Active Ion Transport Across
Canine Blood Vessel Walls

PHILIP N. SAWYER, JOEL LEVINE, ROGER MAZLEN, and
IGNATIUS VALMONT

From the Department of Surgery and Surgical Research of the State University of New York
Downstate Medical Center, Brooklyn

ABSTRACT Experiments giving evidence of active Na and Cl ion fluxes across large canine blood vessel walls (aorta, vena cava) in vitro have been presented. The information has been obtained using ion tracer techniques after Ussing and with diffusion cells of the Hogben type. The available data suggest that the membranes are satisfactorily oxygenated by the bathing solutions saturated with oxygen at atmospheric pressure. Evidence is offered which indicates that active ion transport does occur across the aorta and vena cava in in vitro experiments. Under the conditions of the experiment net Na and Cl flux takes place from intima to adventitia across the aorta, and from adventitia to intima across the vena cava at low measured potential differences. The possible relationships of derangement of active ion transport mechanisms, produced by electric currents and tissue injury potential differences, to intravascular thrombosis are alluded to. It would appear that sodium and chloride fluxes across large blood vessel walls in vitro occur at least in part as the result of metabolic processes and cannot be explained simply on the basis of diffusion across a semipermeable membrane.

Applied electrical fields and those created by currents of injury in injured tissues appear related to intravascular occlusion of blood vessels (1–3). Abramson, Gorin, and Ponder (4), Furchgott and Ponder (5), Ponder and Ponder (6), and others have indicated that the cellular elements of blood at a pH of 7.40 move toward the positive pole in an electrophoretic cell. Vascular occlusion can be potentiated in normal vessels by application of a positive electrode (7, 8). Occlusion is delayed in an injured blood vessel exposed to the field about a cathode (9, 10). Therefore it is postulated that potential differences in the physiologic range and related currents of injury in perivascular tissues may act as causal agents in intravascular occlusion, by means of their electrophoretic or other effects upon the cellular and possibly liquid elements of the contained blood.

As a corollary, it is reasonable to hypothesize that the blood vessel wall
ordinarily performs work in order to neutralize transmural potential differences and adventitious currents occurring in the living organism possibly by the active transport of ions. If this were not possible, minor injuries could result in a chain of events causing progressive, unrelenting intravascular occlusion and widespread ischemia with necrosis. Thus, it seemed reasonable to postulate that one function of the blood vessel wall consists of active transport of ions to control transvascular ion concentration and electric charge. The experiments described in this paper are the first of a series designed to test this possibility.

**EXPERIMENTAL TECHNIQUE, MATERIALS, AND METHODS**

**Experimental Membranes**

It was necessary to measure ion fluxes across blood vessel walls in vitro in order to obtain accurate measurements. The dog blood vessel wall was used as the experi-

| TABLE I  
KREBS SALINE SERUM SUBSTITUTE |
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Mix:</td>
</tr>
<tr>
<td>--------------------------------</td>
</tr>
<tr>
<td>80 parts 0.90 per cent NaCl (0.154 M)</td>
</tr>
<tr>
<td>4 parts 1.15 per cent KCl (0.154 M)</td>
</tr>
<tr>
<td>3 parts 0.11 M CaCl₂</td>
</tr>
<tr>
<td>1 parts 2.11 per cent KH₂PO₄ (0.154 M)</td>
</tr>
<tr>
<td>1 parts 3.82 per cent MgSO₄·7 H₂O</td>
</tr>
<tr>
<td>21 parts 1.5 per cent NaHCO₃ (0.154 M); treated with CO₂ until pH is 7.4</td>
</tr>
<tr>
<td>4 parts 0.16 M Na-pyruvate (or L-lactate)</td>
</tr>
<tr>
<td>7 parts 0.1 M Na-fumarate</td>
</tr>
<tr>
<td>4 parts 0.16 M Na-L-glutamate</td>
</tr>
<tr>
<td>5 parts 0.5 M 5.5 per cent glucose</td>
</tr>
</tbody>
</table>

* Prepared by neutralizing a solution of the acids with M NaHCO₃ solution.

