Renal, Transcapillary, Net Exchange in the Dog

By Edward D. Freis, M.D., Harold W. Schnaper, M.D., John C. Rose, M.D. and Lawrence S. Lilienfield, M.D.

The transcapillary net exchanges of inulin, para-aminohippurate, thiocyanate and heavy water were determined in the renal circulation. It was found that the tracer substances larger than heavy water diffused more freely through renal than through previously studied peripheral or pulmonary capillaries. The results are consistent with the concept of a larger pore size in renal capillaries.

Previous studies have been concerned with the net, transcapillary exchange of diffusible tracer substances in the human forearm, and in the lungs. The patterns of net exchange have been elucidated for heavy water, thiocyanate, sodium, rubidium, S4-labeled sulfate, and inulin. With this experience as a background it seemed of interest to explore the renal circulation using similar technics. There has long been evidence that renal capillaries have a greater permeability than peripheral capillaries.

Methods

Mongrel dogs were used weighing 10 to 16 Kg. The animals were anesthetized with sodium pentobarbital, 25 mg./Kg. A femoral vein was catheterized and then the ipsilateral renal vein. The two catheters were connected so that the entire renal venous drainage on that side was diverted into the femoral vein. The mixture of tracer substances was injected into the renal artery at a constant rate over a 3 to 6 sec. period. Immediately prior to the injection the coupling between the renal and femoral vein was broken and the renal venous outflow collected at 2 sec. intervals into a series of heparinized, paraffined test tubes. Analyses of the injected mixture and of the blood samples and methods of calculation were carried out as described previously. The labeled substances under study which diffuse across capillary walls are mixed with a substance which does not pass through capillary walls to any appreciable extent in a single circulation.

The "expected" concentration of each of the diffusible substances in each sample was then calculated as follows: \( C'_{x} = \frac{C_{x} - C_{m}}{C_{m}} \times 100 \), where \( C_{x} \) is the "expected" concentration of the substance diffusing across the capillary walls, \( C_{x} \) the concentration of this substance in the injecta, \( C_{m} \) the concentration in the injecta of the reference substance which does not diffuse across the capillary wall, and \( C_{m} \) the respective sample concentration of the latter nondiffusing, reference substance. Thus, \( C_{x} \) is the concentration of the diffusing substance that would have been present in the sample if there had been no net transcapillary exchange of the substance. The net per cent transcapillary loss of the substance in any sample can then be calculated as follows: \( \text{per cent loss} = \frac{C_{x} - C_{r}}{C_{x}} \times 100 \).

The "equilibrium time" as defined in this report signifies a point in time rather than a steady state. As the diffusible substance moves across the capillary endothelium the concentration gradient increases in the interstitial fluid. However, as the "bolus" of intravascular material is swept away from the area in the flowing blood stream, the extravascular concentration eventually will exceed the intravascular. When this occurs net return will exceed net loss. The intermediate point at which net loss and net return are in balance (zero per cent loss) is termed the "equilibrium" time. When the curves of actual and expected concentrations are plotted on the same graph the equilibrium time is represented by their point of crossing.

Inulin was analyzed by the method of Schreiner and para-aminohippurate (PAH) by the method of Chasis et al. In some experiments T-1824 was used and in others 1H-labeled human serum albumin (RISA) or Cr51-labeled red cells. A portion of the mixture was saved for later analyses and the remainder injected into the renal artery. The venous samples and the aliquot of the injected mixture were analyzed for their concentrations of the various labeled substances.

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and midpoint of injection. Errors can be introduced by such corrections since time subtractions from curves with short circulation times will have a greater percent effect than the same correction from curves with short circulation times will have a greater percent effect than the same correction applied to curves with longer circulation times. The extent of the error is dependent upon the spread in any single experiment.

RESULTS

The results of simultaneous determinations of the transcapillary exchange patterns of inulin, PAH and thiocyanate in 8 dogs are listed in Table 1. Deuterium oxide also was injected in 6 of these dogs. Dogs B1 and B2 received C14-labeled inulin and samples were analyzed in the same manner as previously reported for S84-labeled methionine.6 Dogs 13, 15, 16, 17 and 21 received Cr51-labeled red cells and RISA. The remaining dogs received

albunin (RISA) and Cr51-labeled red cells (tables 1 and 2).

Time corrections were made for catheter delay and midpoint of injection. Errors can be introduced by such corrections since time subtractions from curves with short circulation times will have a greater percent effect than the same correction applied to curves with longer circulation times. The extent of the error is dependent upon the spread between the circulation times of the compared substances. In the present study the spread between the compared peak times or the "equilibrium" times is small so that the errors are small. The spread is larger, however, in comparing the mean circulation times of Cr51-labeled erythrocyte and inulin and the results of the latter must be regarded as being only semiquantitative. It should be pointed out that comparative differences between the various tracer substances were being measured. This minimizes errors in estimating absolute transit times since the tracer substances were well mixed prior to injection and time corrections were identical for all substances in any single experiment.

