Circulation of Labeled Albumin Through the Aortic Wall of the Dog

By LEROY E. DUNCAN, JR., M.D., JEROME CORNFIELD, B.S., AND KATHERIN BUCK, B.S.

Data are presented which support the concept that albumin enters the inner layer of the aortic wall by passage from the lumen of the aorta across the intimal endothelium. The rate of movement of albumin from serum into the aortic wall is much greater proximally where the aorta is wide than distally where it is narrow. Thus the rate correlates with the circumferential tension to which the aortic wall is subjected. The hypothesis is advanced that circumferential tension acts as a major determinant of transendothelial transfer of proteins into the aorta by widening the endothelial intercellular spaces through which the transfer occurs.

THE MOVEMENT of serum lipoproteins through arterial walls is widely held to be of importance in the development of atherosclerosis. It thus appears of interest to study the movement of proteins in general into and out of arterial walls.

Recently we reported work in which data on the circulation of labeled albumin through the aortic wall of the rabbit were analyzed to yield numerical values for the rates of transfer of albumin into and out of that tissue. We are now reporting similar work on the thoracic aorta of the dog, the larger size of which makes possible investigation of the various layers of the wall at different levels along its length. The data support the concept that albumin enters the inner layer of the aortic wall by passage from the lumen of the aorta across the intimal endothelium, and they demonstrate a progressive decrease in the rate of movement of albumin into the wall of the aorta from the origin of the aorta down to the diaphragm. A hypothesis based on the physical stresses in the aortic wall is presented to explain the progressive decrease in these rates.

METHODS

Human serum albumin labeled with radioactive iodine (RISA, Abbott) was dialyzed in cellophane tubing and then injected intravenously into a series of 27 dogs averaging 12 Kg. in weight. The dogs were killed at intervals following injection. Two were killed at 2 minutes, five at 10 minutes, four at 1.5 hours and at 3 hours, and three at 6 hours, at 18 hours, at 3 days, and at 6 days. The radioactivity of the serum and that fraction of the radioactivity which was dialyzable through cellophane, and the radioactivity of skin and of muscle from the chest wall, of Achilles' tendon, of sclera, of cornea, and of aorta, were determined with a well-type scintillation counter. 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 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 aorta below the left subclavian artery was separated into upper, middle, and lower thirds. Each of these was split into two layers: an inner layer consisting of intima and media, and an outer layer consisting of media and adventitia. The outer ascending aorta and arch were designated as site 1, the inner ascending aorta and arch as site 2, and the upper, middle, and lower thirds of the thoracic descending aorta as sites 3, 4, and 5 respectively. The area of the intimal surface of each portion of aorta removed for analysis was determined by tracing it on paper, and weighing the area traced.

Two dogs were injected intravenously with sodium iodide. One was killed at two hours and the other at four hours following the injection.
The tissue-serum ratio of radioactivity was determined in all the tissues listed above. There was little difference between the two hour and the four hour values, so they were averaged.

Two dogs were given a priming dose of sucrose and then maintained on a constant intravenous infusion of sucrose for four hours. The sucrose concentrations of the three layers of the aortic wall and the serum were determined and the fraction of each that was extracellular water was calculated by dividing the tissue sucrose concentration by the sucrose concentration in serum.

Preliminary Treatment of Data

The data obtained from the dogs killed at two minutes were essentially the same as those from dogs killed at 10 minutes. The two-minute data were used only to establish that fact and did not enter into any other computations.

The injected labeled albumin was gradually broken down, releasing dialyzable activity. There was essentially no dialyzable activity in the sera drawn at 10 minutes. In the sera drawn at subsequent times the percentage of the radioactivity that was dialyzable increased from an average of 0.4 at one and one half hours to an average of 2.7 at six days. The dialyzable activity of the serum was subtracted from the total activity to obtain the activity due to labeled albumin. To determine the labeled albumin in the tissue we subtracted dialyzable activity from total activity. For this computation we treated the dialyzable activity as if it were all ionic iodide. This approximation has been justified previously. The dialyzable activity in each tissue was computed as the product of the serum dialyzable activity and the tissue-activity constant for the inner layer of each of the aortic sites was calculated by the equation

\[ C = c/A_s \]

where

- \( C \) = constant
- \( c \) = count in the sample of tissue
- \( A \) = area of the endothelial surface of the sample.

