Short Communications and Preliminary Notes

EFFECT OF COENZYME A ON CHOLESTEROL FORMATION BY RAT LIVER HOMOGENATES

by

B. B. MIGICOVSKY AND D. M. GREENBERG

Division of Chemistry, Science Service, Canada Department of Agriculture, Ottawa (Canada) and Department of Physiological Chemistry, School of Medicine, University of California, Berkeley, California (U.S.A.)

The formation of cholesterol from acetate by a cell-free adult rat liver homogenate system has been demonstrated by BUCHER[1] and RABINOWITZ AND GURIN[2] who prepared a soluble system from the BUCHER preparation.

A significant feature of the BUCHER system is the fact that when grinding of the liver was prolonged or carried out in a tight fitting homogenizer, the resultant supernate was inactive with respect to cholesterol formation. Investigation of the supernate from thoroughly homogenized livers revealed that its inactivity lay in the presence of an inhibitory factor or factors. This is shown in Table I. As a result of this feature successive preparations of active supernate varied greatly in activity.

TABLE I

EFFECT OF SUPERNATE FROM THOROUGHLY HOMOGENIZED LIVER (I.S.) ON ACETATE* INCORPORATION INTO CHOLESTEROL

Each flask contained 1 ml of supernate from loosely homogenized liver centrifuged for 5 minutes at 2000 r.p.m., 1.3 mg ATP, 3.8 mg DPN, 0.263 ml ^{14}C-acetate solution and buffer to equalize the volume in all flasks to 2.46 ml. The gas phase was O_2, incubation was carried out for three hours at 37° C with constant shaking. Five mg carrier cholesterol was added to each flask prior to saponification. Results are expressed as micromoles acetate incorporated into the total cholesterol times 10^3. This value is equivalent to $\frac{\text{c.p.m./mg digitonide}}{\text{c.p.m./micromole acetate}} \times 20$. The factor of 20 is the cholesterol digitonide equivalent of 5 mg cholesterol.

Exp. No.	*Addition to active system*	*μM acetate incorporated*
1	none	16.1
	I.S. — 0.5 ml	5.2
2	none	1.4
	I.S. — 1.0 ml	0.2
3	none	2.5
	I.S. — 1.0 ml	0.2
4	none	2.6
	I.S. — 0.5 ml	0.4
	boiled liver juice — 0.5 ml	6.6
	boiled liver juice — 0.5 ml plus I.S. — 0.5 ml	0.9

* 1.6 micromoles containing $2.15 \cdot 10^6$ c.p.m. was employed throughout.

The experiments herein described are a preliminary investigation of the role of coenzyme A in cholesterol synthesis. BLOCH[3] indicated the necessity of "Active acetate" now known to be acetyl CoA for sterol synthesis by animals. KLEIN[4] demonstrated the need for coenzyme A by yeast for ergosterol synthesis.

In this study the BUCHER[1] system was employed throughout. Cholesterol was isolated as the digitonide according to the method described by RABINOVITZ AND GREENBERG[5]. The digitonide was plated on paper and counted in a gas flow counter. Decomposition of the digitonide with pyridine and reprecipitation did not alter the specific activity.

Table II shows the results of several experiments. They indicate that coenzyme A behaves as an inhibitor when present in relatively high concentration. In the presence of relatively low acetate concentration a small amount of coenzyme A inhibits cholesterol formation or exerts no effect. When the acetate concentration is raised, the same amount of coenzyme A increases the degree of cholesterol formation.

TABLE II

EFFECT OF COENZYME A* ON ACETATE INCORPORATION INTO CHOLESTEROL

Exp. No.	*Addition to active system*	*Amount of acetate micromoles*					
		0.61	1.6	6.6	11.6	21.6	41.6
5	none		16.1				
	CoA — 1.0 mg		0.8				
6	none		12.0	20.7	20.6	23.1	19.0
	CoA — 0.06 mg			16.3	23.8	34.3	33.0
7	none	12.5	30.4	70.2	76.0	64.9	
	CoA — 0.05 mg	11.0	29.9	85.3	84.3	123.5	
	CoA — 0.10 mg		25.4	83.0	113.2	108.1	
8	none		5.1		5.9		
	CoA — 0.10 mg		1.8		9.5		
	CoA — 0.40 mg		0.5		1.9		
9	none		5.2		8.6		
	CoA — 0.10 mg		5.3		18.2		
	CoA — 0.40 mg		3.0		19.3		

* Product of Pabst

It appears that coenzyme A can be shown to be an activating factor for cholesterol synthesis by homogenate systems, conditional upon the proper concentrations of acetate and cofactor.

A possible explanation of this behaviour lies in the fact that coenzyme A plays a significant role in several of the alternate metabolic routes for acetate and thus alters the competitive ability of the cholesterol system for the available substrate. Experiments to substantiate the possible parallelism between inactive supernate and CoA are in progress.

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