TABLE 1.—Maximum Per Cent Loss and Peak Concentration and Equilibrium Times in Renal Circulation of the Dog

<table>
<thead>
<tr>
<th>Dog</th>
<th>Maximum loss (%)</th>
<th>Time of max. loss (sec.)</th>
<th>Peak concentration time (sec.)</th>
<th>Equilibrium time† (sec.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inulin PAH SCN D₂O</td>
<td>Inulin PAH SCN D₂O</td>
<td>T-1824 or RISA</td>
<td>Inulin PAH SCN D₂O</td>
</tr>
<tr>
<td>11</td>
<td>90 79 81 96</td>
<td>4 4 4 4</td>
<td>6 17 16 11 9</td>
<td>12 12 12 14</td>
</tr>
<tr>
<td>12</td>
<td>82 98 64 92</td>
<td>2.5 4.5 3.5 3.5</td>
<td>3.5 6.5 16 6.5 6.5</td>
<td>8.5 23 18 23</td>
</tr>
<tr>
<td>13</td>
<td>78 96 81 94</td>
<td>1.5 1.5 1.5 1.5</td>
<td>3.5 5.5 5.5 5.5 5.5</td>
<td>8.5 6.5 8.5 16.5</td>
</tr>
<tr>
<td>14</td>
<td>94 93 74 91</td>
<td>3.5 3.5 3.5 3.5</td>
<td>4.5 6.5 6.5 6.5 8.5</td>
<td>10.5 7.5 8.5 7.5</td>
</tr>
<tr>
<td>15</td>
<td>75 93 73 96</td>
<td>1 1 1 1</td>
<td>1 2 2 3 2 2</td>
<td>4 5 5 5 4</td>
</tr>
<tr>
<td>16</td>
<td>77 69 78 92</td>
<td>1 1 1 1</td>
<td>2 4 4 3 4 4</td>
<td>5 4 4 6</td>
</tr>
<tr>
<td>B1</td>
<td>93 94 85 14</td>
<td>1 1 1 1</td>
<td>10.5 13 16 12</td>
<td>18 19 17</td>
</tr>
<tr>
<td>B2</td>
<td>49 94 93 59</td>
<td>2 2 2 2</td>
<td>10 10 12 10</td>
<td>12 14 14</td>
</tr>
<tr>
<td>Av.</td>
<td>79 95 77 93</td>
<td>2.1 2.3 2.2 2.4</td>
<td>5.1 8.1 9.9 7.1 6.6</td>
<td>9.8 11.4 10.9 11.8</td>
</tr>
<tr>
<td>S.D.</td>
<td>15 2 10 2</td>
<td>1.2 1.5 1.3 1.9</td>
<td>3.4 5.4 5.5 3.7 2.0</td>
<td>4.4 6.4 4.9 7.3</td>
</tr>
</tbody>
</table>

* Per cent loss = Expected concentration — actual concentration × 100

Expected concentration

Concentration in inlets

† Equilibrium time is the time at which the expected and actual concentrations are equal.1

‡ Dogs 13 through 16 received 1H2—labeled albumin (RISA). The remainder received T-1824.

TABLE 2.—Mean Circulation Time (T) of Cr51-labeled Red Cells, 1H2-labeled Human Serum Albumin and Apparent T of Inulin in Five Dogs in Which the Three Tracers were Injected Simultaneously

<table>
<thead>
<tr>
<th>Dog</th>
<th>Mean circulation time* (sec.)</th>
<th>TCr51-RBC</th>
<th>TCaso-RBC</th>
<th>TCr51-Inulin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cr51-RBC</td>
<td>RISA</td>
<td>Inulin</td>
<td>TCr51-RBC</td>
</tr>
<tr>
<td>13</td>
<td>4.0 4.5 21</td>
<td>.89</td>
<td>.19</td>
<td>1.5 2.5 3.3 6</td>
</tr>
<tr>
<td>16</td>
<td>2.2 2.8 8</td>
<td>.79</td>
<td>.28</td>
<td>17 6.2 7.3 13</td>
</tr>
<tr>
<td>21</td>
<td>6.2 7.2 14</td>
<td>.86</td>
<td>.44</td>
<td>20 4.2 5.0 14.4</td>
</tr>
<tr>
<td>S. D.</td>
<td>1.7 2.1 6.1</td>
<td>.05</td>
<td>.11</td>
<td></td>
</tr>
</tbody>
</table>

* Mean circulation time was defined as the sum of the products of the sample concentrations multiplied by their times of collection divided by the sum of the sample concentrations. Those integrations were carried out to the time at which the sample concentration had fallen to 5 per cent or less of the peak concentration.

