The lower oxidative capacity of the succinic oxidase system in the abdominal aorta of the dog, as compared to the thoracic, may prove of interest when correlated to its susceptibility to atherosclerosis. Indeed, in the dog on an atherogenic regimen, the abdominal segment is the most susceptible to atherosclerosis, whereas the thoracic aorta is refractory† (13, 14). The decrease in oxidative capacity of human aorta with age may prove equally interesting when correlated to the known greater susceptibility of man to atherosclerosis with advancing age. It is likely that the observed differences between the 3 species studied may be part of a broader difference in metabolic patterns of their arterial tissue. Work now in progress seems to support this view.

Summary. The oxidative capacity of succinic oxidase and cytochrome oxidase systems in 3 aortic segments (ascending and arch, descending thoracic, abdominal), inferior vena cava and liver slices of man, rabbit and dog was studied. A species difference was found to exist: a) man exhibited the lowest oxidative values for both systems; b) the dog, the highest values for the succinic oxidase system in the thoracic aorta; c) the rabbit, intermediate values for the succinic oxidase system in all 3 aortic segments; d) oxidative values for the cytochrome oxidase system were of approximately equal magnitude in the aortic tissue of dog and rabbit. A significant decrease in oxidative capacity of aortic tissue was associated with aging in man, while the vena cava and liver remained unaffected. No appreciable change with age was found in rabbit and dog.

The significance of the above findings in relation to atherogenesis was discussed.

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Metabolism of the Protein Moiety of Rabbit Serum Lipoproteins. (23242)

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Virtually all of the serum lipids exist in combination with proteins(1). On the basis of electrophoretic mobility(2), solubility(3, 4), and density(5) these lipoproteins may be divided into two large groups, the alpha or high density lipoproteins and the beta or low density lipoproteins. Each of these groups may be further subdivided on the basis of density differences(6). In addition to these physical differences, the various lipoproteins differ in their lipid composition(7). Recent

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studies have also demonstrated differences in the N-terminal groups of the proteins in the major lipoprotein fractions(8). That these various lipoproteins may also have different functions has been suggested by changes in their concentrations following fat feeding(9) or heparin induced clearing(10). Studies on the relative turnover rates and metabolic interrelationships of the protein moieties of the major lipoprotein fractions should contribute toward further clarification of the function of the various lipoproteins.

Methods. Labeled materials. l-C^{14}-alanine was obtained from Tracerlab, Inc. $BaC^{14}O_3$ was obtained from Oak Ridge National Laboratories. In certain of the experiments high specific activity C^{14}-labeled proteins were obtained by growing *Chlorella pyrenoidosa*† in an artificial nutrient medium with $NaHC^{14}O_3$ as the sole carbon source(11). The organisms were harvested, extracted with hot ethanol, and dried. l-C^{14}-alanine and labeled lipoprotein fractions were injected into rabbits weighing 3-4 kg. Samples of the protein obtained from C^{14}-labeled *Chlorella* were administered by stomach tube in water suspension. *Plasma Fractionation.* Blood samples (6-12 ml) were drawn into heparinized tubes or syringes and immediately centrifuged. The lipoproteins were fractionated according to the procedure of Havel, Eder and Bragdon (7). The plasma was brought to density 1.063 (D 1.063) by addition of the calculated volume of concentrated salt solution (NaCl + NaBr, D 1.346) and centrifuged at 105,000 x g for 16-18 hours at 12-15° C in a Spinco Model L preparative ultracentrifuge. The lipoproteins concentrated in the upper 2 cm^3 of the tube (*D<1.063 fraction*) were separated from the infranatant by the use of a tube slicing device. The infranatant was brought to D 1.21 by the addition of solid KBr and appropriate adjustment of the final volume. After 20 hours centrifugation at 114,400 x g the top layer (*D 1.063 - 1.21 fraction*) was separated by means of the tube slicer. The infranatant was designated the *residual protein fraction* (D>1.21). The lipoprotein fractions used for injection into recipient rabbits were dialyzed for 16 hours at 4°C against large volumes of 0.9% NaCl in a rocking continuous-flow dialysis apparatus. *Measurements of specific activity.* In the experiments in which l-C^{14}-alanine was administered, the lipoprotein fractions, after dialysis to remove any free amino acids, were hydrolyzed for 16 hours at 110°C in 6 N HCl. CO_2 was liberated from the amino acids by reaction with ninhydrin by the method of Van Slyke, *et al.*(12). The CO_2 was precipitated as $BaCO_3$ and plated for radioassay. In the experiments in which labeled algal protein was the source of C^{14}, the lipoproteins were precipitated with 10% trichloracetic acid, washed with alcohol-ether and then with ether. The washed proteins were hydrolyzed with HCl and the mixture of amino acids was evaporated to dryness repeatedly to remove HCl. The amino acid hydrochlorides were plated directly for radioassay. Radioassays were carried out using the Robinson gas flow proportional counter(13). Results were corrected for self-absorption to a weight of 5 mg using a curve derived for $BaCO_3$. As shown by Karnovsky *et al.*, the self-absorption curves for organic compounds in the Robinson counter differ very little from that for $BaCO_3$(14).

