COMMUNICATIONS TO THE EDITOR

THE FORMATION OF RADIOACTIVE CHOLESTEROL AND FATTY ACIDS FROM C^{14}-LABELED ACETATE BY RAT LIVER HOMOGENATES[1]

Sir:

The formation of cholesterol from acetate has been demonstrated repeatedly in living animals[2] and surviving tissue slices.[3,4,5] Since whole cells are impermeable to many necessary intermediates and other substances that might be added to or removed from an incubation mixture, the development of an effective cell-free system seems essential for more detailed study of the mechanisms involved.

In the present study homogenates derived from adult rat liver were found to be capable of incorporating C^{14}-acetate into cholesterol. The livers were ground for 30 seconds in a very loose-fitting chilled glass homogenizer containing 2.5 volumes of suspending medium. This treatment was insufficient to disrupt all of the tissue, but unbroken cells and tissue debris were eliminated by low speed centrifugation (350 × *g* at 40° for 5 minutes). All preparations were examined under the phase contrast microscope. No intact hepatic cells were seen; the material consisted principally of cytoplasmic granules and fat droplets with an occasional erythrocyte or free hepatic nucleus.

The importance of preparing the homogenate in this manner should be emphasized. When grinding was prolonged or a tight-fitting homogenizer employed, the incorporation fell to near zero.

The results of our experiments with different concentrations of labeled acetate are shown in Table I. The incorporation was equivalent to that obtained with slices incubated with the same amount of acetate.[6]

Radioactive fatty acids could also be recovered from these preparations when α-ketoglutarate (100 micromoles/flask) was added. The specific activity of the fatty acids was of the order of 300–400 counts per minute per milligram, whereas in the absence of added substrate it was only one tenth as high. A similar effect of substrate on fatty acid formation has been reported with liver slices in which the addition of pyruvate produced a threefold increase.[7] In the present study, added α-ketoglutarate had little effect upon the rate of cholesterol synthesis, in contrast to its marked influence upon fatty acid synthesis. Both cholesterol and fatty acid formation were abolished by incubation under nitrogen.

The biosynthesis of fatty acids from acetate has previously been obtained with liver homogenates from pigeons[8] but not from other species. While cholesterol synthesis has heretofore consistently failed to take place in homogenates of adult tissue,[5,9] it has recently been reported to occur in preparations of fetal rat liver, which were free of intact hepatic cells but not of erythropoietic cells[10]; the maximal incorporation was 0.45% of 1.6 micromoles of C^{14}-acetate, or 0.0029 microgram atom of acetate carboxyl carbon incorporated per gram of tissue per hour. The optimum conditions for our system have not yet been determined, but values have been obtained which greatly exceed those reported for fetal liver homogenates and which are equivalent to those obtained with slices of the original tissue.

TABLE I

CHOLESTEROL[a] SYNTHESIS FROM C^{14}-LABELED ACETATE BY RAT LIVER HOMOGENATES[b]

Expt.	Radioactive acetate added per flask: μmoles	Total c.p.m.	% of total c.p.m. incorporated	μ atoms acetate carbon incorporated[c]
20	0.012	1.77×10^4	2.0	0.0003
21	.012	1.77×10^4	1.4	.0002
21	.012	1.77×10^4	2.5	.0004
23	.012	1.77×10^4	1.5	.0002
23	.177	2.58×10^5	2.1	.004
24	.177	2.58×10^5	2.9	.006
23	2.00	2.58×10^5	2.8	.06
23	18.4	2.58×10^5	1.4	.27
24	18.4	2.58×10^5	2.3	.46
24	27.4	2.58×10^5	1.5	.48
24	36.6	2.58×10^5	1.4	.55

[a] Dr. Ivan D. Frantz, Jr., of the Cardiovascular Research Laboratory of the Massachusetts General Hospital has very kindly run a sample of our cholesterol through the dibromide twice with no detectable decline in specific activity. The details of his method and findings will be published in a separate communication. [b] The homogenizing medium was composed of potassium phosphate buffer *p*H 7.4, 0.044 *M*; nicotinamide, 0.028 *M*; magnesium chloride, 0.007 *M*; and sucrose, 0.126 *M*. The incubation mixture contained 2 ml. of the homogenate supernatant, 0.0008 molar AMP or ATP, 0.0015 molar DPN (65% pure) and sodium acetate-1-C^{14} as indicated. The final volume was 2.5–2.7 ml. Incubation was carried out under 100% O_2 at 37° for 3 hours. The cholesterol was extracted with petroleum ether after alkaline hydrolysis, and precipitated as the digitonide following the addition of carrier cholesterol. The petroleum ether soluble material extracted from the hydrolysate after acidification constituted the fatty acid fraction. [c] Expressed as microgram atoms of acetate carboxyl carbon incorporated into cholesterol per gram of tissue per hour. This value = μmoles of acetate added × % of total c.p.m. incorporated in 3 hours × 1/3 × (1/g. of fresh liver per flask).

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