| TABLE II  
BLOOD VESSEL WALL PREPARATIONS |
<table>
<thead>
<tr>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Aorta</td>
</tr>
<tr>
<td>1. Fresh</td>
</tr>
<tr>
<td>2. One day storage at 4°C.</td>
</tr>
<tr>
<td>B. Separated aortic wall layers</td>
</tr>
<tr>
<td>1. Intima (inner layer preparation)*</td>
</tr>
<tr>
<td>2. Adventitia (outer layer preparation)‡</td>
</tr>
<tr>
<td>3. Intimal preparation alone</td>
</tr>
<tr>
<td>C. Vena cava</td>
</tr>
<tr>
<td>D. Control membranes</td>
</tr>
<tr>
<td>1. Dialysis membrane</td>
</tr>
<tr>
<td>2. Parchment paper</td>
</tr>
</tbody>
</table>

* Henceforth called intima for sake of brevity.
‡ Henceforth called adventitia.
mental membrane because it was easily accessible and because oxygen utilization studies had already been performed on the canine aorta by Kirk, Effersoe, and Chiang (10). They report that canine and human aortae consume oxygen for several weeks after being removed from the experimental animal, and give rates for oxygen uptake by the aortic wall.

Vascular membranes for this experiment were obtained from mongrel dogs sacrificed by bleeding, intravenous pentothal, or air embolism. The membranes included canine descending thoracic aorta and thoracic superior and inferior vena cava which were removed within 3 to 4 minutes after death. The vessel was placed immediately in Krebs' saline serum substitute (11) (Table I), and either stored at 4°C, or used immediately. Aorta was incised longitudinally between the paired intercostal vessels. Prepared membranes were placed between ion flux chambers. Most experiments were run in quadruplicate. In the first ninety-seven experiments, the entire thickness of aortic wall was used. In the next series of fifty experiments the intima and subintimal layers were separated from the muscularis and adventitia in the endarterectomy plane (Fig. 1). The inner layers (intima and elastica interna, Table II) alone, or matching separated inner and outer layers of the vessel were used for certain of the differential ion flux experiments. Experiments were also carried out using the vena cava wall as the experimental membrane (Table II).
Experimental Technique

Each of four specimens from the same blood vessel was placed between diffusion cell pairs of the Hogben type (12, 13) (Fig. 2). Ten ml of Krebs' serum saline substitute at pH 7.40 and a temperature of 37°C (11), was placed in each of the cells to bathe each side of the specimen. The solutions were oxygenated with 95 per cent O₂–5 per cent CO₂ which bubbled in at the bottom of the cell. Each membrane had an exposed surface area of 1.0 sq. cm.

After 15 to 20 minutes for equilibration, the radioactive isotopes were added to the donor solution. Experiments run for as long as 10 hours showed evidence of water evaporation with ion concentration. These findings resulted in our using a duration of 4 hours for an experiment.

Radioisotope Measuring Techniques

Sodium movement across the aortic wall was first determined using 1 microcurie of Na¹⁰⁸ in the donor solution. The initial experiments were done cumulatively so that if net unidirectional transport occurred it would be demonstrable after 2 to 3 hours.
because of progressive separation of the curves of Na flux in opposite directions, even though progressive cell asymmetry would result in increasing back diffusion as net transport progressed in one direction. In subsequent experiments 1 microcurie of Na$^{22}$ or Na$^{24}$ and 1 microcurie of Cl$^{36}$ each diluted with 0.9 per cent NaCl were used to determine non-cumulative differential fluxes of the two ions across the same segment of blood vessel wall.

Na$^{24}$ is received as Na$_2$CO$_3$: 5 to 10 mg/10 millicuries. This was diluted by large amounts of 0.9 per cent NaCl in each instance so that the isotope was added to the bathing solution essentially as physiologic saline solution. 250 microcuries of Cl$^{36}$ are received as 2 cc of dilute hydrochloric acid. After neutralization with sodium bicarbonate, the Cl$^{36}$ was further diluted with 250 cc of 0.9 per cent NaCl to make 1 $\mu$C Cl$^{36}$ per cc.

One microcurie of each of the isotopes was placed in the bathing solution on the intimal side of the membrane in two of the cells and on the adventitial side of the membrane in the remaining two pairs of cells. After mixing, 1 cc of donor solution was removed from each cell, dried, and counted, yielding a master donor count for calculation of the per cent of isotope which traversed the membrane per hour.