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Fig. 1. Time-concentration curves of T-1824, C14-labeled inulin, p-aminohippurate, and thiocyanate in dog Bl. The curves for inulin, PAH and SCN have been adjusted (by referring to their relative concentrations in the injected mixture) so that their relationship to the T-1824 concentrations represents the percentage of net loss from or gain to the circulation at any moment in the curve. Thus, the T-1824 curve can also be used to represent the "expected concentration" for each substance and the crossing of each curve with the T-1824 downslope is the "equilibrium time" for that substance. Note delayed peaks and early equilibrium times for inulin, PAH and SCN.

T-1824 as the nondiffusible tracer substance.1

Obvious differences were apparent between the renal and the previously studied forearm or pulmonary circulations.1-8 The first of these was the delay in peak concentrations of all of the diffusible substances as compared to the nondiffusible reference substance (fig. 1). The delay averaged 3.0 sec. for inulin, 4.8 sec. for PAH, 2.0 sec. for thiocyanate, and 3.4 sec. for deuterium oxide (table 1). This was contrary to previous experience in the forearm where, with the exception of deuterium oxide, the peaks of these substances always were simultaneous with the peak of T-1824.

The second striking difference of the renal from the forearm or pulmonary patterns of transcapillary exchange was the marked net losses of all of the diffusible substances in the early portion of the transit curves. During the upslope period of the T-1824 or RISA curves the losses of the diffusible substances averaged 79 per cent for inulin, 95 per cent for para-aminohippurate and 77 per cent for thiocyanate. This compares to average initial period losses in the forearm of approximately 25 per cent for inulin,4 50 per cent for para-aminohippurate4 and 50 per cent for thiocyanate.2 The average loss of thiocyanate in the pulmonary circulation was only 5 per cent.3 The transcapillary loss of deuterium oxide in the dog kidney during this period was comparable to that previously observed in the forearm and lung averaging 93 per cent. The time of these maximum losses was similar for all substances and averaged 2.3 sec. (table 1).

Another difference between forearm and renal circulation was the early marked return of the diffusible substances to the circulation (fig. 1.) Early marked loss followed by a rapid return would be reflected in the delayed peaks which were observed. At the time of the respective peak concentrations (when losses of molecules larger than water are near their maximum in the forearm) the average net per cent transcapillary losses in the kidney had fallen to 29 for inulin, 33 for PAH and 37 for thiocyanate. Heavy water again behaved as it did in the forearm, dropping to a low value of 7 per cent at its peak concentration.

The "equilibrium times" (points in time at which net per cent return equals net per cent loss3) of inulin, PAH and thiocyanate, averaged 9.8, 11.4, and 10.9 sec. respectively (table 1). The ratio of the "equilibrium time" to the peak time of the nondiffusible tracer averaged 1.9 for inulin, 2.2 for PAH, and 2.1 for thiocyanate. In the forearm these ratios for PAH and thiocyanate averaged 10 and 6.7 respectively. The transcapillary exchange of inulin was measured in the forearm of 5 subjects.4 In 2 of these the ratio was greater than either PAH or thiocyanate, while in 3 an equilibrium point failed to occur during the experimental period. This was interpreted as indicating considerable restraint to back flow into forearm capillaries due to the large molecular size of inulin. Thus, in the kidney the "equilibrium time" was essentially the same for all molecules, including
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inulin, and occurred much sooner in the transit curves of these substances than it did in the forearm. The difference was especially pronounced in the case of inulin. In the forearm the ratio of the equilibrium time of deuterium oxide to the peak time of T-1824 averaged 9.2, while in the kidney it averaged 3.3.

In 5 dogs the mixture injected into the renal artery contained not only RISA and RISA and inulin but also Cr^-labeled red cells. The mean circulation time (T) for each of these substances was calculated in order to compare their available volumes. In every case the mean circulation time of albumin was prolonged in comparison to red cells. The ratio of T c^-RBC to T RISA averaged .83 (table 2). In the same animals the ratio of T c^-RBC to T inulin averaged .36. Thus, the rapidly equilibrating space available to inulin appeared to be considerably larger than that available to albumin.

DISCUSSION

The differences noted in this study between the renal circulation of the dog and the human forearm or hind leg of the dog were as follows: (1) delayed rather than simultaneous peak times of the diffusible as compared to the nondiffusible tracer substances, (2) greatly increased net percentage losses of diffusible substances larger than water (especially inulin) in the early portion of the transit curves, and (3) reduced ratio of "equilibrium" to peak times indicating a more rapid rate of return of diffusible substances into the circulation.

