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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(1,2) it has been estimated that the rate of lipogenesis in the kidney is about 10-20% that of the liver; since incubation time and substrate concentrations were arbitrarily chosen in these experiments, their validity for a quantitative estimation must be questioned. Comparing the activity of the fatty acid synthesizing enzymes in beef tissues, Ganguly(3) reported the kidney to have about 40% the activity of the liver.

Since fatty acid synthesis by kidney has not heretofore been the subject of a careful, thorough study, a detailed *in vitro* exploration of renal lipogenesis was undertaken in our laboratory. The following is a report of this investigation.

Methods. Care of rats. Male rats of the Wistar strain were kept at 25°C for 10 days before the experiment and were allowed to eat *ad libitum* the standard diet of the following composition: 25% casein, 10% lard, 50.95% glucose, 3% salt mix(4), 5% cellulose, 6% brewer's yeast, 0.04% cod liver oil concentrate, and 0.01% alpha tocopherol. The rats weighed about 250 g.

Experiments with tissue slices. The rats were killed by decapitation and the liver and kidneys rapidly excised and placed in ice-cold Krebs-Henseleit bicarbonate buffer solution (5). Tissue slices were prepared free-hand. The slices were collected in Petri dishes containing ice-cold buffer. They were then blotted free of excess solution on filter paper and aliquots of approximately 100 mg of tissue were weighed and transferred to 25 ml incubation flasks(6). The main compartment of the incubation flask contained 100 mg of slices, 2.25 ml of Krebs-Henseleit bicarbonate buffer, and 0.25 ml of solution containing the labeled substrate. The slices were incubated at 37.5° with continuous shaking. $C^{14}O_2$ formed during incubation was recovered by the method of Chernick *et al*(1) and its C^{14} content was determined(7).

The tissue slices were dissolved and the lipid esters saponified by heating for 3 hours in 30% KOH made up in alcohol:water 1:1. The alcohol was removed under a N_2 stream and the hydrolysate was acidified to pH 2 or less with HCl. The mixture was allowed to stand for 12 hours at 0°C. Then it was filtered through No. 43 Whatman filter paper and the paper and precipitate thoroughly washed with dilute acetic acid. The precipitate on the filter paper was then extracted with hot $CHCl_3$:CH_3OH (2:1) and the fatty acids isolated free of the non-saponifiable lipids as previously described(8). The C^{14} content of the fatty acids was measured(9).

Experiments with cell-free preparations. The rats were killed by decapitation. The liver and kidneys were perfused *in situ* with 0.25 M sucrose to free them of blood and were then excised and placed in an ice-cold solution of 0.25 M sucrose. The tissues were minced with scissors and the mince rinsed 3 times with the sucrose solution. The tissues were ground in a glass homogenization tube by 4 strokes of a motor-driven Teflon pestle. The tissue suspensions were diluted with sucrose to yield a 20% suspension.

The 20% tissue suspensions were used to make the following preparations:

a) Homogenate. The tissue suspensions were centrifuged for 10 minutes at 600 $\times$ *g* to remove nuclei, unbroken cells and debris. The supernatant from this centrifugation step will hereafter be called the homogenate.

b) Cytoplasmic particles. The homogenate was centrifuged for 60 minutes at 105,000 $\times$ *g*. The pellet at the bottom of the centrifuge tube was rinsed with sucrose. This pellet is referred to as the cytoplasmic particles, *i.e.*, the mitochondria, microsomes and other cytoplasmic particles such as lysosomes.

c) Supernatant. The supernatant obtained from centrifuging the homogenate for 60 minutes at 105,000 $\times$ *g* is called the supernatant. It is essentially particle-free.

All of the above homogenization and centrifugation steps were carried out at about 2°C.

Combinations of cytoplasmic particles and supernatant were prepared by resuspending the particles in supernatant by means of a motor-driven Teflon pestle homogenizer.

One and one-half milliliters of a tissue preparation was added to 1 ml of special medium experimentally determined to provide maximum fatty acid synthetic activity for the particular tissue preparation being studied. For liver homogenate, the incubation flask contained 120 μmoles KCl, 7.5

