Passage of Labeled Cholesterol into the Aortic Wall of the Normal Dog

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With the assistance of Almorris Lynch

In the normal dog the rate of movement of cholesterol from the serum into the inner layer of the aortic wall is greatest at the proximal end of the aorta and decreases progressively along the length of the aorta until in the terminal aorta the rate is only about one sixth that in the proximal aorta. A similar gradient of rates was previously demonstrated for albumin which enters the aortic wall about three times as fast as cholesterol. The similarity of the gradients and the relative magnitude of the rates for cholesterol and albumin support the concept that cholesterol is carried into the aortic wall of the normal dog by the passage of the lipoproteins of which it is a part.

The general concept of the passage of serum proteins through the interstitial fluid of tissues is based on a large body of data1 and has been developed theoretically in considerable detail.2 Although the general features of this passage are probably everywhere the same, in specific tissues the process is modified by local conditions. Due to its possible connection with the development of atherosclerosis, the passage of proteins through arterial walls is of particular interest. We have previously3 studied the circulation of albumin through the aortic wall of the dog. This paper describes the passage of labeled cholesterol into the aortic wall of the normal dog. It provides evidence supporting the concept that in the normal dog cholesterol is carried into the aortic wall as a part of the normally occurring lipoproteins.

METHODS

Mongrel dogs weighing about 12 Kg. were fed a mixture of commercial dog chow (protein 24 per cent, fat 5 per cent, carbohydrate 44 per cent) and horsemeat. Gelatin capsules containing cholesterol-4-C\(^{14}\) dissolved in sesame oil were forced down the throats of 15 dogs. The doses ranged from 5 \(\mu\)e./dog for the dogs killed after a short period of time to 20 \(\mu\)e. for those killed after a long time. Serial determinations of serum labeled cholesterol were made and the dogs were killed at intervals of from 2 hours to 80 days after ingestion of the labeled cholesterol. Four dogs were killed at 2 hours, 3 at 3 days, 3 at 10 days, 4 at 20 days, 2 at 40 days, and 2 at 80 days.

The aorta from the valve ring to the brachiocephalic artery was separated into the outer side of the arch and the inner side of the arch. Each of these sites was split into an inner layer which contained intima and some media, a middle layer consisting of media, and an outer layer consisting of media and adventitia. The descending thoracic aorta below the left subclavian artery was divided into upper, middle, and lower thirds. Each of these was split into two layers; an inner layer of intima and media, and an outer layer of media and adventitia. The abdominal aorta was also separated into these two layers. The outer ascending aorta and arch were designated as site 1, the inner ascending aorta and arch as site 2, the upper, middle, and lower thirds of the thoracic descending aorta as sites 3, 4, and 5 respectively, and the abdominal aorta as site 6.

The weight and radioactivity of each layer of each site were determined. Since radiocarbon is not removed from the cholesterol nucleus4 and since cholesterol is present in serum and in the tissues we studied in much greater concentration than any of its derivatives, it was not necessary to isolate it chemically before determination of its radioactivity. Measurement of radioactivity was made by the following procedure. One half milliliter of serum or 0.1 to 2.0 Gm. of tissue were digested at 80 C. in 4 ml. of a 3.6 per cent solution of NaOH in 50 per cent ethanol. This solution was extracted with 15 ml. of dichloromethane. The dichloromethane was washed with water until neutral and then transferred to a vial where it was...
evaporated at 40 C. The small amount of water left in the vial was removed by the addition and evaporation of ethanol. To the vial was added 5 ml. of toluene containing 0.6 per cent diphenyloxazole and 0.02 per cent of 1,4-di[2-(5-phenyloxazolyl)] benzene. The vial was placed in a Packard Tri Carb Liquid Scintillation Spectrometer and its contents counted. Serum concentration was expressed as the fraction of the administered dose per kilogram of body weight present in 1 ml. of serum. The data for tissue were expressed as the ratio of the amount of labeled cholesterol in 1 Gm. of tissue to that in 1 ml. of serum.

The cholesterol concentrations of the layers of each site of the aortas of 7 dogs not fed labeled cholesterol were determined. The tissues were digested, extracted, and washed by the procedure described above for labeled cholesterol. The total cholesterol content of the dried extract was measured by the method of Schoenheimer and Sperry.

RESULTS

The average serum concentrations of labeled cholesterol are shown in figure 1. The serum concentration rose rapidly, reached its peak between 1 and 3 days after ingestion of labeled cholesterol and thereafter decreased gradually.

