# Alteration of Healing Responses in Experimental Wounds in Arteries and Other Tissues by Hypercholesterolemia

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THE HEALING of wounds and, more recently, regeneration or repair of vascular tissue have continued to be important fundamental and practical surgical problems. A number of years ago a pattern of arterial repair and regeneration in normal rabbits was described[3,8]; a profound alteration in the quality of arterial repair in the presence of hypercholesterolemia[7] was also reported (Fig. 1). Later, in studies in monkeys, localization of lipids at sites of injury with impaired repair was reported in all animals with serum cholesterol levels exceeding 250 mg. per 100 cc. When serum cholesterol levels were below 250 mg. per 100 cc. lipids did not accumulate at sites of injury.[9] The general subject of arterial repair has been reviewed elsewhere.[10]

Since such marked changes were observed in the repair of arteries in the presence of hypercholesterolemia, it was decided that studies of a similar nature should be carried out to observe the effects of hypercholesterolemia on healing of other tissues. While the present studies were in progress, a related study concerning the effect of hypercholesterolemia on inflammatory responses was reported.[11]

## MATERIALS AND METHODS

Two groups of young adult male albino rabbits were used. A control group (four animals) received a stock diet of Purina rabbit pellets throughout the period of study. The experimental group (seven animals) was fed the same basic diet plus 5 per cent corn oil and 2 per cent cholesterol. Levels of total serum cholesterol were determined periodically in both groups of animals; the method of Abell and others[1] was employed. After sixty days on these diets, each animal was anesthetized with ether and areas of local aseptic necrosis were produced in the aorta, skeletal muscle, liver, spleen, and kidney by a freezing technique developed in our laboratories.[5] Incisional wounds were made in skin and muscle of the thigh and in the abdominal wall. Dietary regimens were maintained and animals were sacrificed sequentially during 120 days after the production of the experimental wounds. Healing processes were studied grossly and microscopically. The following staining techniques were employed: hematoxylin and eosin, Mallory's aniline blue for connective tissue, Weigert's elastic tissue stain, and the Sudan IV stain for lipids.

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## RESULTS

*Serum Lipids.*—The control total serum cholesterol levels of all animals ranged between 35 and 100 mg. per 100 cc. Animals kept on the control diet maintained serum cholesterol levels below 100 mg. per 100 cc. At the time the experimental wounds were produced, the group of rabbits on the cholesterol and oil supplement had been on this diet for sixty days and their serum cholesterol levels had increased to between 715 and 1350 mg. (average 1130 mg.) per 100 cc. Subsequent determinations at thirty-day intervals were generally higher, ranging between 1265 and 2280 mg. (average 1780 mg.) per 100 cc.

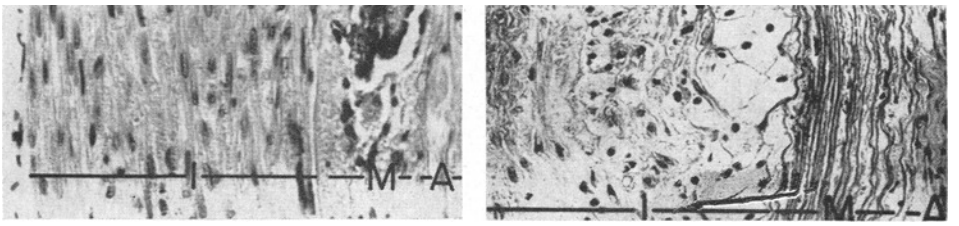


Fig. 1. *(left)* Cross-section of a healed arterial lesion, twelve weeks old, produced by freezing the abdominal aorta of a normocholesterolemic rabbit ingesting a vegatarian diet. The intima (I) at this stage is as thick as the original undamaged media. There is no localization of lipids in the lesion. It is composed principally of parallel smooth muscle cells lying between thick lamellas of elastic tissue. The badly damaged media (M) is largely occupied by masses of calcium which are surrounded by macrophages and dense fibrous tissue. The adventitia (A) is composed of dense bundles of fused callogenous fibrils. *(right)* Lesion, twenty-six weeks old, produced by freezing the abdominal aorta of a rabbit with an average blood cholesterol level of 1,090 mg. per 100 cc. In hypercholesterolemic animals, lesions less than nine weeks old usually showed this "mucinous" degeneration of the proliferated intima. The proliferated intima (I), the degenerate media (M), and the adventitia (A) are indicated in the illustration. Note lipophages in the inner one-half of the proliferated intima. Comparison with the left view demonstrates marked alteration in the intimal repair following freezing of the aortic wall of the hypercholesterolemic animal. Fibroblasts are less numerous and the newly-formed fibroelastic tissue has a peculiar loose reticular character resembling that found in "mucinous" degeneration of proliferated intima in human arteriosclerosis.

