Lipoprotein Movement through Canine Aortic Wall

Abstract. *Low-density (1.019 to 1.063) lipoprotein labeled with radioiodine enters the inner layer of the canine aortic wall directly from the aortic lumen. Its rate of entry is greatest in the proximal aorta and decreases progressively down the length of the aorta. A similar gradient was observed previously for the accumulation of cholesterol early in experimental atherosclerosis.*

The movement of plasma lipoproteins through arterial walls is of interest because of the possibility that they are trapped there and produce atheromata. This is the filtration theory of atherosclerosis (*1*). Several workers have studied the passage into arterial walls of lipids, labeled with isotopes, which are present in the plasma as part of lipoprotein molecules, but, because of exchange phenomena (*2*), their results are difficult to interpret in terms of the movement of intact lipoprotein. The difficulty due to exchange was avoided by Okishio (*3*) who used radioiodine as a label for the protein portion of the lipoprotein and demonstrated that lipoprotein enters the aortic wall of the rabbit. This paper describes the passage into the normal canine aorta of low-density lipoprotein labeled with radioiodine. The label studied was that on the protein portion of the lipoprotein.

Lipoprotein with a density between 1.019 and 1.063 was isolated from normal canine serum. The density of the serum was adjusted with NaCl. All ultracentrifugal separations were made in a Spinco 40.3 rotor at 40,000 rev/min and 18°C for 16 hours. The lipoprotein was isolated by two ultracentrifugations and purified by an additional ultracentrifugation. It was dialyzed against saline buffered to *p*H 7. The dialyzed lipoprotein was labeled with elemental iodine (*4*). Throughout the isolation and iodination, the solutions contained ethylenediaminetetraacetate to inhibit oxidation of the lipoprotein (*4*). Less than 10 percent of the activity on the lipoprotein could be extracted with hot ethanol. Calculations based on an assumed molecular weight of one million indicated that there was about half an atom of iodine per molecule of lipoprotein. The specific activity was about 1 μc/mg.

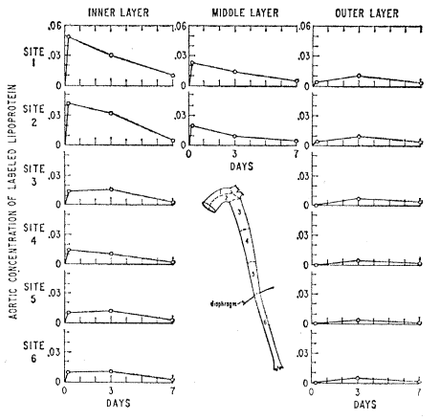

Fig. 1. The concentration in the aortic wall of labeled lipoprotein injected intravenously. The amount of labeled lipoprotein per gram of tissue is expressed as a fraction of the amount per milliliter of plasma drawn 2 minutes after injection. A diagram of the sites into which the aorta was dissected is shown in the center of the figure. The subdivision of sites into layers is described in the text.

Labeled lipoprotein was injected intravenously into 12 dogs. Three were killed at each of the following intervals after injection: 30 seconds, 6 hours, 3 days, and 7 days. Blood was drawn at 30 seconds from the dogs killed then, and from the other dogs at 2 minutes after injection and just before death. Part of each sample of serum was adjusted to a density of 1.063 and then split into two parts by ultracentrifugation. One dog killed at 6 hours and one killed at 3 days were fed 6 g of potassium iodide daily, from 4 days before injection until death, to inhibit the formation of radioactive thyroglobulin by their thyroids.

The aorta of each dog was dissected into the sites shown in Fig. 1. Site 1 was the convex side and site 2 the concave side of the ascending aorta, sites 3, 4, and 5 were the upper, middle, and lower thirds of the descending thoracic aorta, and site 6 was the abdominal aorta. Sites 1 and 2 were split into three layers, and the remaining sites were split into two layers. The thickness of the inner layer was determined from its area and weight at the time of dissection, and made to fall between 0.22 and 0.28 mm.

Twenty-percent trichloroacetic acid was used to precipitate the protein of the serums, serum fractions, and tissue specimens. The tissue specimens were homogenized in this solution. Normal serum was added when necessary to increase the bulk of the precipitate. The precipitates were transferred to extraction thimbles of filter paper and thoroughly dried at 105°C. They were then extracted for 20 minutes with gently boiling ethanol to remove any radioiodine incorporated in the lipid portion of the lipoprotein molecule. It was found that further extraction with hot ethanol, a hot ethanol-ether-chloroform mixture, or hot ether, removed very little additional radioiodine. All the precipitates for each dog were counted on the same day in a scintillation counter.

The concentration of labeled lipoprotein in the plasma is shown in Fig. 2. In the final samples of plasma from the three dogs killed 6 hours after injection, 3, 4, and 15 percent of the labeled lipoprotein did not float on ultracentrifugation after the density of the serum had been adjusted to 1.063. Feeding potassium iodide did not decrease the fraction that did not float.