μmoles $MnCl_2$, 15 μmoles sodium acetate-1-C^{14}, 2.5 μmoles succinate, 4 μmoles TPN, 47 μmoles isocitrate, 6 μmoles DPN, 0.25 μmole CoA, 12 μmoles phosphate buffer, and 20 μmoles $KHCO_3$. For kidney homogenate, the flask contained 120 μmoles KCl, 1.9 μmoles $MnCl_2$, 15 μmoles sodium acetate-1-C^{14}, 2.5 μmoles succinate, 4 μmoles TPN, 47 μmoles isocitrate, 6 μmoles DPN, 0.125 μmole CoA, 12 μmoles phosphate buffer and 20 μmoles $KHCO_3$. For liver supernatant the flask contained 120 μmoles KCl, 7.5 μmoles $MnCl_2$, 15 μmoles sodium acetate-1-C^{14}, 4 μmoles TPN, 94 μmoles isocitrate, 0.25 μmole CoA, 10 μmoles ATP, 12 μmoles phosphate buffer and 20 μmoles $KHCO_3$. For kidney supernatant the flask contained 120 μmoles KCl, 3.75 μmoles $MnCl_2$, 15 μmoles sodium acetate-1-C^{14}, 4 μmoles TPN, 141 μmoles isocitrate, 0.25 μmole CoA, 15 μmoles ATP, 12 μmoles phosphate buffer and 20 μmoles $KHCO_3$. The pH of each system was approximately 7.0.

The incubation procedure, the isolation of the fatty acid and the determination of the fatty acid-C^{14} and acetate-C^{14} were carried out as described earlier(10).

Protein content of tissue slices and cell-free preparations. The tissue slices and cell-free preparations were dissolved by heating in 5 N NaOH. The relative protein content of the solubilized proteins was measured by the method of Lowry *et al*(11).

Results. Liver and kidney slices were incubated for 2 hours with varying concentrations of acetate-1-C^{14}; in the range of 20 μmoles of acetate-1-C^{14} per flask, the amount of acetate carbon incorporated into fatty acids was independent of the acetate concentration. Therefore, in all tissue slice experiments reported below, a concentration of 20 μmoles acetate-1-C^{14} per flask was used.

In Fig. 1 the time course of acetate-1-C^{14} incorporation into fatty acids is plotted for liver and kidney tissue slices derived from the same rats. Clearly, during the first hour of incubation the amount of labeled fatty acid formed by the liver is considerably less than during the second or third hours of incubation. Fatty acid synthesis by the kidney slices was less than that of the liver at all time intervals; in the kidney there was also some increase in rate of lipogenesis during the later time interval.

TABLE I. Comparison of Acetate-1-C^{14} Incorporation into Fatty Acids by Liver and Kidney.

Incubation time, hr	Ratio: Ac-1-C^{14} incorp. into FA per mg liver protein / Ac-1-C^{14} incorp. into FA per mg kidney protein
½	3.3 ± .95*
1	4.5 ± 1.11
2	11.1 ± 3.02
3	7.8 ± 1.56

* Avg value from 3 experiments ± SEM.

Since the rate of fatty acid synthesis is changing throughout the incubation period, it is difficult to draw a conclusion regarding the relative rates of lipogenesis in liver and kidney slices. However, in Table I a comparison between kidney and liver slices is made regarding the amount of C^{14} incorporated into fatty acids for each of the 4 incubation time intervals studied. Using these data as an index, the lipogenic activity of kidney tissue can be said to vary from 9 to 30% that of the liver, depending upon the time interval chosen for calculation.

The comparatively low rate of lipogenesis in kidney as compared to liver cannot be due to an inability of kidney to activate acetate because kidney slices oxidize acetate to CO_2 at a rate about 4 times greater than that observed with liver slices (Fig. 2). It should also be noted that, unlike hepatic lipogenesis, acetate oxidation to CO_2 by liver slices occurs at a constant rate throughout the 3-hour incubation period.

To explore kidney lipogenesis further, a comparison was made of the lipogenic activity of liver and kidney homogenates. Acetate-1-C^{14} was added at a concentration of 15 μmoles per flask because at this range of concentration the rate of lipogenesis is independent of the concentration of acetate. Liver homogenates, like liver slices, exhibit an incubation time lag (Fig. 3) before the maximum rate of fatty acid synthesis occurs, reaching the maximum rate by ½ hour of incubation. On the other hand, kidney homogenates formed very little fatty acid from acetate at any time during the incubation.

