Arterial Synthesis of Cholesterol in vitro from Labeled Acetate

Various studies, with isotopes, of the synthesis of cholesterol by living tissues have shown that the liver is a principal site of synthesis (*1*), but that almost all tissues possess the ability to some extent (*2*). Recent work with tissue homogenates indicates that the synthesis of cholesterol does not require the intact cell (*3, 4*). The present investigation was undertaken to study cholesterol synthesis *in vitro* from C^{14}-labeled acetate by aorta minces.

The aortas of male and female Broad-Breasted Bronze turkeys, approximately 7 mo old, were used. Hog aortas were also used in some preliminary experiments. The excised aortas were ground approximately 1 min in a Micro Model Latapie Grinder. From 2 to 4 g (average, 3 g) of moist tissue was ground with 50 ml of the buffer developed by Bucher *et al.* (*3, 5*).

Various concentrations of C^{14}-(carboxyl) labeled sodium acetate were added to the tissue minces. Controls (buffer plus radioactive acetate, and buffer plus aorta) were treated in the same way as the samples. In three experiments, slices of aorta were used. All samples were incubated at 37°C for 24 hr. After incubation the cholesterol was extracted and precipitated as the digitonide by the method of Siperstein *et al.* (*6*). The precipitates were thoroughly washed to remove all unused radioactive acetate. Counts were made by placing the filter paper carrying the dried cholesteryl digitonide between two metal plates, the upper one having a hole slightly smaller than the diameter of the paper. The plates were always placed at the same distance from the window of the Geiger-Müller counter.

The data in Table 1 indicate that C^{14}-labeled sodium acetate can be converted into cholesterol by minced turkey aorta, for a significant fraction of the radioactive acetate was recovered in the form of cholesteryl digitonide. The amount of cholesterol synthesized—as expressed in terms of the weight of the digitonide, the total counts per minute, and the counts per minute, per milligram of carbon—varied directly with the weight of aorta and with the concentration of acetate added. The percentage conversion (counts per minute of digitonide as a percentage of total counts per minute of acetate added), however, varied inversely with the amount of acetate, especially in the lower ranges of acetate concentration.

Table 1. Cholesterol synthesis from C^{14}-labeled acetate by turkey aorta minces (experiments 1 to 16) and slices (experiments 17 to 19). The average weight of aorta minces was 3 g; of slices, 3.3 g. Samples were counted 10 min.

Expt.	Sex	Concn. of radioactive acetate added (μmole)	Recovered cholesterol*		Percentage of total counts per min converted	"New" cholesterol synthesized from acetate (μg)
			Digitonide (mg)	10^3 counts/min mg C		
1	F	20	8.8	14.6	0.19	0.95
2	F	15	6.6	11.5	0.12	0.46
3	F	10	4.8	12.0	0.18	0.46
4	F	8	5.1	10.6	0.20	0.41
5	F	6	4.8	11.4	0.28	0.43
6	F	4	7.2	7.4	0.40	0.41
7	F	2	7.2	7.7	0.84	0.43
8	F	1	4.8	8.8	1.29	0.33
9	F	0.8	4.4	8.0	1.32	0.27
10	F	0.6	5.5	5.1	1.42	0.22
11	F	0.4	7.3	3.4	0.18	0.08
12	F	0.2	6.3	3.2	3.03	1.55
13	M	0.08	5.0	2.2	4.36	0.09
14	M	0.06	5.8	2.4	7.07	0.11
15	M	0.04	4.8	0.8	2.74	0.03
16	M	0.02	0.53	4.5	3.66	0.02
17	M	0.6	4.4	6.4	1.41	0.21
18	M	0.4	3.1	9.0	2.11	0.22
19	M	0.2	0.90	2.1	0.29	0.02
1–16 avg.		3.06	5.7	5.8	0.31	0.23
17–19 avg.	M	0.4	2.8	6.7	1.43	0.15
10–12 avg.	F	0.4	6.4	2.6	3.63	0.38
Controls		0.04	0.3			
Controls		0.40	0.4			
Controls		1.00	0.3			
Controls		0.00	5.5 avg.			
Standard		1.00	4.4			
Standard		1.00	4.4			

* 1 μmole acetate = 2051×10^8 disintegrations/min.

Apparently the efficiency of conversion of acetate to cholesterol increased as the concentration of acetate decreased. With a few exceptions, the amount of "new" cholesterol synthesized from acetate decreased as the amount of acetate added decreased.

The ability of the aorta to synthesize cholesterol without any added acetate—that is, from precursors in the tissue itself—is obvious from the fact that practically the same amount of cholesteryl digitonide was obtained from the tissue to which acetate was added as was obtained from the tissue (controls) not containing added acetate. The aorta of the hog is evidently more active than that of the turkey in the synthesis of cholesterol, for 26 to 34 mg of digitonide was obtained with the former, and only 5 to 6 mg from approximately the same weight of turkey aorta. Since only a very small amount of precipitate was obtained from the control samples without aorta, the cholesterol resulted from the metabolic activity of the tissue. No claim for the synthesis of cholesterol in the absence of living cells can be made, because the tissue mince was not shown conclusively to be free from intact cells.

Mincing the tissue evidently increases the metabolic activity, for the amount of cholesterol obtained—in terms of either weight of precipitate or percentage conversion of acetate—was considerably greater with the minced tissue than it was with the slices.

The results of this study verify earlier investigations that implicated the aorta in the synthesis of cholesterol (*2, 6*). The conversion of C^{14}-labeled acetate into cholesterol by aorta mince ranged from 1 to 69 percent of that produced by liver homogenates from the same amounts of acetate (*3–5*). Although the aorta appears to be less active than the liver in cholesterol synthesis, it may nevertheless be an important factor in the development of atherosclerosis.

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References and Notes

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12 April 1955