4°C. The antigen was solubilized by the use of butanol at 20% by volume from suspensions of such liver cell-fragments, if the suspensions were previously maintained at 4°C for some time. Butanol extraction could also be applied to Triton-extracted lipoprotein of cell-fragments obtained from normal rabbit lymph node and spleen with serologically active material obtained in solution in about 10 times the yield obtained from liver, relative to mass of tissue. When the butanol-extracted soluble antigen from rabbit lymph node was injected twice into normal rabbits, the sera of the latter showed the property of suppressing antibody formation by transferred lymph node cells on incubation with the cells *in vitro* prior to transfer.

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Beta-hydroxyacyl Dehydrogenase in Human Arterial Tissue.* (30051)

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Beta-hydroxyacyl dehydrogenase is an enzyme of the fatty acid cycle which catalyzes the following diphosphopyridine nucleotide (DPN) dependent reaction:

Acetoacetyl-Coenzyme A + DPNH + H^+ ⇄ Beta-hydroxybutyryl-Coenzyme A + DPN^+

Because beta-hydroxyacyl dehydrogenase is involved in the biosynthesis and catabolism of lipids, it was considered important to study its activity in human arterial tissue. The enzyme assays were made separately on normal and arteriosclerotic tissue. For comparative purposes, a limited number of venous samples was included in the investigation.

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Materials and methods. Vascular samples were obtained fresh at autopsy from subjects ranging in age from 0 to 85 years and were immediately frozen. A total of 215 specimens was included in the study. The determinations of beta-hydroxyacyl dehydrogenase activities were made by a modification of the procedure described by Michal and Bergmeyer(1).

The acetoacetyl-coenzyme A substrate was prepared fresh daily through treatment of coenzyme A (Sigma Chemical Co., St. Louis) with thioglycolic acid and diketene(2). Ten % aqueous homogenates of intima-media layers of the vascular samples were made at 4°C with a Kontes Duall type tissue grinder; the homogenates were subsequently centrifuged at 25,000 r.p.m. and the supernatants immediately used for enzyme determination.

Nitrogen measurements were performed by the Kjeldahl procedure(3) on portions of the same tissue specimens from which the homogenates were prepared.

The final millimolar concentrations employed in the test were: acetoacetyl-coenzyme A, .075; DPNH, .110; and sodium pyrophosphate, pH 7.3, 85.0. The reaction was conducted at 25°C. The DPNH reagent dissolved in pyrophosphate buffer and .2 ml homogenate supernatant were first placed in a silica cuvette. When unspecific oxidation of DPNH was no longer detectable, acetoacetyl-coenzyme A substrate was added and optical density readings were made at 1-minute intervals over a period of 20 minutes. The enzyme activity was calculated on the basis of the linear part of the curve. A reagent blank was run with each tissue test. The validity of the prepared reagents was

TABLE I. Beta-Hydroxyacyl Dehydrogenase Activities of Human Vascular Tissue.