These observations are consistent in many respects with those of Chinard, Vosburgh and Ennis who utilized almost identical experimental technics. In the dog kidney they observed early losses of inulin, PAH, thiocyanate and deuterium oxide that were very similar in percentage to those reported here. They also noted a significantly lower percentage loss of inulin in the dog's hind limb, similar to our observations in the forearm, but did not comment on its significance.

Whereas Chinard did not interpret his data as indicating any fundamental difference in the structure of the capillary walls in different regions of the body, we believe that the various data collected by our group and also by Chinard strongly suggest differences in the size of the openings through which diffusion occurs in the capillaries of the lungs, forearm and kidney.

The delay in peak concentrations of the diffusible molecules as compared to T-1824 or I^131-labeled albumin in the renal circulation indicates not only marked early loss but also a rapid and marked return of these substances to the circulation. This could have occurred either by back diffusion across the glomerular endothelium, or the endothelium of peritubular capillaries, or interstitial tissue capillaries, or by tubular reabsorption. The rate and extent of return was the same for inulin as it was for the other substances. Since there is good evidence to support the view that inulin is not reabsorbed in the tubules the observed early return of the diffusible substances signifies back diffusion rather than tubular reabsorption.

In the forearm there was a direct relationship between early net losses from the circulation and molecular size. This ranged from 90 to 95 per cent in the case of the smallest molecule, heavy water, and 50 per cent for thiocyanate, to 20 to 25 per cent for the largest molecule, inulin. In the renal circulation, however, no such relationship was found. The early net percentage losses of inulin, PAH and thiocyanate were much higher than in the forearm and were approximately equal. It seems reasonable to assume that the capillaries of the interstitial tissue of the kidney are similar in structure to those in interstitial tissues elsewhere, and that if the high rate of net loss was occurring through the renal interstitial tissue capillaries it also should have been observed in the forearm. This difference and other evidence discussed below suggests that the massive early net losses occurred in the glomerular and peritubular capillaries.

An additional argument favoring the con-
cept of large openings in certain renal capillaries is the observation that the rate of return of noncolloids to the circulation also was not influenced by molecular size. The "equilibrium times"\(^2\) were no different for inulin than for thiocyanate. This relationship was strikingly different than in the forearm where the "equilibrium time" for inulin was absent or greatly delayed compared to thiocyanate reflecting inability of this larger molecule to return back readily into circulation. The shortening of equilibrium times for the smaller molecules larger than water (thiocyanate and PAH) in the kidney as compared to the forearm also indicates larger openings for diffusion. Obviously the larger the openings in the diffusion membranes the more readily back flow can occur.

These physiologic observations correlate well with anatomic data obtained with the electron microscope. Gautier, Bernhard and Oberling\(^1\) were the first to note the unusually attenuated and porous nature of the glomerular endothelium. The openings measure approximately 0.1 \(\mu\) m.\(^1\) They also discovered that interdigitating processes arose from the visceral epithelium forming slit-pores variously measured at 0.04 \(\mu\) m and 100 \(\AA\) wide\(^1\) which open into the glomerular space. Pease\(^1\) found that the endothelium of cortical peritubular capillaries is similarly attenuated and porous; the pore size being approximately half that of the glomerular capillaries. Pease did not see such capillaries elsewhere in the body; he believes that they represent a specialization to facilitate diffusion processes.

In the present studies the comparative mean circulation times indicated that the rapidly equilibrating space available to inulin was almost three times larger than that available to red cells and more than twice as large as that available to albumin. This large rapidly equilibrating space for inulin is far greater than can be accounted for by the total volume of all the glomerular spaces suggesting relatively unhindered diffusion of inulin through peritubular as well as glomerular capillaries.

**Summary**

A mixture of tracer substances was injected into the renal artery of the dog. Time-concentration curves derived from collection of renal venous blood permitted estimation of net transcapillary exchange. The data were consistent with the concept of a larger pore size in renal as compared to peripheral capillaries. This is in agreement with anatomic information obtained from electron microscopy. The magnitude of net exchange indicated relatively unrestricted diffusion of molecules as large as inulin but smaller than albumin.

**SUMMARIO IN INTERLINGUA**

Un mixtura de substantias tracıatori esseva injicite in le arteria renal de canes. Curvas de tempore: concentration pro le sanguine reno-venose permitteva le estimation del nette excambio transcapillar. Le datos obtenite supportava le concepto que le capillares renal es characterisate per plus grande poros que le capillares peripheric. Isto es de accordo con le information anatomic obtenite per microscopia electronic. Le magnitude del excambio nette indicava un relative-mente libere diffusion de moleculas con dimensiones usque a illos de inulina sed inferior a illos de albumina.

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