By use of this proportionality constant, the data for inner layers of aorta obtained at subsequent intervals were corrected for labeled albumin on the endothelial surface by the equation

\[ y_s = y_i - a_i R \]

where

\( a_i \) = due to radiiodine on albumin

Mathematical Analysis of Data

The data for the aortic sites expressed for the full thickness of the aortic wall, and the data for the other tissues, are analyzed by methods described elsewhere. We assume that the rate of movement of labeled albumin into each tissue is proportional to the serum concentration of labeled albumin, that the rate of movement out of each tissue is proportional to the tissue concentration of labeled albumin, and that mixing of labeled albumin within the tissue is instantaneous. These assumptions lead to the equation

\[ dy/dt = k_s - k_y \]

where

- \( y \) = amount of labeled albumin per gram of tissue
- \( s \) = amount of labeled albumin per milliliter of serum
- \( k_s \) = amount of labeled albumin transported into a gram of tissue per day expressed as a fraction of the amount in one milliliter of serum
- \( k_y \) = fraction of the amount of labeled albumin in tissue leaving that tissue per day.
FIG. 1. The serum radioalbumin concentration plotted against time. The concentration is expressed on the ordinate as 1,000 times the fraction of the injected dose per kilogram present in 1 ml. of serum. The curve is that obtained by graphical analysis of the data into two exponential components.

The serum concentration can be expressed as a function of time by the equation

\[ s = A_1 e^{-\lambda_1 t} + A_2 e^{-\lambda_2 t} \]  

(2)

where

A and \( \lambda \) = parameters that can be determined from the data

Equation 2 can be substituted in equation 1 and the resulting expression integrated to obtain an integral equation for \( y_s \). Dividing this integral equation by equation 2 yields an expression for \( y_s \) as a function of time. 2 This equation can be fitted to the data so as to obtain least-squares estimates of \( k_1 \) and \( k_2 \), and the tissue-serum ratio due to albumin in serum in capillaries of the tissue and on the endothelium of the aortic intima.

RESULTS

The serum data are shown in figure 1. Graphic analysis of the serum data gave the following values for the parameters of equation 2 which describes the serum concentration as a function of time. \( A_1 \) and \( A_2 \) are respectively 0.0151 and 0.0076 of the dose injected per kilogram of body weight. The estimates of \( \lambda_1 \) and \( \lambda_2 \) are respectively 0.191 and 6.48 per day.

The tissue-serum ratios for skin, tendon, sclera, cornea, and muscle are shown as functions of time in figure 2. The values of \( k_1 \), \( k_2 \), the tissue-serum ratio due to albumin in serum in the capillaries of the tissue and \( k_1/k_2 \) are given in table 1. The ratio \( k_1/k_2 \) equals the steady state ratio of the concentration of albumin in the tissue to that in serum. 2

Immediately after injection of the labeled albumin the amounts of labeled albumin per square centimeter of intimal surface expressed as fractions of the amount in 1 ml. of serum were, for sites 1 through 5 respectively, 0.00092, 0.00080, 0.00074, 0.00063, and 0.00054. These are the values designated by the symbol C earlier. The tissue-serum ratios as functions of time are shown in figure 3 for each layer of each site, and in figure 4 for the full thickness of the aortic wall at each site. The values of \( k_1 \), \( k_2 \), the tissue-serum ratio
CIRCULATION OF ALBUMIN THROUGH AORTIC WALL

Fig. 3. The ratios of radioalbumin in the various sites of the aortic wall to serum radioalbumin plotted against time. Site 1 is the outer ascending aorta and arch, site 2 the inner ascending aorta and arch, and sites 3, 4, and 5 the upper, middle, and lower thirds respectively of the thoracic descending aorta. The curves were fitted by eye.

due to albumin in serum or on endothelium, and of \( k_1/k_2 \) are given in table 1. The data for the layers of aorta are also given in figure 5 as ratios of concentrations in the layers. Before the ratios shown in this figure were computed, the data were corrected for labeled albumin on the intimal endothelium or in capillaries, and expressed as concentration in the extracellular fluid of the aortic layer.

The fraction of each tissue that was extracellular water as measured by the distribution of sucrose was: skin 0.52, tendon 0.40, sclera 0.57, cornea 0.17, muscle 0.14, aortic inner layer 0.76, aortic middle layer 0.61, and aortic outer layer 0.75.