At the end of each hour after the experiment had begun, the entire recipient solution was removed from the flux cells to limit the amount of back diffusion. One cc samples of recipient solution were dried in aluminum planchets and counted.

**Counting Techniques**

Counts of the beta emission from both Na$^{24}$ and Cl$^{36}$ were taken using a Baird atomic model 132 scaler and a shielded gas flow Geiger-Muller counter. Duplicate counts have been found accurate to 2 per cent. The determination of Na$^{22}$ counts was carried out by a single count. Initial and repeat determinations for differential Na$^{24}$, Cl$^{36}$ counts were made several days apart. After an initial count, immediately after drying, which effectively measured the emission for both isotopes, the Na$^{24}$ emission was allowed to decay through ten to eleven half-lives (6 days to 1 week) leaving only Cl$^{36}$ as the material to be counted. The initial Na$^{24}$ determinations were corrected for decay during the experiment because of its short half-life (15.2 hours).

Flux was calculated in micromoles, per square centimeter of membrane, hour of experiment. Mean hourly fluxes for each experiment were calculated. The duplicate mean fluxes in each direction were determined, and net flux determined by subtracting efflux from influx. A "T" test for significance of the experimental data in each group of experiments was calculated.

The sign test was occasionally used to obtain a quick comparison of experiments in any given group.

In an attempt to check the magnitude of absorption of radioactive isotope by the membrane itself, as a source of discrepancy in the determinations, the experimental membranes were themselves counted at the end of 50 experiments. At no time did radioisotope concentrations within the membrane at the end of a 4 hour experiment, exceed 0.1 to 0.5 per cent of the total isotope placed in the solution on the donor side of the cell (14). Does the isotopic loss caused by hourly removal of the isotope in the
recipient solution appear to affect the experiment? In the aortic experiments less than 2 to 5 per cent of total isotope traversed the membrane per hour from the donor to the recipient solution. At the end of 4 hours, this amounted to 8 to 20 per cent of the total isotopic and non-isotopic Na and Cl in the donor solution.

In the vena caval experiment net fluxes were five to ten times those of the aortic experiment and this possibility appears more possible. However, the Na and Cl flux curves flatten out following the 1st hour and it would appear that the decrease in available donor ion does not result in an appreciable decrease in flux. Thus the Na and Cl flux remains fairly constant once the isotopes of Na and Cl reach equilibrium across the membrane.

The possibility of Na or Cl leak out of the blood vessel wall membrane in sufficient quantity to cause an effective change in Na and Cl concentrations in the bathing solutions must be considered. In a recent article Bevan (15) (Table III) has indicated that rabbit aorta has an Na concentration of 87.1 m.eq/kg, which after bathing 4 hours in Krebs' saline solution increased to 115.0 ± 3.7 m.eq/kg. Based on these figures and assuming that dog and rabbit aortae do not contain grossly different amounts of Na, the total Na content within the exposed aortic membrane (1 cm²) is 17.8 to 23.5 μ eq/cm² of exposed membrane and for the vena cava if the membranes are comparable, 12.6 to 16.6 μ eq/cm² (Table III). In addition the available information indicates that Na⁺ and by inference Cl⁻ increases rather than decreases within the membrane during an experiment. Since each side of the diffusion cell contains 1694 to 1848 μ moles of NaCl (depending on whether 11 or 12 cc of Krebs' solution are placed in the cells), even total unidirectional extravasation of non-isotopic NaCl from membrane into the bathing solution would change isotope concentration less than 1 per cent.

During experiments duplicate isotope flux determinations in each direction, intima to adventitia, and adventitia to intima, were obtained. Gross discrepancies between the duplicate determinations were considered as an indication of poor mixing, cell leak, or defect in the membrane, and these required that the experiment be discarded.

