cisely the methods of deposition of lipid substances by this means, there are at least 2 possible routes by which foam cells in the newly formed intima may derive their lipid content. One is by direct contact with blood plasma in the lumen of the vessel. The other is by way of capillaries in the connective tissue surrounding fibers of the fabric of the synthetic vessel. It may be significant that the atheromatous changes did not occur in animals treated for a short period. The grafts used here were made of orlon or nylon fabric which is porous. Gross and microscopic studies revealed fixation of the neointima to the graft by fibroblasts that penetrated between the synthetic fibers from the external surface. Consequently, it is possible that a period of capillary ingrowth along these fibrous bands must occur to allow deposition of cholesterol by this route. Further information concerning the pathogenesis of such lesions might be obtained by using materials which are essentially impervious to the ingrowth of fibroblasts, and studies of this type are under way.

Summary. Atheromatous changes developed on the intimal surface of nylon and orlon thoracic-aortic vascular replacements in 2 of 6 dogs with induced chronic hypercholesterolemia. In both animals high serum cholesterol concentrations were maintained for over one year before the changes became evident. This is the first report of induced atheromatous change in a synthetic vascular prosthesis and may shed some light on the mechanism by which such changes occur.

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Response of Lactic Acid Bacteria to Amino Acid Derivatives. IV. *Lactobacillus casei* 280-16A and Phenyllactic Acids.* (24393)

MERRILL N. CAMIEN AND MAX S. DUNN
Chemical Laboratory, University of California, Los Angeles

Ability of *Lactobacillus casei* 7469 to utilize either L- or D-phenyllactic acid (but not D-phenylalanine) in place of L-phenylalanine (1) has suggested the presence of α-hydroxy acid racemase in this organism. Independent evidence from this laboratory of α-hydroxy acid racemase activity in *L. casei* 7469 (data to be published) prompted the present investigation of phenyllactic acid utilization by *L. casei* 280-16A, a strain which was derived originally from *L. casei* 7469 and which can be demonstrated to be incapable of racemizing phenyllactic acid.

Methods. Procedures were essentially the same as those employed previously(1) except that *L. casei* 280-16A† was substituted for *L. casei* 7469 and 2 test media were employed. Medium I was the same as the earlier test medium(1) except that L-phenylalanine was included at final concentration of 20 mg %. Medium II was the same as Medium I except that L-phenylalanine was omitted and D-lactic acid was included at final concentration of 1.5 μM/ml. Tests with 2 media were set up simultaneously, employing a single inoculum preparation for both media in each test. Responses to L-, DL-, and D-phenyllactic acids and to L-phenylalanine were determined after varying periods of incubation at 35°.

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† Strain 280-16A was isolated(4) from a culture of strain 280-16 which had spontaneously dissociated. Strain 280-16 had been isolated(5) from an ultraviolet irradiated culture of strain 7469. The D-α-hydroxy acid requirement described for strain 280-16 (6) appears to be identical with that of strain 280-16A.

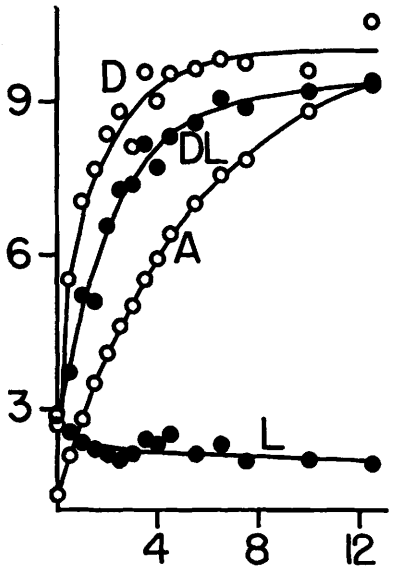

FIG. 1. Response of *L. casei* 280-16A to D-, DL-, and L-phenyllactic acids after 30 hours in 2 synthetic media. Curves D, DL, and L, which correspond to the 3 forms of phenyllactic acid, were obtained with Medium I (complete with respect to amino acids but lacking a D-α-hydroxy acid source). Curve A, representing any of the 3 phenyllactic acids (diameters of circles correspond to average spread of the 3 sets of data), was obtained simultaneously with Medium II (complete with respect to a D-α-hydroxy acid source, supplied in the form of D-lactic acid, but lacking phenylalanine). Values on horizontal scale represent phenyllactic acid test concentrations in μg/ml. Values on vertical scale represent ml of 0.01N NaOH required to titrate each ml of test culture.

Results. Medium I (containing L-phenylalanine) supported normal growth of *L. casei* 7469 without further supplementation but failed to support growth of *L. casei* 280-16A unless a D-α-hydroxy acid source was included. D-phenyllactic acid but not L-phenyllactic acid had growth promoting activity in this respect, and DL-phenyllactic acid had approximately half the activity of D-phenyllactic acid. Typical 30 hour responses to these acids in Medium I are illustrated by curves D, DL and L, Fig. 1.