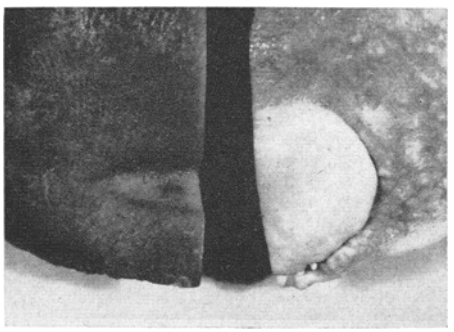

Fig. 2. *(left)* A healed, retracted scar in the liver of a normocholesterolemic rabbit. *(right)* A large xanthomatous lesion in the liver of a hypercholesterolemic rabbit. Forty-five days earlier, identical areas of necrosis were produced in both livers.

Since all animals were young adults, there was a 30 to 40 per cent increase in weight of both control and experimental groups of animals; there was no demonstrable difference between the rate of growth of the two groups.

*Arterial Repair.*—In the hypercholesterolemic animals, repair or regeneration of aortic lesions was adversely affected presumably by the marked propensity for lipids to accumulate at sites of arterial injury. The findings in arterial tissue were similar to those

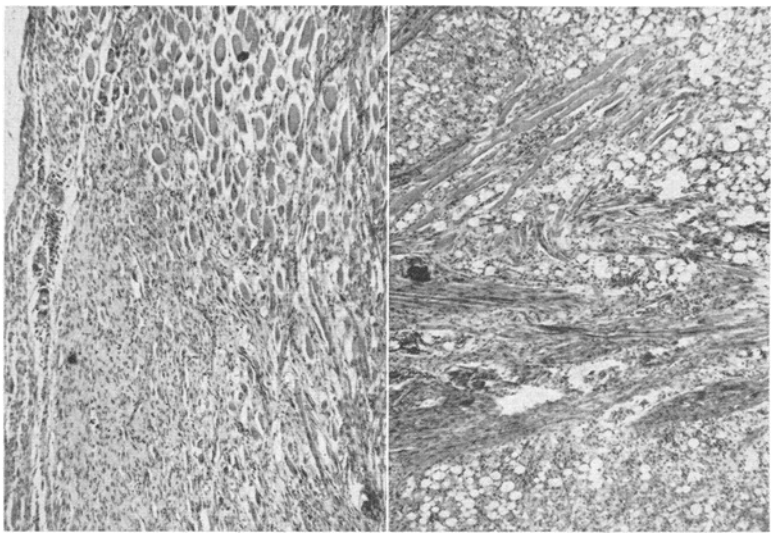

Fig. 3. *(left)* Photomicrograph of a healed wound in skeletal muscle of a control animal. This hematoxylin and eosin stain shows fibrocytes and well-oriented collagen bundles filling in the defect in the skeletal muscle shown in the left lower corner. Lesion made by local freezing. *(right)* Photomicrograph showing large groups of fat-laden macrophages infiltrating defect in skeletal muscle produced in hypercholesterolemic rabbit by freezing.

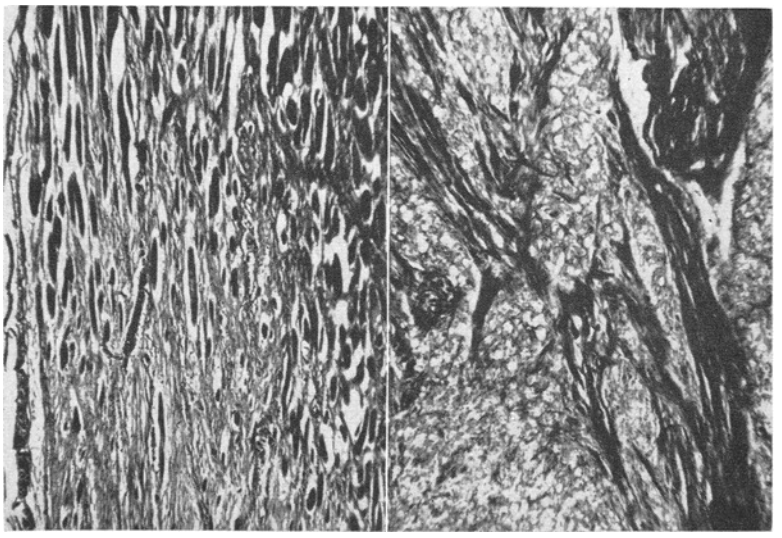

Fig. 4. *(left)* Photomicrograph of Mallory's connective tissue stain showing dense bundles of collagen (in left lower corner) bridging defect in skeletal muscle. *(right)* Photomicrograph of Mallory's connective tissue stain showing poor bridging of defect in skeletal muscle. Note large masses of fat-laden macrophages with only a delicate reticular network lying between these cells.

reported earlier[3,7,8]—and later summarized and well illustrated in another publication.[10] In addition, it should be pointed out that it has been demonstrated in the monkey,[9] the rabbit,[6] and the dog,[4] that there is a critical level of total serum cholesterol (about 250 mg. per 100 cc.) above which lipids invariably accumulate in regenerating arterial tissue at sites of injury or degeneration. When the total serum cholesterol is below 250 mg. per 100 cc., lipids do not accumulate in regenerating vascular tissue and the vascular scar is not adversely affected.