### Table III

<table>
<thead>
<tr>
<th></th>
<th>Concentration of Na in rabbit blood vessel bathed in Krebs saline, m.eq/kg</th>
<th>Average wet net weight, 1 cm² of canine blood vessel wall (Sawyer)</th>
<th>Total Na content for canine blood vessel</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Beginning</td>
<td>After 4 hrs.</td>
<td>Beginning</td>
</tr>
<tr>
<td>Aorta</td>
<td>87.1</td>
<td>115.0</td>
<td></td>
</tr>
<tr>
<td>Vena cava</td>
<td>No direct data available</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Figure in parantheses is number of determinations
‡ Assuming that vena cava has approximately the same chemical composition per unit weight as aorta.
From Bevan (15).
Control flux experiments were performed using a 5 μm/pore dialysis semipermeable membrane between the cells.

At the beginning and end of the first fifty experiments pH's were measured to ascertain the extent of change during the course of the experiments. The largest pH changes found during the course of an entire experiment were in the range of 0.1 pH unit.

**Electrical Measurements**

Electrode bridges containing Krebs' agar were placed in close approximation on each side of the membrane and connected via two calomel half-cells to a high impedance recording potentiometer for determination of transmembrane potentials.

The spontaneous, electrical potential of the aortic wall in this arrangement was measured on approximately one hundred occasions. The measured asymmetry of the circuit (liquid junction asymmetry due to the fluid interface and calomel cell asymmetry) at the bridge junction was at most 0.1 mv in any given experiment after calibration. This asymmetry was measured by placing the bridge tips in a common Krebs' solution pool. The potentiometer was calibrated daily to 0.05 mv against a standard calibrator accurate to 0.01 mv. The stated random instrumental error of the Leeds and Northrup recorder is 1 percent of the full scale reading. The potentiometer was set at zero before and after each measurement of potential difference. The electrode asymmetry was monitored continuously in instances when potential differences were recorded for an entire experiment, since spontaneous membrane potential differences were small.

**Experimental Variables**

In an effort to determine the physical chemical characteristics of blood vessel walls with respect to ion fluxes across them, several experimental variables were introduced. These included experiments to determine the effects of:

A. Using vena cava as well as aorta for the flux determinations (Table II).
B. Aerobic and anaerobic conditions on matched pairs of specimens from the same aorta, or the effects of intermittent aerobic and anaerobic periods on ion fluxes across the same specimens of aorta and vena cava. Duplicate experiments were not run in this group of experiments. Two cells were run under aerobic conditions, two under anaerobic conditions.
C. Varying temperatures on ion flux rates and direction (16).
D. Separating the blood vessel wall into layers to determine which, if any, are predominantly responsible for ion fluxes. The experiments in this group have been limited to the aortic wall.

**Results**

The combined results for several groups of studies completed during the first three hundred paired flux experiments using aortic and vena caval wall
are shown in Table IV. No statistical evidence of net transport was found in the control experiments using dialysis membrane as the control membrane (Table IV, row 8).

Net transport of both sodium and chloride ions occurs from intima to adventitia across the aortic wall \textit{in vitro} under the conditions of this experiment (Table IV, row 1).

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|c|}
\hline
Experimental conditions & No. of experiments* & Net Na flux & No. of experiments & Net Cl flux & \\
Vessel & Gas & Direction & Flux magnitude & P value & Direction & Flux magnitude & P value & \\
\hline
1. Fresh aorta & O$_2$ & 15 & 1 $\rightarrow$ A & 1.4 & 0.004 & 10 & 1 $\rightarrow$ A & 1.25 & 0.015 \\
2. External aorta & O$_2$ & 8 & 1 $\rightarrow$ A & 1.3 & 0.03 & — & — & — & — \\
1 day & & & & & & & & & \\
3. Aorta & N$_2$ & 6 & A $\rightarrow$ I & 3.9 & 0.001 & — & — & — & — \\
4. Aorta & O$_2$ & 15 & A $\rightarrow$ I & 9.8 & 0.001 & 6 & A $\rightarrow$ I & 15.8 & 0.005 \\
intima & & & & & & & & & \\
5. Aorta & N$_2$ & 6 & None & — & 0.35 & — & — & — & — \\
intima & & & & & & & & & \\
6. Aortic adventitia & O$_2$ & 8 & None & — & 0.21 & — & — & — & — \\
7. Vena cava & O$_2$ & 8 & A $\rightarrow$ I & 15.5 & 0.02 & 8 & A $\rightarrow$ I & 18.0 & 0.013 \\
8. Dialysis membrane control & O$_2$ & 11 & None & — & 0.98 & 11 & None & — & 0.50 \\
\hline
\end{tabular}
\caption{Table IV}
\end{table}

* All membrane preparations from one blood vessel are considered one experiment.
‡ Mean hourly flux from four hourly determinations.