The lipogenic activity of the "particle-

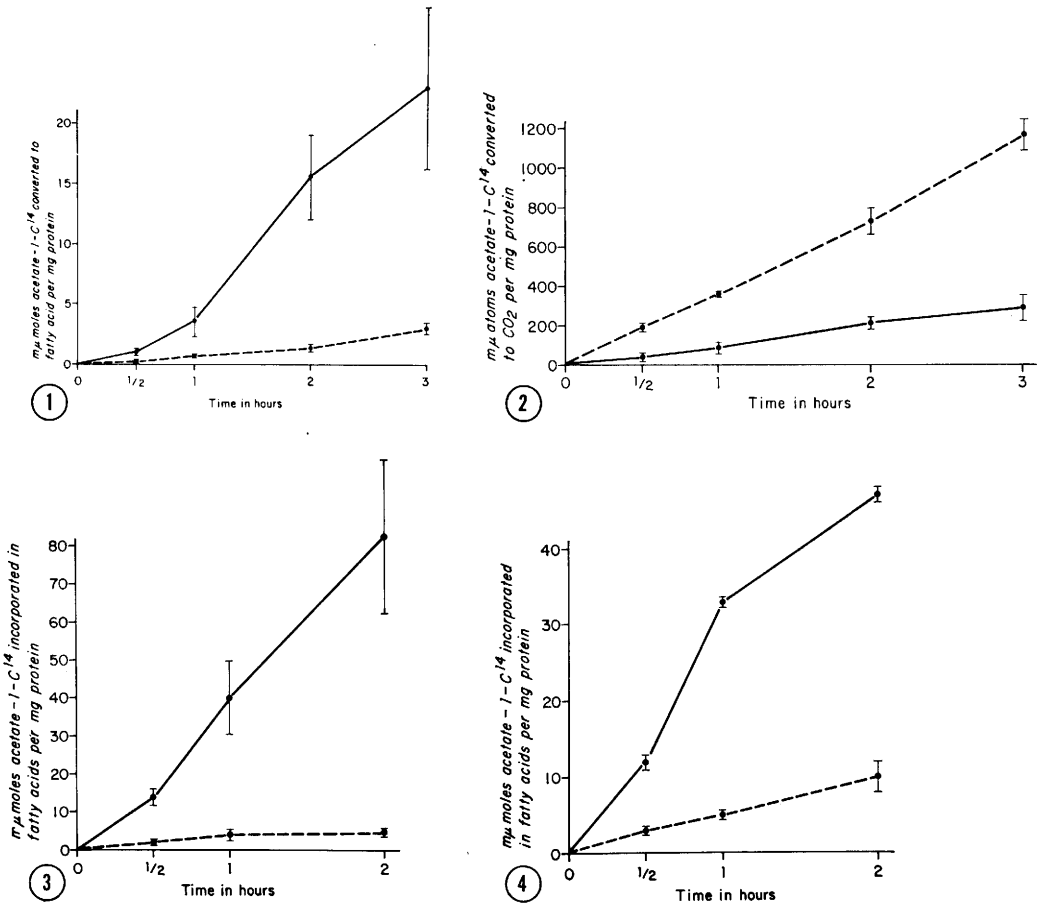

FIG. 1. Fatty acid synthesis from acetate-1-C^{14} by liver and kidney slices: ●– – –● refers to kidney; ●———● refers to liver. Average values from 3 experiments ± SEM.

FIG. 2. CO_2 production from acetate-1-C^{14} by liver and kidney slices. ●———● refers to liver; ●– – –● refers to kidney. Average values from 3 experiments ± SEM.

FIG. 3. Fatty acid synthesis from acetate-1-C^{14} by liver and kidney homogenates. ●———● refers to liver; ●– – –● refers to kidney. Average values from 5 experiments ± SEM.

FIG. 4. Fatty acid synthesis from acetate-1-C^{14} by liver and kidney supernatant. ●———● refers to liver; ●– – –● refers to kidney. Average values from 5 experiments ± SEM.

free" supernatant of liver and kidney homogenates was studied next. This fraction of the cell is considered the major site of fatty acid synthesis(12). Fifteen μmoles of acetate-1-C^{14} per flask was used for the reason described above. Kidney supernatant lipogenesis was much lower than that of liver (Fig. 4). A comparison of the rates of lipogenesis reveals the activity of the kidney to be 15 to 25% of that of the liver system. The rate of lipogenesis observed with kidney supernatant is greater than that observed in homogenates while with the liver the homogenate has considerably greater lipogenic activity than does the supernatant.

To study this problem further, cytoplasmic particles of liver and kidney were added to kidney supernatant (Table II). In previous reports(10,13) it was found that addition of liver cytoplasmic particles to liver supernatant markedly increased fatty acid synthesis. It should be noted that when liver cytoplasmic particles were incubated in this medium without supernatant, no lipogenesis occurred(14). In the present study the addition of liver cytoplasmic particles to kidney supernatant caused a marked increase in acetate-1-C^{14} incorporation into fatty acids, while addition of kidney cytoplasmic par-

TABLE II. Effect of Kidney and Liver Cytoplasmic Particles on Lipogenesis by Kidney Supernatant.

Tissue system*	mμmoles acetate-1-C^{14} conv. to fatty acid
Kidney supernatant	31 ± 4.6†
Kidney supernatant + liver cytoplasmic particles‡	123 ± 14.4
Kidney supernatant + kidney cytoplasmic particles‡	6 ± 4.3

* The medium described as being optimal for fatty acid synthesis by kidney supernatant was used for this experiment.

† Average values for 5 animals ± SEM.

‡ On the basis of a paired comparison method (18), the difference between kidney supernatant and kidney supernatant + liver cytoplasmic particles has a $P < 0.01$ and the difference between kidney supernatant and kidney supernatant + kidney cytoplasmic particles a $P < 0.001$.

ticles to kidney supernatant depressed the incorporation.