*Repair of Wounds In Other Tissues.*—The areas of aseptic necrosis produced by freezing local areas in the liver, spleen, kidney, and skeletal muscle resembled infarcts. Fifteen days after injury, lesions in both groups of animals were white, swollen masses of necrotic tissue undergoing early organization; however, even at this time, lesions in the hypercholesterolemic animals were larger and had a slight yellow, xanthomatous color. At forty-five days, lesions in control animals consisted of firm but flexible, small, gray-white scars (Fig. 2, left) while those in the hypercholesterolemic animals appeared much larger, were more yellow and resembled atheromata (Fig. 2, right). Lesions from control and experimental animals at seventy, ninety-five and 120 days after their production remained grossly similar to those lesions forty-five days old.

The incisional wounds in the control animals presented the gross appearance of well-healed wounds with small pliable scars. In the experimental animals dermal scars appeared poorly healed and stiff with raised yellow margins; cross-sections revealed yellow grumous scars in the dermis and subcutaneous tissue. Incisional scars in skeletal muscle presented a similar picture.

All wounds in control animals healed in the usual manner and the defects, due either to necrosis following freezing or incisions, were filled with fibrocytes and an abundance of well-oriented collagen bundles (Fig. 3, left and Fig. 4, left). At fifteen days, discoid lesions produced by freezing (diameter, 15 mm. and thickness, 5 mm.), were still undergoing resorption and organization. After forty-five days, they were completely healed (Figs. 2, 3 and 4, left). In the hypercholesterolemic animals, there was a profound alteration in healing of the experimental wounds which were filled with fat-laden macrophages. Defects in muscle or fascia were bridged by masses of these lipophages with only thin reticular fibrils between nests of these cells (Figs. 3, right and 4, right). Wounds of six and fifteen days contained an abundance of extracellular, stainable lipids in the interstitial fluids in and adjacent to the wounds. Lesions forty-five days old looked much like early arterial atheromas with most of the lipids in large macrophages. In a few areas, lesions forty-five days old showed areas of degeneration of macrophages with crystallization of lipids. Older lesions (seventy, ninety-five and 120 days) demonstrated areas that looked like degenerated arterial atheromata with numerous cholesterol crystal clefts. Hypercholesterolemia had no apparent adverse effect on regeneration of squamous epithelium.

## DISCUSSION

The solubility of lipids in the aqueous milieu of plasma and interstitial

fluids is dependent upon conjugation with proteins. Maximal stability of lipids appears to exist in interstitial tissues when plasma lipids are low. We have presented evidence to indicate that, when serum cholesterol levels exceed 250 mg. per 100 cc., lipids in interstitial fluids in injured arterial walls are no longer in stable solution. The present studies also demonstrate that when plasma cholesterol levels are about 1100 mg. per 100 cc., there is an increased concentration of lipids in interstitial fluids which results in their deposition at sites of injury of other tissues. This local excess of lipids has an adverse effect on the healing of wounds. It appears that, in hypercholesterolemia, newly-proliferated totipotent mesenchymal cells are diverted from normal differentiation into fibrocytes (which form collagen) and become large fat-laden macrophages.

The xanthomatous lesions in the experimental wounds in the rabbits resemble those which over-lie tendons and bony prominences of patients with congenital hypercholesterolemia. We have not determined the reversibility of the experimental lesions but separate studies in our laboratory have demonstrated reversibility of cutaneous xanthomas in patients and Rhesus monkeys when their serum cholesterol levels are reduced by diet from 1100 to below 500 mg. per 100 cc. Reversibility of atheromatous deposits in arteries has been demonstrated experimentally.[2]

## SUMMARY

When the serum cholesterol is below 250 mg. per 100 cc., the interstitial lipoproteins do not accumulate at sites of arterial injury and repair. When levels exceed 250 mg. per 100 cc., lipids do accumulate in scars at sites of arterial injury.

When the serum cholesterol is about 1100 mg. per 100 cc., lipids accumulate as xanthomatous lesions in experimental wounds of the liver, spleen, kidney, muscle, and skin.

In hypercholesterolemic animals, lipid accumulation at sites of injury adversely affects healing of wounds.

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