Dominant sodium ion flux across the 1 day old canine aortic wall stored at 4°C is also from intima to adventitia and of similar magnitude (Table IV, row 2). Experiments to determine chloride flux across stored aortic wall have not been carried out.

Results with paired aerobic and anaerobic specimens from the same aorta indicate that anaerobic conditions cause reversal of net sodium flux (Fig. 3), (Table IV, row 3), (16). Under anaerobic conditions net sodium flux occurs from adventitia to intima (Table IV, row 3).

\textit{Aortic Inner Layer and Outer Layer Experiments}

In an additional series of experiments, matched pairs of separated inner and outer layers of the aorta were run in an effort to determine, which if any, layer was predominantly responsible for net sodium and chloride flux. In
another series the inner layer preparation alone was used. One notes that net transport for both sodium and chloride across the inner (intimal and subintimal) layers occurs from the adventitial to intimal side, just the op-

![Graph](image)

**Figure 3.** Plot of mean hourly sodium flux across the aortic wall of fourteen pairs of matched aerobic, anaerobic experiments. Note the reversal of net aerobic Na flux when anaerobic conditions are imposed. Net flux is also greater under anaerobic conditions. This increase has not been explained.

- A → I, indicates flux from adventitia to intima (influx).
- I → A, indicates flux from intima to adventitia (efflux).
- N₂ → I, indicates perfusion of cells with 95 per cent N₂-5 per cent CO₂.
- O₂ → I, indicates perfusion of cells with 95 per cent O₂-5 per cent CO₂.

posite of that which occurs across the intact aortic wall. There is no net flux on either an individual experimental or on a statistical basis across the outer (medial and adventitial) layers of the aortic wall in these experiments (Table IV, rows 4 and 6), (Fig. 4).

If the experiment is carried out under anaerobic conditions, the aortic
intima displays no statistical evidence of net sodium flux in the first small series of six experiments (Table IV, row 5). Determinations for chloride ion have not yet been completed.

**Figure 4.** Comparison of net Na flux across matched inner and outer layer aortic preparations. These studies indicate that the inner histologically intact aortic layers are predominantly responsible for net Na$^+$ and probably Cl$^-$ flux.

**Vena Cava**

Net transport of both sodium and chloride across the vena cava occurs from adventitia to intima (Table IV, row 7). The magnitude of this net flux is approximately five to ten times that of the aortic flux with respect to both
ions, and takes place, in the opposite direction (Table IV: compare aortic magnitude and direction, rows 1–2, with vena caval magnitude and direction, row 7).

One also notes that the net flux across the inner layers of the aortic wall is similarly from adventitia to intima with a magnitude approximately five times that for the intact aortic wall. Thus, under identical conditions the inner aortic layers seem to parallel the vena cava both with respect to direction and magnitude of net ion transport of both sodium and chloride ions.

The voltage determinations indicate that the spontaneous in vitro P.D. of aortic and vena caval wall is very low, of the order of 1 mv for aorta and even smaller for vena cava. The data indicate, as originally demonstrated in vivo (14), that the intima is slightly negative to the adventitia under the

<table>
<thead>
<tr>
<th></th>
<th>Mean P.D.</th>
<th>No. of determinations</th>
<th>T test for significance</th>
<th>Probability that number is not significantly different from zero</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aorta</td>
<td>0.995</td>
<td>42</td>
<td>1.879</td>
<td>0.032 (3.2 per cent)</td>
</tr>
<tr>
<td>Vena cava</td>
<td>0.019</td>
<td>19</td>
<td>0.457</td>
<td>0.030 (30 per cent)</td>
</tr>
</tbody>
</table>

conditions of the experiment; i.e., with the vessel bathed with identical solutions on each side. The spontaneous measured aortic transmural P.D.'s have ranged from 8 to 0.9 mv with a mean P.D. = 0.99 mv. Vena caval P.D.'s average much closer to and not statistically different from zero with a mean calculated P.D. = 0.019 mv (Table V).