No radioactivity could be detected in any of the aortic layers 2 hours after the ingestion of labeled cholesterol. From 3 days on radioactivity was present in the aortic wall in measurable amounts. The top chart of figure 2 displays the tissue-serum concentration ratios of labeled cholesterol for the inner layer during the first 10 days of the experiment. Of the ratios for the inner layer those in the ascending aorta rose most rapidly initially. The initial rate of rise was progressively less down the length of the aorta. The next to the top chart of figure 2 displays the ratios for the inner layer during the entire 80 days of the experiment. Between 10 and 40 days the rates of increase of the ratios changed so that the ratios became greater distally than proximally. They became constant at about 40 days. The final ratios formed a gradient with the highest value at the distal end and the lowest at the proximal end of the aorta.

The ratios for the middle and outer layers are shown in the lower charts of figure 2. The ratios rose with time until they became constant at about 40 days. No systematic differences between sites were apparent.

The concentrations of unlabeled cholesterol in the aorta are given in table 1. A gradient exists in the inner layer. The concentration is least proximally and increases along the length of the aorta. The concentration is 62 per cent greater in the abdominal aorta than in the ascending aorta. In the outer layer the concentration of cholesterol is greatest in the ascending aorta. In the rest of the outer layer there is little difference in cholesterol concentration between sites.

DISCUSSION

We have dealt elsewhere with the problem of determining from data such as we have here the rate of passage of a labeled substance from serum into a tissue and the rate of passage out of that tissue. That analysis was based on the assumptions (1) that the rate of transfer of the labeled substance from serum into the tissue was proportional to its concentration in the serum, (2) that mixing of the labeled substance with its unlabeled form in the tissue occurred instantaneously in a single compartment, and (3) that the rate of movement of the labeled substance out of the tissue was proportional to the amount in the tissue. These assump-
tions were satisfactory for the specific problem we faced, the analysis of the movement of labeled albumin into and out of tissues. The first and third assumptions were appropriate since a substance, labeled so as not to change its properties and present as a minute fraction of the same unlabeled substance, always moves from a compartment at a rate proportional to the amount of labeled material in the compartment. The second assumption seemed an adequate approximation. In the analysis of our data for labeled cholesterol we face a more complex problem: the second assumption is not appropriate. The mixture of labeled cholesterol within a tissue cannot be considered an instantaneous process in a single compartment. Rather it involves the movement of labeled cholesterol from the interstitial fluid of the tissue into a number of intracellular compartments. This limits the analysis we can make: specifically it prevents us from computing the rate of movement of labeled cholesterol out of the tissue. However, by an analysis similar to that previously made it can be shown that no matter how many compartments a tissue may contain, the initial slope of the plot of the tissue-serum ratio of a labeled substance against time is the rate of passage of that substance into the tissue. If the data are expressed in the units we have chosen, that rate is the amount of labeled substance entering a gram of tissue per day expressed as a fraction of the amount in a milliliter of serum. We have used the initial slopes derived from our data to estimate the rates of passage of labeled cholesterol from serum into the various portions of the inner layer of the aortic wall. These rates are much faster proximally than distally. They are 0.21, 0.16, 0.16, 0.07, 0.04, and 0.04 for sites 1 through 6 respectively.

From these rates what can we infer about the mechanism of movement of cholesterol into the aorta of the normal dog? Several possibilities are apparent. Lipoproteins containing cholesterol as a constituent may move past the endothelial barrier into the aortic wall. The endothelial cells may remove cholesterol from the lipoprotein molecule and transport it into the aortic wall. Another possibility is that labeled cholesterol may pass into the tissue as the result of a chemical exchange between serum and tissue. By chemical exchange we mean in this case the exchange of a molecule of cholesterol in serum lipoprotein with one in tissue without any net movement of cholesterol. This type of process which occurs
TABLE 1.—Concentration* of Cholesterol in the Aorta of the Dog

<table>
<thead>
<tr>
<th>Site</th>
<th>Inner</th>
<th>Middle</th>
<th>Outer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.14</td>
<td>1.27</td>
<td>1.54</td>
</tr>
<tr>
<td>2</td>
<td>1.15</td>
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<tr>
<td>3</td>
<td>1.32</td>
<td></td>
<td>1.87</td>
</tr>
<tr>
<td>4</td>
<td>1.38</td>
<td></td>
<td>1.31</td>
</tr>
<tr>
<td>5</td>
<td>1.60</td>
<td></td>
<td>1.40</td>
</tr>
<tr>
<td>6</td>
<td>1.85</td>
<td></td>
<td>1.31</td>
</tr>
</tbody>
</table>

*The data are expressed as milligrams of cholesterol per gram of aorta.
†The location of the sites and the composition of the layers is described in the section on methods.

