Lipid Changes Produced by Chronic Hypercholesterolemia in Nylon and Orlon Replacements of Canine Thoracic Aorta.* (24392)

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Our experiments have demonstrated that canine aortic homograft develops atherosclerotic changes under conditions of induced chronic hypercholesterolemia more readily than does the host aorta(1). The changes were similar in thoracic and abdominal homografts. No atheromatous lesions, however, occurred in nylon and orlon fabric tubes used as vascular replacements in similar experiments(2). Duration of cholesterol administration in this latter group of animals varied from 3.25 to 13.5 months. Although it was considered doubtful that longer periods of cholesterol administration would produce different results, it seemed desirable to resolve the problem by further experiments and the present study was therefore undertaken.

Methods. Adult mongrel dogs were anesthetized with Nembutal sodium intravenously, and the left hemithorax was entered through an intercostal incision. The proximal segment of the descending aorta was mobilized, dividing one or 2 pairs of intercostal arteries. After occluding clamps were placed on the aorta, a segment was removed measuring approximately 2.5 cm. The defect was replaced by a nylon or orlon fabric tube of comparable size using running sutures of black silk for the end-to-end anastomoses. About 2 weeks following operation, radioactive iodine, 1 millicurie/kg of body weight, was administered parenterally. Animals were then placed on a diet of horse meat 70%, Purina chow 25%, and cholesterol 5%. Serum cholesterol determinations were made weekly by the method of Abell and associates(3). The animals were sacrificed after varying periods and the heart with the entire aorta, including nylon or orlon vascular replacement, was removed. The aorta was opened longitudinally to note number and distribution of lesions. The specimen was fixed in formalin and blocks of tissue were obtained for microscopic study from the "graft" and from portions of host aorta. These were imbedded in paraffin, cut serially from each block, and sections stained with hematoxylin and eosin, Masson's trichrome, and Weigert's elastic tissue stains.

Results. Of 10 dogs, 3 did not survive for a sufficient time to be included, and one that had maintained a high serum cholesterol concentration for more than one year was unavoidably destroyed. Four surviving animals had nylon prostheses and 2 had orlon. The high cholesterol diet was administered for 15, 18, 28, 58, 60, and 62 weeks, respectively. Average serum cholesterol concentrations ranged from 358 mg % to 880 mg % (Fig. 1). All prostheses remained patent, and there was no evidence of thinning or dilatation. Two of the 6 dogs with highest serum cholesterol concentrations developed intimal changes in nylon or orlon grafts, somewhat similar to those previously observed in homografts(1). However, there was no morphologic evidence that these changes had in any way damaged the integrity of the plastic fabric. These prostheses were 2 of the oldest, one orlon having been in place for 25 months in an animal which was on the high choles-

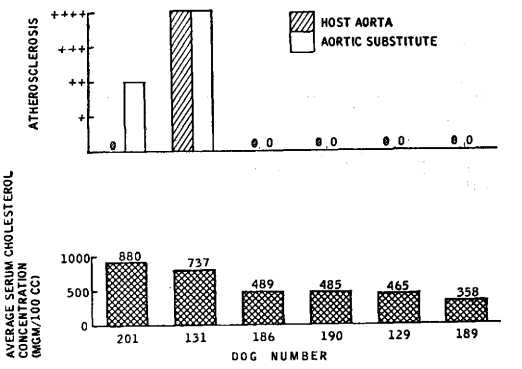

FIG. 1. Graph showing average serum cholesterol concentrations and atheromatous changes in aortas and synthetic prostheses.