**Discussion**

These experiments indicate that active transport of Na and Cl ion does occur across large canine blood vessel walls. When statistical analysis of results of every experiment is made, a net flux of sodium and chloride is found for each of the vessel membranes studied. This flux occurs in the virtual absence of an electrical gradient.

For any univalent ion when ion movement across the membrane is entirely passive:

\[
\frac{f_i}{f_o} = \frac{C_i}{C_o} Z
\]
where $f_i = \text{flux in}$

$f_o = \text{flux out}$

$C_i = \text{concentration of ion in question bathing the inside of the wall}$

$C_o = \text{concentration of ion bathing the outside of the wall}$

$Z = e^{BF/RT}$

$C_i = C_o$ in these experiments.

At 37°C the P.D. in millivolts = 61 log $f_o/f_i$, if ion movement across the membrane is passive (17).

For both the sodium and the chloride ions, the ratios of outflux to influx differ in the three preparations, the aorta, the aortic inner layer, and the vena cava, from ratios predicted on the basis of passive diffusion and the measured potential differences (Table VI). Therefore, according to the definition of Rosenberg (18), Na and Cl must be crossing the membrane by active transport.

It is possible that other ions such as K, Ca, PO$_4$, and Mg are being transported by the vessel wall. No experiments to measure fluxes of these ions in opposite directions have yet been carried out. If active transport is going on, either ions with like charge must be transported in opposite directions, or those of opposite charge in the same direction to keep transmural P.D.'s at a low level. Since Na and Cl most frequently appear to be transported in the same direction, their charges would tend to cancel each other.

Experimental evidence that the measured net flux is not an artifact due to membrane asymmetry or non-mixing, is obtained from various elements of the experiments. Measured sodium and chloride net fluxes are shown to occur in opposite directions when intact aortic wall is compared to either vena caval wall or the aortic inner layer preparation; the magnitude of net flux is greater for both inner aortic wall preparations and vena cava than could be

**Table VI**

<table>
<thead>
<tr>
<th>Sodium</th>
<th>Chloride</th>
</tr>
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<tbody>
<tr>
<td>Mean P.D.</td>
<td>Mean P.D.</td>
</tr>
<tr>
<td>calculated</td>
<td>measured</td>
</tr>
<tr>
<td>from flux ratios</td>
<td>P value</td>
</tr>
<tr>
<td>Aorta</td>
<td>43.1 (39)†</td>
</tr>
<tr>
<td></td>
<td>1.0*</td>
</tr>
<tr>
<td>Vena cava</td>
<td>-74.05 (16)</td>
</tr>
<tr>
<td></td>
<td>0.01*</td>
</tr>
</tbody>
</table>

* Possibility that the calculated P.D. values, based on Ussing’s equation, and the measured P.D.’s for aorta and vena cava are not significantly different.

† Numbers in parenthesis are the number of determinations.
explained on the basis of differences in thickness between these membranes and the intact aortic wall. Net transport across the aortic wall occurs in opposite directions in matched aerobic and anaerobic experiments. It would appear improbable that the various differences listed would occur consistently if the membranes studied were only passively permeable.

Calculations have been made concerning the possibility that the aortic wall might be too thick for satisfactory oxygenation and that the observed phenomenon of reversal of flux occurs for this reason.

**TABLE VII**

<table>
<thead>
<tr>
<th>Wall thickness</th>
<th>QO₂</th>
<th>Min.</th>
<th>Max.</th>
<th>Mean</th>
<th>Min.</th>
<th>Max.</th>
<th>Max. permissible thickness, cm‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Warburg (19), Stevenson (20), Hill (21)</td>
</tr>
<tr>
<td>Aorta</td>
<td>0.20</td>
<td>0.06</td>
<td>0.10</td>
<td>0.030</td>
<td>0.62</td>
<td>QO₂ = 0.03</td>
<td>QO₂ = 0.62</td>
</tr>
<tr>
<td></td>
<td>1.15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.15 cm</td>
<td>0.245</td>
</tr>
<tr>
<td>Vena cava</td>
<td>0.038</td>
<td>0.025</td>
<td>0.030</td>
<td>0.49</td>
<td>0.59</td>
<td>QO₂ = 0.49</td>
<td>QO₂ = 0.59</td>
</tr>
<tr>
<td></td>
<td>0.29</td>
<td></td>
<td></td>
<td>0.29</td>
<td></td>
<td>0.29</td>
<td>0.26</td>
</tr>
</tbody>
</table>

* No of determinations.
† Maximum permissible thickness in centimeters as calculated from:
Warburg's (19) equation:
\[ d' = \sqrt{8 C_o \times D / A} \]

Where \( d' \) = limiting thickness of the membrane.