The movement of labeled cholesterol into the inner layer of the aorta appears to be without physiologic significance. There are two pieces of auxiliary information that we can use in trying to decide which of the above mechanisms is the major one involved in the movement of labeled cholesterol into the inner layer of the aorta. The first is that the gradient of slopes along the length of aorta is similar to that that exists for the movement of albumin into the aorta. The second piece of auxiliary information is the quantitative relationship between the rates of movement of albumin and the rates of movement of labeled cholesterol into the inner layer of the aortic wall. In a previous paper the rates given for albumin described its movement into the entire thickness of the aortic wall. However, from the data on which that paper is based we can also estimate the rates of entrance of albumin into the inner layer of the aortic wall. These rates are 0.80, 0.67, 0.35, 0.20, and 0.16 for sites 1 through 5 respectively. Thus albumin enters the inner layer 2 to 4 times as fast as labeled cholesterol does. The fact that the rates of movement of labeled cholesterol into the inner layer of the aortic wall at the various sites along its length vary directly with the rates of movement of albumin into these sites and are slower but of the same order of magnitude as those for albumin suggests that labeled cholesterol and albumin move in by a similar process. It suggests that most of the cholesterol moving into the inner layer of the aortic wall is carried in as a part of lipoprotein molecules passing into the aortic wall in a manner similar to albumin.

We have previously supported the concept that albumin enters the inner layer of aorta by passage across the intimal endothelium. It thus appears probable that in the normal dog cholesterol enters in the same fashion as part of normal serum lipoproteins which in the dog are largely a-lipoproteins. The rates we have determined appear to be estimates of the rates of entrance of a-lipoproteins into aortic wall. They should be considered approximations rather than precise estimates since they may have been affected somewhat by the behavior of labeled cholesterol once it was within the aortic wall.

Why proteins move into the aortic wall more rapidly proximally than distally has not yet been experimentally studied. The gradient of rates correlates positively with the circumferential tension exerted on the aortic wall. Circumferential tension is directly proportional to the aortic radius (Laplace's law) and thus is greater proximally. We previously suggested that the greater circumferential tension proximally tends to separate the endothelial cells between which protein flows and thus to permit a more rapid entrance of protein into the wall. The more rapid proximal entrance of proteins into the inner layer of the aortic wall does not appear to be related to the vasa vasorum. In fact in the outer layer of the aortic wall, where these small vessels are concentrated, no gradient of entrance rates for labeled cholesterol exists.

The conclusion that cholesterol enters the inner layer of aortic wall in the normal dog primarily by transport in lipoprotein molecules cannot be generalized to other tissues. The rates of entrance of protein as judged by albumin are much faster for the inner layer of aorta than for many other tissues. In these other tissues labeled cholesterol may enter more rapidly by cellular trans-
The concentration ratios for labeled cholesterol stopped rising about 40 days after ingestion of the tracer and thereafter remained constant. In an experiment such as this if labeled cholesterol exchanges completely with the cholesterol of the tissues and if the serum level of labeled cholesterol is maintained constant, then a gradient of cholesterol concentrations in tissue sites will be exactly repeated by a gradient in the control values of labeled cholesterol. However, if the cholesterol is not everywhere completely exchangeable or if the serum level of labeled cholesterol is falling, the final gradient for labeled cholesterol may differ from that for cholesterol. In our study the final gradient of labeled cholesterol in the inner layer was more extreme than that for cholesterol. The variation in cholesterol concentrations in the outer layer was not repeated by the final concentrations of labeled cholesterol.

A gradient of cholesterol concentrations in aortic wall similar to that we have described was previously found by Werthessen et al. in swine and cattle. The cause of the gradient is not known.

**Summary**

In the normal dog labeled cholesterol passes from serum into aortic wall most rapidly proximally. It enters the wall progressively less rapidly distally along the length of the aorta. It enters the abdominal aorta only one sixth as fast as it enters the thoracic aorta.

A similar gradient of entrance rates into aortic wall is known to exist for albumin. At each level along the aortic length, labeled cholesterol enters about one third as fast as albumin.

This relationship supports the concept that cholesterol is carried into the aortic wall of the normal dog as part of normally occurring lipoprotein molecules.

**ACKNOWLEDGMENT**

We are grateful to Dr. Daniel Steinberg for helpful comments on this work.

**SUMMARIO IN INTERLINGUA**

In canes normal, le passage de cholesterol marcate, ab le sero a in le pariete aortic, es le plus rapide in sitos proximal. In sitos de plus in plus distal al longo del aorta, su passage a in le pariete aortic deveni progressivemente plus lente. Le rapiditate con que illo passa a in le aorta abdominal es solmente un sexto del rapiditate con que illo passa a in le aorta thoracic.

Il es cognoseite que un simile gradation del rapiditate del passage ab le sero a in le aorta existe in le caso de albumina. In omne sito al longo del aorta, le rapiditate del passage de cholesterol marcata e es circa un terio del rapiditate del passage de albumina.

Iste relation supporta le conception que cholesterol es transportate a in le pariete aortic de canes normal como parte de moleculas lipoproteinica de occurrentia normal.

**REFERENCES**


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