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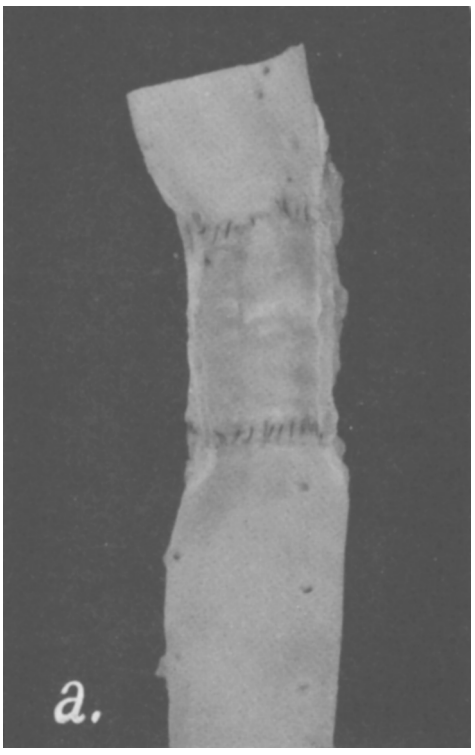
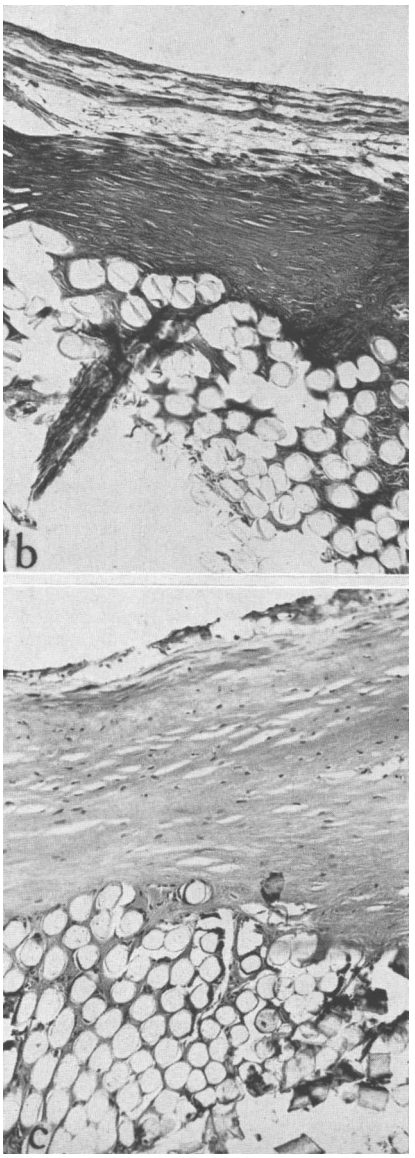
terol diet for 60 weeks (dog 131), while the other, nylon, had been in place for over 14 months in a dog on cholesterol diet for 57 weeks (dog 201). Atheromatous changes developed in the host aorta of only one of these animals (dog 131).

Structural alterations observed in dog 201 with the highest cholesterol concentration maintained for 57 weeks were only in the graft portion. Grossly, the nylon fabric was encased in a newly formed fibrous connective tissue (Fig. 2a). The intimal surface was undulating with scattered yellow plaques slightly elevated above the surface. The sutures were glazed with a delicate film of tissue covering them but clearly visible. No change was noted on the intimal surface of the remainder of the aorta. Microscopic examination disclosed a zone of hyalinized fibrous connective tissue about one-fourth the width of the anisotropic clear round threads of the nylon prosthesis bordering intimal and adventitial surfaces. On the intimal surface between delicate fibrils were large foam cells with clear cytoplasm and eccentrically placed small nuclei (Fig. 2b and 2c).

In dog 131 with the second highest average cholesterol concentration maintained for 60 weeks, there were marked atheromatous changes in the coronary arteries, over the sur-

FIG. 2. (a) Photograph of specimen showing atheromatous change on intimal surface of nylon thoracic prosthesis (dog 201). (b) Photomicrograph (× 100) showing zone of hyalinizing fibrous connective tissue bordering intimal surface. Toward the lumen between delicate fibrils are large foam cells (Masson's trichrome stain). (c) Photomicrograph (× 100) revealing similar change in preparation taken from mid-portion of prosthesis (hematoxylin-eosin stain).