TABLE I. Specific Activity of Serum Fractions after Intravenous l-C^{14}-alanine.

Time (hr)	D <1.063 Fraction	D 1.063-1.21 Fraction	D >1.21 Fraction
	Counts/min./mM $BaCO_3$*		
2	3300		545
6	3780	680	771
24	840	1090	963
72	610	562	548
120	250	360	417
168	193	140	527
264			242

* Spec. activity of ninhydrin-labile $C^{14}O_2$ counted in the form of $BaC^{14}O_3$ (see *Methods*).

Results. Injection of 1-C^{14}-alanine. 100 μc (8.9 mg) of l-C^{14}-alanine was injected intravenously. The specific radioactivities of the various fractions as a function of time are shown in Table I. The D<1.063 fraction showed an initial specific activity considerably higher than that in the other two fractions and reached maximal activity earlier.

† We are indebted to Dr. Rodney A. Olson for the Chlorella strain used.

The D 1.063 - 1.21 lipoproteins and the residual proteins attained lower levels of activity and maximum activity was reached between 6 and 24 hours. Half-time values estimated on the basis of the points beyond 24 hours were: D<1.063 fraction, 60-70 hours; D 1.063-1.21 fraction, 50-60; D>1.21 fraction, 120-140 hours. During the early period the specific activity of the D<1.063 fraction decreased rapidly with half maximal activity reached at 9 hours. Because the repeated bleedings necessary for these studies disturbed the steady state, the true disappearance rates are probably somewhat lower. It is clear, however, that the circulating lipoproteins turn over more rapidly than the remaining serum proteins considered as a group. In a subsequent experiment the D<1.063 fraction was subfractionated into a D<1.006 and a D 1.006-1.063 fraction. The specific activities of the protein in the two fractions were identical at the two points studied.