\( C_o = \) oxygen tension in atmospheres.

\( D = 1.64 \times 10^{-4} \) cc of O₂/min. through 1 cm² of tissue with a pressure gradient of 1 atm/cm at 37°C. Value from Krogh's original calculation.

\( A = \frac{QO₂ \times 1000}{1000 \times 60 \times \text{wet weight/dry weight ratio}} \)

Stevenson and Smith (20)

Wet weight ratio for canine and human aorta = 5.00 (26).

Equations from Warburg (19), Stevenson (20), and Hill (21) indicate that the aortic wall of maximum thickness equal to 0.245 to 1.15 cm is thin enough to be oxygenated satisfactorily by a bathing solution on each side (saturated with O₂ at one atmosphere pressure) at the QO₂ for canine aortic wall determined by Kirk et al. (10) and for canine vena cava by Lee, Yu, and Sawyer (22), Table VII.

Since net Na flux across the aorta is not abolished but reversed on switching from aerobic to anaerobic conditions, we must postulate at least two opposing active transport mechanisms. Studies by Kirk et al. (10) show that approximately 60 per cent of the energy produced by aerobic metabolism is produced by anaerobic glycolysis, indicating that energy for reversal of active transport is available under anaerobic conditions.
Paradoxically net Na and Cl aortic flux occurs in opposite directions across the intact aortic wall when compared to the aortic inner layer preparation. Two possible explanations for reversal of transport can be postulated. One of these would indicate that since no active transport of Na or Cl takes place across the media and adventitia of the aortic wall in these experiments (Table IV, row 6), these layers act as a limiting factor in ion influx, since ions must diffuse through the aortic media to get to the intima and become available for transport.

The available evidence indicates that the aortic inner layers act as the effective flux membrane. Blood vessels appear to penetrate the outer layers of the arterial wall in vivo to the level of the inner media where they form a capillary plexus (Fig. 1). It would appear probable that ion transport in vivo need only occur across the intact inner layer membrane of the arterial wall, the necessary ions being transported to and from these layers through the vasa vasorum and capillaries (23). This perfusion source is lost when the vessel is removed from the experimental animal. When one separates the inner layer from the remainder of the aortic wall, the amount of sodium and chloride which can diffuse to the inner intimal cellular membrane through the adventitial side of the inner layer preparation probably approximates more closely that which occurs in vivo. Ion flux rates for the aortic inner layer preparation approach ion flux rates across the vena cava. This explanation implies that Na and Cl flux in vivo takes place from adventitia to intima.

A second possible explanation for aortic sodium and chloride ion efflux when compared to the aortic inner layer preparation influx suggests that upon separating the layers, considerable injury is produced in the aortic wall. The injury so produced may result in reversal of the net transport. It has not yet been possible to determine which if either of these two arguments is more valid. Additional studies are being carried out.

Do these experiments in any way relate to normal ion transport across the blood vessel wall in vivo? The experiments are carried out under conditions which do not approximate those of the vessel in vivo. It would be helpful if the results could be compared to those of in vivo experiments carried out to study the same problem. Evidence from several investigators indicates that the cerebral capillary wall does not obey simple Donnan equilibrium requirements (24, 25). This may be an indication that the phenomena herein described occur in vivo. Whether or not transvascular active transport is actually related to vascular homeostasis and the prevention of intravascular thrombosis due to injury currents or injury potentials in surrounding tissue, or other resultant thrombotic mechanisms has still to be determined.

The phenomena described obviously relate to ion movement between the intravascular-extravascular space. If transport occurs in vivo as it does in vitro, it can be postulated that ion efflux on the arterial side of the vascular
tree and ion influx on the venous side of the vascular tree are at least partially due to active transport.

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