Medium II (containing D-lactic acid as a D-α-hydroxy acid source) failed to support growth of either *L. casei* 7469 or *L. casei* 280-16A unless a phenylalanine source was included. L-phenylalanine, L-phenyllactic acid, DL-phenyllactic acid and D-phenyllactic acid had equal growth promoting activities in this respect for both strains. Curve A, Fig. 1, illustrates the 30 hour response of *L. casei* 280-16A to any one of the 3 (L-, DL- and D-) phenyllactic acids in Medium II. These data (curve A, Fig. 1) were obtained simultaneously and with the same inoculum preparation as those illustrated by curves D, DL, and L, Fig. 1.

Essentially the same results were obtained in repeated experiments in which incubation periods up to 43 hours were employed (strain 280-16A is capable of D-α-hydroxy acid-free growth when the incubation period is prolonged to more than 48 hours).

Discussion. The failure of L-phenyllactic acid to support growth of *L. casei* 280-16A in Medium I (curve L, Fig. 1) appears to indicate either that an α-hydroxy acid racemase is not available in this organism (since such a racemase would convert L-phenyllactic acid to D-phenyllactic acid, which is nutritionally active as shown in curve D, Fig. 1) or that L-phenyllactic acid cannot penetrate the cell wall. The latter is evidently not the case, however, since L-phenyllactic acid is readily utilizable as a phenylalanine source for this organism (curve A, Fig. 1), and it may be concluded, therefore that *L. casei* 280-16A is incapable of racemizing phenyllactic acid under the present experimental conditions.

The equivalence of L-, DL- and D-phenyllactic acids in replacing L-phenylalanine (in Medium II as shown in curve A, Fig. 1), on the other hand, appears to indicate that *L. casei* 280-16A (as well as *L. casei* 7469) can readily convert either D- or L-phenyllactic acid to L-phenylalanine. Since an α-hydroxy acid racemase is evidently not present in *L. casei* 280-16A, it seems likely that this organism (and probably *L. casei* 7469 as well) contains a D-phenyllactic dehydrogenase together with an L-phenyllactic dehydrogenase permitting formation of phenylpyruvic acid, which also can replace L-phenylalanine in the nutrition of *L. casei*(3), from either hydroxy acid. The postulated dehydrogenation of D-phenyllactic acid is evidently not reversible in *L. casei* 280-16A, however, since phenylpyruvic acid will not replace D-phenyllactic acid as a D-α-hydroxy acid source for this organism(5).

Summary. L- and D-phenyllactic acids were

shown to be interchangeable in replacing L-phenylalanine for *L. casei* 280-16A, but not interchangeable in functioning as a D-α-hydroxy acid source for this organism. It was concluded that *L. casei* 280-16A cannot racemize phenyllactic acid, and it was suggested that utilization of L- and D-phenyllactic acids in place of L-phenylalanine for this organism is mediated by separate (L- and D-) phenyllactic dehydrogenases.

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Studies Indicating Inactivation of Post-Heparin and Endogenous Human Plasma Lipoprotein Lipase During Triglyceride Lipolysis.* (24394)

H. ENGELBERG
Cedars of Lebanon Hospital, Los Angeles

Circumstanstial evidence has been obtained (1,2) which suggested that endogenous plasma lipemia clearing factor (lipoprotein lipase) was inactivated or became unstable as triglyceride lipolysis occurred. Further investigation was important since considerable data now indicate that lipoprotein lipase normally functions in clearing of post-alimentary fat from the bloodstream. However, investigators have been unable to demonstrate endogenous lipoprotein lipase in plasma of most healthy individuals. This fact is less puzzling, however, in view of data here presented which strongly support the concept that plasma lipoprotein lipase loses its activity during the lipolytic process. In addition other studies will be shown which indicate that heparin is a component of the enzyme.

Methods. Three sources of lipoprotein lipase were used: 1. fasting post-heparin human plasma; 2. fasting human plasma containing endogenous lipemia clearing activity; 3. citrate eluates(3) of tricalcium phosphate adsorbates of 1 or 2. Thus little or no neutral fat was available for lipolysis until it was added *in vitro*. The fat substrate used was human low-density lipoproteins.† The following approach was adopted: aliquots of plasma or of citrate eluates were placed in 2 tubes incubated at 37°C for 2 hours. Lipoproteins were added in excess (.2 ml/ml plasma, at which concentration the substrate does not effect rate of reaction(4)) to tube 1 at the start of first and second hours of incubation, and to tube 2 only at start of second hour. Thus any possible inactivating effect of incubation at 37°C was identical for both sets of tubes. Bovine albumin was also added to tube 1 for the second hour in some experiments to guarantee adequate fatty acid acceptor, although it was probably not necessary as the plasma itself contained ample albumin. Infranatant serum‡ was used as source of acceptor protein and necessary ions in the citrate eluate studies. In several experiments oleic acid was added to tube 2 as a control together with lipoproteins since release of unesterified fatty acids in tube 1 during first hour could possibly inhibit subsequent lipolysis. Aliquots of plasma were removed from each tube every 15

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‡ Infranatant serum obtained after ultracentrifugation contains no low density lipoproteins (supplied by Inst. of Medical Physics).

† Supplied by Inst. of Medical Physics, Belmont, Calif. through the courtesy of Mr. D. Spector. Lipoproteins were obtained by ultracentrifugation from sera of individuals with high concentration of Standard Sf 12-400 lipoprotein. They represent a 5-fold concentration of lipoprotein resuspended in their original sera, and predominately consist of Sf 12-400 and higher lipoprotein classes whose chief constituent is neutral fat.