Fate of C^{14}-labeled lipoprotein fractions. In the first series of experiments the labeled lipoproteins were prepared from a donor rabbit given 480 μc (68.1 mg) of 1-C^{14}-alanine intravenously. Six hours later the animal was exsanguinated and the D<1.063, D 1.063-1.21, and D>1.21 fractions were isolated and prepared for intravenous injection into recipient rabbits, as described previously. Enough of each fraction was injected to double the circulating level of this fraction in the recipient animal. Blood samples were taken at frequent intervals up to 96 hours. In each animal the administered protein disappeared rapidly from the circulation. By 30 hours virtually all of the injected D<1.063 protein had disappeared from the circulation of the recipient animal. The D 1.063-1.21 and D>1.21 fractions disappeared at a slower rate and remained at low levels in the circulation for over 96 hours. In each recipient animal protein fractions other than the one injected showed some activity, but the relative activities of these other fractions did not vary consistently with time, as would have been the case had interconversion of one protein species to another occurred. Experiments were therefore undertaken to test for the completeness of fractionation under the conditions of ultracentrifugation used. A labeled D>1.21 fraction, which was presumably free of lipoproteins, was mixed *in vitro* with normal whole rabbit plasma and the mixture was refractionated by ultracentrifugal flotation. The D<1.063 and the D 1.063 - D 1.21 fractions, which should have been free of radioactivity, contained protein of specific activity respectively 22% and 28% of that in the D>1.21 fraction. In the rabbit only 26 mg per 100 ml of total serum protein is found in the D<1.063 fraction(6). Consequently, the presence of less than 0.2% of the protein of the D>1.21 fraction remaining in the D< 1.063 fraction would give the observed specific activity.

TABLE II. Relative Specific Radioactivities of Lipoprotein Fractions after Mixing Labeled D >1.21 Fraction with Whole Serum.

	D <1.063	D 1.063-1.21	D >1.21
Procedure A	2.4	7.2	100
B	.9	2.1	"
C	.6	1.7	"

To see if further centrifugation would eliminate this apparent contamination, rabbit serum labeled by feeding C^{14}-Chlorella was centrifuged twice at D>1.21 and the infranatant was then mixed with whole unlabeled rabbit serum. The mixture was fractionated as in the routine preparative procedure. From the D<1.063 and the D 1.063 1.21 fractions aliquots were removed for determination of protein specific activities (Table II, Procedure A). The remainder of each fraction was divided into two aliquots. One aliquot of each fraction was again centrifuged overnight (Procedure B). The other was mixed with unlabeled D>1.21 fraction and centrifuged overnight (Procedure C). As shown in Table II, the centrifugations, both with and without addition of the unlabeled D>1.21 fraction, markedly reduced the contamination of both lipoprotein fractions with D>1.21 proteins but did not completely remove it. Apparently the sedimentation of proteins of density greater than 1.21 is not complete under these conditions. When labeled D<1.063 or labeled D 1.063 1.21 lipoproteins were added to whole serum and fractionated as above, cross contamination between the frac-

TABLE III. Fate of C^{14}-Labeled Serum Fractions in Recipient Rabbits.

Labeled fraction	Time after inj. (hr)	Specific radioactivity of protein (cpm/mg) D <1.063 fraction	D 1.063-1.21 fraction	D >1.21 fraction
D <1.063	*In vitro**	4,592 (100)†	233 (5.1)	.0 (0)
	Inj. 1	280 (")	10 (3.7)	.0 (0.1)
	4	154 (")	16 (10.0)	.1 (")
	24	36 (")	1 (3.9)	.6 (2.5)
D 1.063-1.21	*In vitro*	23 (7.3)	318 (100)	.6 (0.2)
	Inj. 4	7 (2.9)	331 (")	1.0 (0.3)
	24	1 (1.0)	155 (")	1.2 (0.8)
D >1.21	*In vitro*	3 (1.1)	36 (14)	262 (100)
	Inj. 1	4 (5.1)	54 (28)	191 (")
	24	7 (8.0)	14 (15)	89 (")

* 1-C^{14}-alanine labeled D <1.063 lipoprotein from another experiment was used in this fractionation control and centrifuged only once.

† Figures in parentheses are relative specific activities with that of the fraction used for inj. arbitrarily assigned a value of 100.

tions also occurred, but to a lesser extent (Table III, "*in vitro*"). In view of these findings, all fractions used for injection in the subsequent experiments were centrifuged twice.

