free and esterified sterols. When the synthesis of free sterol was stimulated corresponding stimulation of esterified sterol formation also occurred. The greatest esterification of cholesterol-4-C^{14} occurred when DPN, TPN, ATP and CoA in various combinations were added to the homogenate. Addi-

tion of oleic acid did not stimulate esterification of cholesterol. The significance of these findings is discussed.

1. Friedman, M., Byers, S. O., St. George, S., *Am. J. Physiol.*, 1956, v184, 141.
2. Friedman, M., Byers, S. O., *J. Clin. Invest.*, 1955, v34, 1369.
3. Harper, P. V., Jr., Neal, W. B., Jr., Hlavacek, G. R., *Metabolism*, 1953, v2, 69.
4. Swell, L., Trout, E. C., Jr., Field, H., Jr., Treadwell, C. R., *J. Biol. Chem.*, 1958, v230, 631.
5. Swell, L., Law, M. D., Field, H., Jr., Treadwell, C. R., *ibid.*, 1960, v235, 1960.
6. Swell, L., Treadwell, C. R., *ibid.*, 1950, v182, 479.
7. Swell, L., Byron, J. E., Treadwell, C. R., *ibid.*, 1950, v186, 543.
8. Byron, J. E., Wood, W. A., Treadwell, C. R., *ibid.*, 1953, v205, 483.
9. Schotz, M. C., Rice, L. I., Alfin-Slater, R. B., *ibid.*, 1954, v207, 665.
10. Krishnamurthy, S., Seshadri Sastry, P., Ganguly, J., *Arch. Biochem. Biophys.*, 1958, v75, 6.
11. Mukherjee, S., Kunitake, G., Alfin-Slater, R. B., *J. Biol. Chem.*, 1958, v230, 91.
12. Fredrickson, D. S., *ibid.*, 1956, v222, 109.
13. Frantz, I. D., Jr., Bucher, N. L. R., *ibid.*, 1954, v206, 471.
14. Swell, L., Trout, E. C., Jr., Hopper, J. R., Field, H., Jr., Treadwell, C. R., *ibid.*, 1958, v232, 1.
15. Bucher, N. L. R., McGarrahan, K., Gould, E., Loud, A. V., *ibid.*, 1959, v234, 262.
16. Fluck, E. R., Pritham, G. H., *Metabolism*, 1961, v10, 444.

Received October 9, 1961. P.S.E.B.M., 1962, v109.

Vitamin B_{12} Unsaturated Binding Capacity of Sera from Various Animals.* (27144)

HAROLD L. ROSENTHAL AND SHIRLEY AUSTIN
Dept. of Physiological Chemistry, Washington University School of Dentistry, St. Louis, Mo.

The native vitamin B_{12} content of serum varies widely among animal species(1-4) and may range from 0.04 mμg/ml in alligator serum to 31 mμg/ml in rabbit serum. Other reports(5-7) have shown that the native vitamin is non-dialyzable and is bound to serum proteins. However, serum is apparently unsaturated and will bind additional cyanocobalamin added *in vitro*. This phenomenon has been termed the serum "unsaturated B_{12} binding capacity" or UBBC by Miller and Sullivan(8).

Gregory and Holdsworth(2) have recently reported that the UBBC of chicken serum is 1100 mμg/ml as compared with that of human serum (2 mμg/ml). Furthermore, it has been shown that the UBBC of serum from patients with chronic myelogenous leukemia is also elevated(8-10). These results suggest a relationship between high concentrations of nucleated blood cells and the UBBC of serum, and it was of interest to determine the UBBC of serum from a wide variety of animal species in order to test this possibility.

Materials and methods. Sera from many animal species were maintained frozen until analyzed by an exhaustive dialysis procedure previously described by Rosenthal *et al.*(10). In brief, 0.3 ml of sample was placed in visking tubing containing 7 ml of Co^{60} cyanocobalamin ($Co^{60}B_{12}$) or Co^{60} dimethylbenzimidazolyl cobamide coenzyme (DMBC) in M/10 phosphate buffer at pH 7.4. Samples with very high UBBC were diluted in saline. In all cases, a 2-3-fold excess of radioactive material was available for binding to serum pro-

* We are grateful to George J. Ridgeway, U. S. Fish and Wildlife Service, Seattle, Wash.; Carl J. Sindermann, U. S. Bureau Commercial Fisheries, Boothbay Harbor, Maine; Arthur M. Phillips, U. S. Fish and Wildlife Service, Cortland, N. Y.; Carl O. Schweidenback, Marine Biol. Lab., Woods Hole, Mass.; Harold S. Olcott, Univ. of California, Berkeley; L. Angelone and F. Orrego, Washington Univ., St. Louis, Mo. and R. A. Coulson, Louisiana State Univ., New Orleans, La. for many of the samples used in this study. We are also grateful to N. S. Ritter, Merck Sharp and Dohme, Rahway, N. J. and D. Perlman, Squibb and Co., New Brunswick, N. J. for generous supplies of Co^{60} cyanocobalamin and Co^{60} 5,6 - dimethylbenzimidazolyl cobamide coenzyme respectively. This study was supported by grant from Am. Cancer Soc.