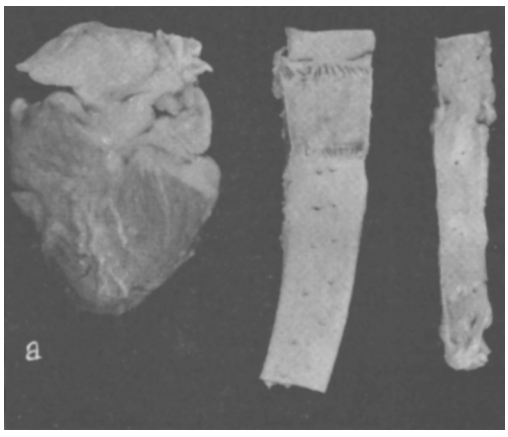




face of the orlon graft portion of the thoracic aorta, and in the abdominal aorta (Fig. 3a). In microscopic preparations the intimal plaques were composed of a loose fibrillar substance with few cell nuclei and between them large clearly delineated foam cells (Fig. 3b and 3c). The anisotropic oval stippled orlon fibers were surrounded by delicate or broad bands of hyaline fibrous connective tissue. This contained varying numbers of capillaries. Surrounding these, occasional foam cells could be seen. In the coronary arteries the changes in the intima and media were those of frank atheromatous change that seemed to follow the course of the vasa vasorum. A study of these changes will be reported elsewhere.

Comment. In previous studies we reported that synthetic aortic substitutes did not develop atherosclerotic changes(2). Our current observations indicate that atheromatous changes may occur, but only after long periods of hypercholesterolemia. It remains true, however, that the synthetic vascular substitutes are less susceptible to these changes than are homografts, and that these changes do not impair the integrity of the plastic fabric graft as they do that of the homograft.

Although the mechanism for production of these atheromatous changes in the synthetic aortic grafts is not entirely clear, their occurrence under these circumstances would seem to have significance in several respects. It suggests that while there may be some inherent metabolic or enzymatic capacity of the normal tissues of the vessel wall to form atheroma, this factor is apparently not essential to their production. It seems to lend support to the filtration concept of atherosclerosis. While these experiments do not provide sufficient evidence to determine pre-



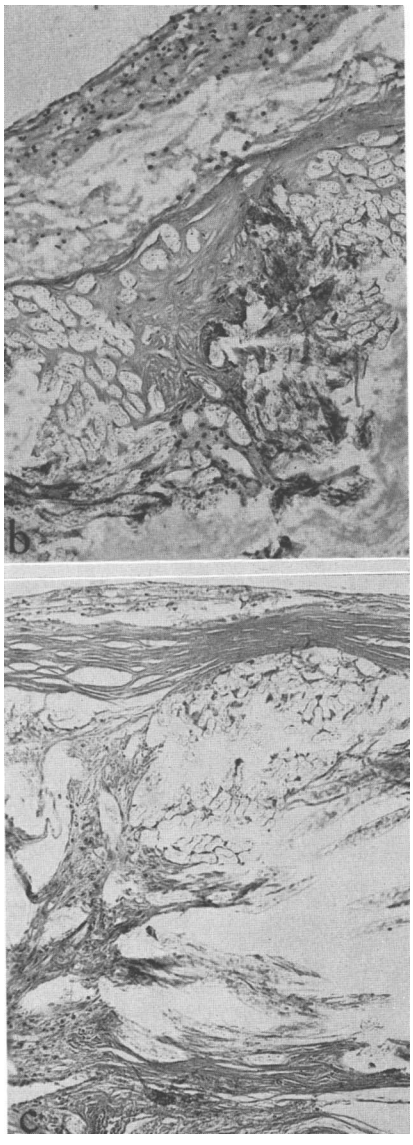


FIG. 3. (a) Photograph of specimens showing atheromatous change on intimal surface of orlon thoracic prosthesis (dog 131). Atheromatous changes may also be noted in abdominal aorta and coronary arteries. (b) Photomicrograph (× 100) showing zone of hyalinizing fibrous connective tissue bordering intimal surface. Toward the lumen between delicate fibrils are large foam cells (hematoxylin-eosin stain). (c) Photomicrograph (× 100) showing similar change in preparation taken from midportion of prothesis (Weigert's elastic tissue stain).

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)

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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.