**Discussion**

The transfer rates that we have determined describe the movement of iodinated human albumin through canine tissues at the naturally occurring concentration of albumin. Since the physical properties of albumin from different species of mammals are similar and since light iodination produces no great change in these properties, the transfer rates of iodinated human albumin should be approximations of those of canine albumin. This view receives some support from the finding of Wasserman et al.\(^4\) that iodinated human albumin disappears from serum and appears in lymph at the same rate in dogs as does iodinated canine albumin.

The values for the rates of transfer of albumin into and out of canine skin, tendon, sclera, cornea, and muscle are given in table 1. An interpretation of these rates in terms of transport by filtration and diffusion was given earlier.\(^2\)

There was a demonstrable tissue-serum ratio of labeled albumin for each layer of the
TABLE 1.—Numerical Data on Circulation of Labeled Albumin

<table>
<thead>
<tr>
<th>Tissue</th>
<th>R*</th>
<th>k†</th>
<th>k‡</th>
<th>k.§/k.§</th>
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<td>Aorta #</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>.125</td>
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<tr>
<td>site 2</td>
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<td>.31</td>
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<td>site 4</td>
<td>.031</td>
<td>.19</td>
<td>3.32</td>
<td>.057</td>
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<tr>
<td>site 5</td>
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<td>.12</td>
<td>2.22</td>
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<td>.117</td>
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<td>.106</td>
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<tr>
<td>Tendon</td>
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<td>.042</td>
<td>.76</td>
<td>.055</td>
</tr>
<tr>
<td>Sclera</td>
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</tr>
<tr>
<td>Cornea</td>
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<td>.030</td>
<td>.35</td>
<td>.086</td>
</tr>
<tr>
<td>Muscle</td>
<td>.006</td>
<td>.038</td>
<td>1.04</td>
<td>.036</td>
</tr>
</tbody>
</table>

*Ratio of the amount of albumin in 1 Gm. of tissue to that in 1 ml. of serum due to albumin in serum or on endothelium.

†Amount of albumin entering 1 Gm. of tissue per day expressed as a fraction of the amount in 1 ml. of serum.

‡Amount of albumin leaving 1 Gm. of tissue per day expressed as a fraction of the amount in that gram of tissue.

§Ratio of the amount of albumin in 1 Gm. of tissue to that in 1 Gm. of serum which would exist under steady-state conditions.

Site 1 = outer ascending aorta and arch; 2 = inner ascending aorta and arch; and 3, 4, and 5 = upper, middle, and lower thirds respectively of the thoracic descending aorta.

The aortic wall at each site when the tissues were first obtained two minutes after injection of the albumin. Essentially the same ratios were present 10 minutes after injection of the albumin. There is nothing to suggest that these early ratios were due to transeptal transfer of labeled albumin. The subsequently observed rates of transfer were much too slow to account for them. Probably the 10 minute values of the inner layer are primarily due to labeled albumin on the intimal endothelium, and the 10 minute values for the middle and outer layers are probably due to labeled albumin in capillaries in those layers. The 10 minute values for the inner layer were highest at the proximal aortic sites and decreased progressively as the sites became more distal. Presumably this difference is due to some change along the length of the aorta in the endothelial surface which is known to be complex with evaginations of lumen into the cell surfaces and with a layer of serum protein applied to the surface of the intercellular cement joining the cell margins. Anatomical work has never established to everyone’s satisfaction the presence or absence of capillaries in the middle layer of canine aorta. Our finding of labeled albumin in that layer almost immediately after injection supports the view that such capillaries do exist. As shown in figure 3, the 10 minute values for the outer layer were lowest proximally and increased progressively along the length of the aorta. This probably was due to a denser capillary supply to the distal portion of the outer layer of the aorta.

The ratios of the concentrations of labeled albumin in the aortic layers plotted in figure 5 show that the concentration of labeled albumin rose most rapidly in the inner aortic layer, less rapidly in the middle layer, and least rapidly in the outer layer. This sequence indicates that albumin enters the inner layer directly from serum, and thus supports the concept that albumin passes from the lumen of the aorta across the intimal endothelium into the inner layer of the aortic wall. The data do not exclude the possibility that albumin also enters the middle and outer layers directly from the capillaries in those layers. Indeed, the fact that capillaries are definitely present in the outer layer and probably present in the middle layer makes direct entrance of albumin into these layers from serum seem probable.

From figure 5 it is apparent that no large gradient of albumin concentration is maintained between the various layers of the aortic wall at any one site. The major barrier to the flow of albumin into and through aortic tissue appears to be at the endothelial surfaces.