The concentrations of labeled lipoprotein in the aortic walls of the dogs killed 30 seconds after injection were about the same as those for labeled albumin and slightly more than those for labeled erythrocytes in an earlier study (*5*). Some reasons for believing that the concentrations of labeled al-

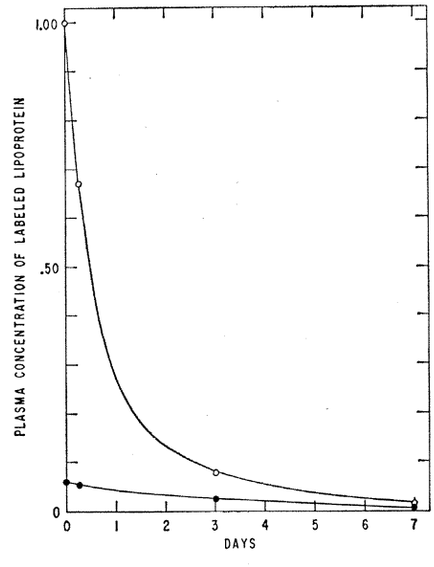

Fig. 2. The concentration in plasma of labeled lipoprotein injected intravenously. The top curve represents total labeled protein. The bottom curve represents labeled lipoprotein that did not float on ultracentrifugation after the density of the serum was adjusted to 1.063. All concentrations are expressed as a fraction of the concentration of total labeled protein 2 minutes after injection.

bumin in the aorta at 30 seconds were due to intravascular albumin on intimal endothelium or in vasa vasorum were given in that study. The similarity of the concentrations for labeled albumin to the concentrations for labeled lipoprotein suggests that the latter concentrations also are due to intravascular protein.

The concentrations in the aortic wall of labeled lipoprotein that had penetrated beyond the endothelium into the tissue (Fig. 1) were computed from the concentrations at 30 seconds after injection and the data at subsequent intervals. The concentration of labeled lipoprotein increased more rapidly in the inner layer than in the outer layer ($p < .05$ by a two-tailed t-test). Since the inner layer is almost free of vasa vasorum (*6*), this suggests that lipoprotein enters the inner layer directly across the intimal endothelium rather than from the vasa vasorum of the outer layer.

In the inner aortic layer the concentration of labeled lipoprotein increased faster in the ascending thoracic aorta than in the descending thoracic aorta, and faster in the descending thoracic aorta than in the abdominal aorta (by a two-tailed t-test $p < .02$ for the difference between the ascending aorta and the abdominal aorta). Thus, the rates of entry of lipoprotein into the inner layer of the aorta formed a gradient. Similar gradients of the rates of entry into the inner layer have been observed both for labeled cholesterol fed to normal dogs (*7*) and for labeled albumin injected intravenously into such dogs (*5*). All these gradients are similar to the gradient formed by the accumulation of cholesterol in the inner layer of the aorta during the first month of the development of atherosclerosis in dogs fed thiouracil and cholesterol (*8*).

No evidence of trapping of lipoprotein in arterial tissue was found in the present study. It seems unlikely, of course, that there is much trapping of lipoprotein in the aorta of the normal dog. If there were, the concentration of some constituent of lipoprotein should increase in the aorta with age. The only evidence for this is a very slow increase in the concentration of cholesterol with age (*9*).

The similarity of the gradient formed by the accumulation of cholesterol early in experimental atherosclerosis to the gradient formed by the rates of entry of lipoprotein is compatible with the filtration theory of atherosclerosis, but does not prove that the theory is correct. The present data offer no explanation of the fact that later in the course of experimental atherosclerosis, the concentration of cholesterol in the abdominal aorta exceeds that in the thoracic aorta. Some possible explanations of that fact were suggested earlier (*8*).

Many factors may have contributed to the failure of a small fraction of the labeled protein in the plasma to float at a density of 1.063. The possibility that the fraction not floating was thyroid hormone labeled in the thyroids of the injected dogs was excluded by the results in the dogs fed potassium iodide. The amount fed should have almost completely blocked the utilization of radioiodine by their thyroids. The failure of a small fraction to float at 1.063 does not affect any of the conclusions drawn from the data. A contaminating labeled protein would have affected the validity of the conclusions most of all if it had been a rapidly diffusing protein like albumin. The concentrations of labeled albumin in the aorta following its intravenous injection are known (*5*). Even if all the labeled protein not floating at 1.063 had been albumin, that albumin would have made an insignificant contribution to the concentrations found in aortic tissue.

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Convulsant Drug Action on Neuronally Isolated Cerebral Cortex

Abstract. *After topical application of thiosemicarbazide, semicarbazide, or isoniazid to the surface of an isolated region of cortex, convulsive responses to electrical stimulation appeared only after a finite number of normal responses had been elicited, and disappeared again if stimulation was interrupted for about 10 minutes. With any of the other convulsant drugs tested, development of the convulsive pattern was independent of whether or not the cortex was stimulated. The two groups of drugs evidently have different modes of action.*

Many different substances are known to produce convulsive neuronal activity when topically applied to the cerebral cortex. In the intact spontaneously active cortex they all produce much the same kind of regional alterations in electrical activity, but it is unlikely that they all act by the same pharmacological mechanism. This has been inferred from the fact that some of the drugs, when systemically administered, produce different topographic distributions of convulsive activity within the central nervous system (*1*). This study affords a demonstration of differences in their action on the cortex itself. These differences became apparent when a number of these drugs were applied to the unanesthetized neuronally isolated cortex, in which neuronal activity does not usually occur spontaneously but can be elicited by electrical stimulation. Under these conditions it could be shown that the drugs could be divided into two groups according to whether or not the frequency with which neuronal activity was elicited influenced the development of convulsive effects.

The experiments were performed on a total of 18 cats. In each case, a high midbrain section was carried out under ether anesthesia, and then the anesthetic was discontinued (cerveau isolé preparation). A region of cortex about 5 by 15 mm in area was isolated in the suprasylvian gyrus, with the subpial incision technique described by Burns (*2*). Agar-saline wick electrodes were used for monopolar surface re-