In Table III is summarized an experiment in which protein-labeled fractions were injected into recipient animals. Control data are shown giving results on the distribution of radioactivity in each fraction after *in vitro* mixing of an aliquot of the injected fraction with whole serum. The injected fractions were prepared from the sera of animals fed C^{14} algal proteins. Sera from the recipient animals were obtained at the indicated time intervals after injection of the labeled protein, and the specific activity of the protein in each of the three fractions was determined. The rate of disappearance of the injected protein was rapid. In the animal receiving the D<1.063 fraction the activity in the other two fractions was not significantly different from that found after *in vitro* mixing. This was true also of the animal receiving the D 1.063 - 1.21 fraction. In the animal receiving the D>1.21 fraction, the activities in the other two fractions were higher than found with *in vitro* mixing.

Discussion. The findings that the protein moiety of the lipoprotein fractions turns over at a rate faster than that of the other plasma proteins and that the protein of the low density lipoproteins turns over at a rate greater than that of the high density lipoproteins is in agreement with the observations of others. Volwiler, *et al.*(15) labeled serum lipoproteins in man by feeding S^{35} cystine and observed a $t_{1/2}$ for the beta lipoproteins ranging from 3 to 7 days, with a median value of 6.1 days. Using proteins labeled with I^{131} Gitlin and Cornwell(16) found a $t_{1/2}$ for the S_f 3-8 class of lipoproteins of about 3 days and for the alpha lipoproteins of over 4 days.

Most studies on the metabolic relationships between lipoproteins have been indirect. In the ultracentrifugal studies of Graham *et al.* (10), falling concentrations of low density lipoproteins (S_f 20-100) during heparin induced clearing were correlated with simultaneous increases in higher density lipoproteins (S_f 12-20). Recently, direct evidence for transfer of proteins from the S_f>8 class to the S_f 3-8 class has been presented by Gitlin and Cornwell(16), using I^{131} labeled lipoproteins. They found no evidence for conversion of these classes of lipoproteins to alpha lipoproteins. The present studies are not exactly comparable since interconversion within the D<1.063 fraction was not studied. This present study shows no evidence for significant conversion of D<1.063 lipoproteins to D 1.063 - 1.21 lipoproteins or vice versa. These results are consonant with previous studies showing that the proteins in these fractions have distinctly different chemical structure(8) and physical properties

(1,17). Studies of conversion of D>1.21 proteins to lipoproteins are inconclusive because of the necessity of making almost complete separation between these proteins and the lipoproteins. Furthermore, in the *in vivo* studies where these proteins are injected, relatively large amounts of labeled amino acids are present and these may well be reutilized in the synthesis of lipoproteins.

Summary. Alanine-1-C^{14} was administered to rabbits and its incorporation into the lipoproteins of D<1.063 and D 1.063 - 1.21 fractions and into the remaining serum proteins was measured. The D<1.063 fraction reached a higher specific activity than did the D 1.063-1.21 lipoprotein fraction or the D>1.21 fraction. Both lipoprotein fractions turned over at a faster rate than did the remaining serum proteins. C^{14}-labeled lipoprotein fractions were injected into rabbits and there was no significant interconversion between the two major density classes of lipoproteins studied. These data suggest that the D<1.063 and D 1.063 - 1.21 classes of lipoproteins are metabolically as well as chemically distinct.

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Anti-fibrillatory Activity of 17-(2-Piperidylmethyl)-3β, 17β-androstane diol. (23243)

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A number of basic steroids have been found to possess cardiac activity. Krayer, Uhle and Ourisson(1) showed that 20-(5'-methyl-2'-piperidyl)-5-pregnene-3, 20 diol had anti-accelerator action on the heart-lung preparation of the dog. Margolin, Lu, Yelnosky and Makovsky(2) demonstrated both anti-accelerator and anti-fibrillatory activity for 16-cyclohexylamino-allopregnandiol in the dog, while Robson and Trounce(3) found that 16-dimethylaminomethyl - epi-dehydroisoandrosterone antagonized ventricular arrhythmias in the cat. The present report describes the anti-fibrillatory activity of 17-(2-piperidylmethyl)-3β, 17β-androstane diol (Ro 2-7302). This compound has the following