For determination of the transfer rates into and out of each site, we have analyzed the data expressed for the full thickness of the aortic wall. The transfer rates estimated on this basis are given in table 1. The value for the inflow rate (k1) was greatest at the proximal aortic sites, and decreased progressively
as the sites became more distal, until in the terminal portion of the thoracic aorta it was about a third of the proximal value. The values for the outflow rate \( k_2 \) varied somewhat from site to site, but there was no trend similar to that for the inflow rate. Thus the value of the ratio \( k_1/k_2 \) which equals the steady state ratio of the concentration of albumin in the aorta to that in the serum was highest proximally and decreased progressively as the sites became more distal. The progressive decrease in the inflow rates from the proximal to the distal end of the aorta is not caused in any way by differences in the lateral blood pressure, since this is the same along the length of the aorta. The decrease in inflow rates does correlate positively with a progressive decrease in the radius of the aorta and thus with a progressive decrease in circumferential tension as described by Laplace's law. The decrease in the radius of the aorta is somewhat less than that in the inflow rate for albumin. For example, in a dog of the size we studied, aortic radii after
death at sites 1 and 2 (which are at the same level) and at sites 3, 4, and 5 were respectively 5.4, 3.8, 3.2, and 2.9 mm.

The circumferential tension to which the aortic wall is subjected tends to separate the endothelial cells from each other, thus widening the spaces between them. Albumin flows through the intercellular cement which bridges these spaces and should flow more rapidly where they are wider. Thus it appears possible that in those portions of the aortic wall where circumferential tension is high the endothelial cells are pulled farther apart, permitting a more rapid inflow of albumin. If this is in fact the case, the thicker arterial walls which exist in the regions of higher circumferential tension must not quite overcome the effect of the tension in separating the cells. This hypothesis that we have offered is similar to that advanced by Wasserman and co-workers\textsuperscript{11} to explain their studies of the movement of macromolecules from serum into lymph. They postulated that expansion of the blood volume increased capillary permeability to macromolecules by stretching the capillary walls and thus the pores in the intercellular cement between endothelial cells.

**Summary**

The rates of movement of albumin into and out of the aorta, skin, tendon, sclera, cornea, and muscle of the dog have been determined by analysis of the time course of serum and tissue concentrations following intravenous injection of labeled albumin.

Data obtained by splitting the aorta into inner, middle, and outer layers support the concept that albumin enters the inner layer of the aorta from the lumen of the aorta by passage across the intimal endothelium. No large concentration gradients for albumin are maintained within the aortic wall.

The rate at which albumin enters the wall of the thoracic aorta decreases progressively from its proximal to its distal end. Since the rate at which albumin leaves the aorta does not decrease along the length of the aorta, the steady state concentration of albumin in the aortic wall decreases in proportion to the decrease in inflow rate.

The inflow rates along the length of the aorta correlate positively with the circumferential tension. To explain this finding, the hypothesis is advanced that circumferential tension acts as a major determinant of the rate of transendothelial transfer by widening the endothelial intercellular spaces through which the transfer occurs.

**Acknowledgment**

We are indebted to Mr. Elliott Cramer for having programmed for the IBM 650 the computations required to obtain least-squares estimates of \( k_1, k_2, \) and \( B \).

**Sommario in Interlingua**

Le intensitate del movimento de albumina in e ex le aorta, pelle, tendine, sclera, cornea, e muscolo canin esseva determinate per le analyse del progresso in le tempore del concentrationes serum e histal de albumina a marcage post injectiones de illo per via intravenose.

Datos obtenite per finder le aorta in stratos interne, medio, e exterior supporta le concepcion qne albumina penetra le strato interne del aorta ab le passage del aorta per cruciar le endothelio intimal. Nulle grande gradientes de concentration de albumina es mantenite intra le pariete aortic.

Le intensitate con qne albumina penetra le pariete del aorta thoracique decresce continuemente inter su extremitates proximal e distal. Viste que le intensitate con qne le albumina quita le aorta non decresce in le curso del aorta, le concentration de stato constante del albumina in le pariete aortic decresce in proportion con le reduction del intensitate de influxo.

Le intensidades de influxo al longo del aorta es positivemente correlationate con le tension circumferential. Pro explicit iste constata- tion, le hypotheses es formulate que le tension circumferential age como un major determinante del intensitate del transferimento trans- endothelial per allargar le spatios intercellular endothelial via le quales le transferimento es effectuate.
REFERENCES

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_Circ Res._ 1959;7:390-397
doi: 10.1161/01.RES.7.3.390

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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