	Age group years	No.	Units/g wet tissue	S.E.M.	Units/g tissue nitrogen	S.E.M.
Normal aorta, thoracic	0- 9	7	.069	.015	1.52	.30
	10-19	2	.123	—	2.71	—
	20-29	16	.252	.014	6.01	.44
	30-39	19	.257	.021	6.70	.54
	40-49	15	.214	.025	5.92	.77
	50-59	24	.209	.021	5.55	.56
	60-69	12	.234	.033	6.10	.87
	70-85	5	.133	.051	3.72	1.32
Total		100	.214	.010	5.54	.28
Arteriosclerotic aorta, thoracic	10-19	1	.074	—	1.65	—
	20-29	5	.194	.050	4.75	1.31
	30-39	1	.162	—	4.21	—
	40-49	6	.171	.036	5.22	1.18
	50-59	10	.161	.043	4.70	.98
	60-69	2	.214	—	5.88	—
	70-85	1	.251	—	6.70	—
Total		26	.174	.020	4.86	.51
Abdominal aorta	20-39	2	.342	—	9.98	—
Pulmonary artery	0- 9	2	.094	—	2.41	—
	20-29	7	.195	.034	5.42	1.14
	30-39	7	.279	.033	6.90	1.07
	40-49	3	.231	—	6.89	—
	50-59	13	.225	.026	6.60	.84
	60-69	4	.176	—	5.28	—
Total		36	.217	.017	6.14	.53
Normal coronary artery	0- 9	1	.098	—	2.51	—
	10-19	2	.123	—	3.72	—
	20-29	6	.241	.071	6.27	1.88
	30-39	8	.346	.061	9.66	1.60
	40-49	2	.297	—	10.21	—
	50-59	10	.208	.044	6.54	1.58
	60-69	2	.260	—	11.35	—
Total		31	.250	.028	7.52	.86
Cerebral arteries	30-59	5	.152	.025	4.53	.62
Vena cava inferior	20-29	5	.059	.016	1.53	.40
	30-39	4	.064	—	1.86	—
	40-49	2	.111	—	2.87	—
	50-59	3	.142	—	4.00	—
	60-69	1	.034	—	1.00	—
Total		15	.082	.015	2.25	.46

verified daily by separate experiments in which a known quantity of purified beta-hydroxyacyl dehydrogenase (Sigma Chemical Co., St. Louis) was added to the substrate buffer solution.

Results. The average enzyme activities recorded for various types of blood vessels are listed in Table I. The values are expressed in units per gram of wet tissue and per gram of tissue nitrogen, 1 unit representing the metabolism of 1 micromole of substrate per minute(1). A tendency was noted for the beta-hydroxyacyl dehydrogenase activity of aortic tissue to increase from early childhood to the age of 30 years; no significant variation in enzyme activity was observed over the 30- to 60-year range. A comparison of activities exhibited by inferior vena cava and aortic specimens from the same subjects revealed distinctly lower values for the venous samples, the mean beta-hydroxyacyl dehydrogenase of the fresh venous tissue being only 31.4% (t of difference = 7.75) of that recorded for the aorta. When calculated on the basis of tissue nitrogen content, the corresponding percentage value was 33.0 (t of difference = 6.65).

Assays performed on normal and lipid-arteriosclerotic aortic tissue samples (N = 26) showed significantly lower activities for the pathological specimens both when expressed per gram of wet tissue (72.7%, t of difference = 3.78) and per gram of tissue nitrogen (76.3%, t of difference = 3.30).

Discussion. The beta-hydroxyacyl dehydrogenase enzyme has recently received increasing attention because it is involved in the metabolism of lipids. No investigations have previously been reported of this enzyme in vascular tissue; the finding that one gram of human aortic tissue is capable of metabolizing .214 micromole of substrate per minute indicates a notable activity in the aortic wall. The difference between the beta-hydroxyacyl dehydrogenase activities of the aorta and vena cava observed in the present study is conspicuous.

Summary. Determinations were made of the beta-hydroxyacyl dehydrogenase activities exhibited by intima-media layers of various types of human blood vessels. A total of 215 vascular specimens was included in the study. The average activity of the thoracic descending aorta, expressed in units (micromoles of substrate metabolized per gram tissue per minute) was .214; the corresponding values recorded for the abdominal aorta, pulmonary artery, coronary artery, cerebral arteries, and inferior vena cava were, respectively, .342, .217, .250, .152, and .082. The mean enzyme activity exhibited by atherosclerotic aortic tissue samples was 72.7% of that observed for normal tissue of the same arterial specimens.

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A Comparison of Fatty Acid Synthesis by Liver and Kidney.* (30052)

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That the kidney is capable of synthesizing fatty acids from glucose and acetate has been established by *in vitro* experiments with surviving rat kidney slices(1,2). Furthermore, evidence indicates that the fatty acid synthetic enzymatic pathway in kidney is similar to that established for liver(3).

On the basis of the tissue slice experiments

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