Discussion. It is difficult to come to a definite conclusion concerning the rate of renal lipogenesis as compared to hepatic lipogenesis because of the ever changing rate of hepatic lipogenesis during the incubation. The conclusion of Chernick *et al*(1) and Medes *et al*(2) that the lipogenic activity of kidney is 10 to 20% that of the liver seems to be confirmed by our experiments in which substrate concentration and time of incubation are also considered. On the basis of the supernatant studies, it would appear that there is a lower level of fatty acid synthesizing enzymes in kidney than in the liver, but it is also clear that lack of these enzymes is not the only reason for the low rate of lipogenesis in kidney tissue.

The cytoplasmic particles of the kidney are inhibitory to renal lipogenesis. It is possible that this inhibitory action is similar to that found with cytoplasmic particles from livers of fasted rats(10), or the kidney particles may be inhibiting lipogenesis by utilizing the labeled acetate rapidly for oxidative metabolism. Whatever the mechanism, this inhibitory action may be part of the reason for a low rate of lipogenesis in the kidney cell.

Liver cytoplasmic particles markedly promote lipogenesis in both kidney and liver supernatant. The mechanism by which these particles stimulate lipogenesis is not known but it may be related to lipogenin(15,16). The absence of such stimulatory particles in the kidney cytoplasm further explains the lower rate of lipogenesis in the kidney as compared to the liver.

Therefore, the results of the present investigation indicate: a) The kidney is probably not an important site of fatty acid synthesis. Since the kidney, like most organs, is composed of more than one cell type, it is possible that certain kidney cells have a high lipogenic activity. If this be so, such cells could make up only a small minority of the total cell population. b) The kidney probably has a lower level of fatty acid synthesizing enzymes than the liver. c) The kidney contains a lipogenic inhibitory system or systems in its cytoplasmic particles. d) The marked ability of liver cytoplasmic particles to promote fatty acid synthesis is not seen with kidney cytoplasmic particles.

The time course of lipogenesis from labeled acetate in liver slices deserves further comment. To the authors' knowledge, this is the first study in which the time course of fatty acid synthesis by liver slices incubated in a bicarbonate buffer has been measured. Medes *et al*(2) did carry out a time curve with liver slices in a phosphate buffer and found the lipogenic rate to be linear for 3 hours of incubation. However, it is well established that the rate of lipogenesis is much greater in a bicarbonate buffer(17) and thus, this buffer has been the one chosen for most experiments with liver slices. The reason for the increasing rate of lipogenesis as incubation progresses has not been established. There could be a time lag in penetration of acetate to the active site of synthesis; however, since a time lag was also noted with the homogenates, this possibility does not seem likely. Time may be required to build up the concentration of labeled intermediates; since our knowledge of the nature of the intermediary compounds in fatty acid synthesis is still rudimentary, this possibility cannot be considered further at this time. Possibly under *in vivo* conditions the rate of lipogenesis is held in check by a humoral agent which disappears during the incubation.

Summary. The lipogenic activity of liver and kidney was compared. Kidney slices

synthesize fatty acids at a considerably slower rate than do liver slices; an approximate estimate would place the rate of kidney slice lipogenesis at about 10-20% that of the liver slice. Kidney tissue appears to have a lower level of fatty acid synthesizing enzymes than liver but the difference in lipogenic rate of the intact cell is probably related to other factors as well. Kidney cytoplasmic particles suppress lipogenesis in kidney particle-free supernatant while liver cytoplasmic particles stimulate kidney particle-free supernatant lipogenesis. Thus, the presence of cytoplasmic particles in the kidney that inhibit rather than stimulate fatty acid synthesis probably is also related to difference in lipogenic activity between liver and kidney slices.

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Sequestration of Serum Low-Density Lipoproteins in the Arterial Intima by Complex Formation.* (30053)

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Low density serum lipoprotein has recently received considerable attention because of its probable role in the genesis of atherosclerosis. In general it is thought that it may act as a carrier of cholesterol and other lipids that accumulate in the diseased vessel. Previous studies have indicated that this class of protein, alone of all the serum proteins, is selectively bound in the aortic intima(1). But the material extracted from the intima, although antigenically identical with low density serum lipoprotein, was not entirely characteristic of it. Its immunoelectrophoretic pattern was unique. This study is concerned with the nature of this alteration of lipoproteins in extracts of arterial intimas.

The serum lipoproteins with molecular density 1.006 to 1.063 in the ultracentrifuge are subdivided into beta and alpha-2, named for their electrophoretic mobilities in numerous media including agar gel. Each of these has its own immunoelectrophoretic pattern, yet the protein portions of these subclasses have eluded all attempts to distinguish them antigenically with the more recently developed immunological techniques(1,2). They

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