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□ Original Article □

Compartmental Analysis of RBC Circulation through the Rabbit Kidney

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This experiment involved 12 rabbits of both sexes, weighing 2.1 kg. After anesthesia, the kidneys were exposed, isolated and cannulated in the renal artery, ureter and sometimes in the vein as well. The kidney were perfused through the renal artery with Krebs-Henseleit solution, which were then filtered to be free of particles, gased with 95% O_2-5% CO_2, and kept at 37℃. We measured RBCs concentrations by means of Coulter Counter in the venous outflow collected, and plotted them against the volume perfused. Using 2 different flow rates, 9 ml/min (group I) and 19 ml/min (group II), we found that the RBCs decreased in a multiexponential decay fashion and a biophysical model for each flow rate was constructed. These models indicated that there were more cell stores (2.20×10^{10}) in the fast compartment of group II than in group I (1.72×10^{10}). This difference is not statistically significant, but certainly coincides with urine flow collected from ureter cannula during perfusion. Our present data clearly suggest that in order to clear 99% blood cells out of 10~12 gm rabbit kidneys, at least 3~6 ml of cell free perfusate is required while clearing the whole blood cells out of human kidneys (200~240 gm) may need 600 ml or more. Thus, we recommend that at least 600 ml of perfusate should be used to clear most of the blood cells in the renal vasculature before renal transplantation is performed.

Key Words: Compartmental analysis, renal vasculature, washout kinetics

The kidney is a unique organ with respect to blood circulation and urine formation. Its major functions are to excrete metabolic waste products from the body fluid and to maintain a constant internal milieu. In order to carry out these functions, the kidney requires sufficient blood flow (25% of cardiac output) and performs i) filtration (GFR) ii) absorption (Tm) and iii) secretion (active pump, counter current systems). Therefore, the renal microcirculation is built to meet the specifications of its complicated functions.

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Anatomically, renal vasculatures are well known to scientists (Thurau 1964; Beeuwkes 1980; Pallone *et al.* 1990). However, the reports on blood flow between cortex and medulla or RBCs flow through vasa recta and afferent-efferent systems are scarce because the kidney is a solid organ (Jones 1983). In the early 1950s, Pappenheimer and Kinter (1956) proposed a hypothesis called "PLASMA SKIMMING" by which the hematocrit ratio varied in different segments of kidney tissues. Therefore, hemoconcentrated parts such as Henle's loop receive slow blood flow, while hemo-diluted part may receive faster flow because of low viscosity. However, this separation of blood flows at the microcirculation level was only hypothetical. Because the kidney is a solid organ, it is very difficult to study the internal blood flow distribution. Tracer methods were applied using radioisotopes (McNay and Abe 1970; Katz *et al.* 1971; Bankir *et al.* 1979; Aukland 1980; Karberg *et al.* 1982) but the range of detectable degrees in the low magnifications of

microcirculation was limited.

In our present study, we have attempted RBC (red blood cell) washout kinetics assuming the whole kidney as a black box unknown and giving an input (0 cells in infusate) signal and collecting the outflow (cell counts of various rate constants). Thus in building a systematic model, we may characterize the black box as a collection of compartments showing various washout kinetics. This method has been applied on many organs such as spleens (Song and Groom 1971), hearts (Song 1975) and skeletal muscle (Groom *et al.* 1973) but not on renal circulation. Therefore, out report is the first one in presenting a wide range of cell counts from 10^3 to 10^{10} with 0.0000001 accuracy. In addition to the biophysical model, we have also intended to prove histologically the presence of slow compartments in the renal tissue.

MATERIALS AND METHODS

Animal preparation

We used 12 rabbits of both sexes whose body weights were approximately 2.1 kg (see table 1). All the subject animals were anesthetized through a IV butterfly needle into an ear vein with Ketamine hydrochloride (initial dose 25 mg/kg; and thereafter intermittently until the termination of experiments).

After midline laparotomy, both kidneys were exposed and the ureter was cannulated with a plastic cannula (id, internal diameter: 1 mm). A polyethylene tube (id: 1 mm) filled with heparin (100 IU) was also inserted into renal artery. Sometimes the renal vein was also cannulated but most of the time the renal vein was cut open to collect venous outflow by draining gravimetrically. As soon as cannulation had been set, the minipump (Renal system, Inc. Minneapolis, MN 55441, RS-7800) began to pump the cell-free perfusate into renal artery and venous outflow from the renal vein was coollected continuously for cell counts and volume measurements. The perfusate and the kidney were kept at 37°C throughout the experiment and the infusion pressures were recorded by means of a physiological pressure transducer (Gould) and physiography. The typical tracings of the pressure recordings are shown in Fig. 1 as obtained during 3 different flows.

We infused the kidneys with 2 different flow rates, 9 ml/min and 19 /ml/min and compared the results between 2 groups. Since the flow rate is dependent on hydrostatic pressures, we examined the flow rate at different pressures (9 ml/min and 19 ml /min). After perfusing for over an hour, the kidneys were preserved in 10% Formalin solution for histological examinations.

Table 1. Physical characteristics of rabbits

	Group I	Group II
Body wt. (kg)	2.1±0.67	2.1±0.67
Kidney wt. (g)	11.5±1.82	12.1±2.30

Values are mean±SE.

Perfusate

Krebs-Henseleit solution is composed of NaCl 118, KCl 4.8, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 24, Glucose 11 mmoles in 1L of distilled water. The solution was filtered through a millipore filter (GSWP 04700, 0.22 μm) sometimes more than twice, using a mixed gas (95% O_2+5% CO_2) throughout the experimental period.

Cell counting

We have counted RBCs from venous outflow along with the volume collected by means of Coulter Counter (Model ZM) from which we have also recorded volume distribution curves via pulse height analyzer (Coulter channelyzer 256) and printer (Fujitsu DX 2100). Fig. 6 shows mostly RBCs at the peak range and small fractions of larger volume as WBCs and smaller volume for platelets and some particles much smaller than RBCs. We have also corrected the cell counting by subtracting background counts of the cell-free infusate from each count.

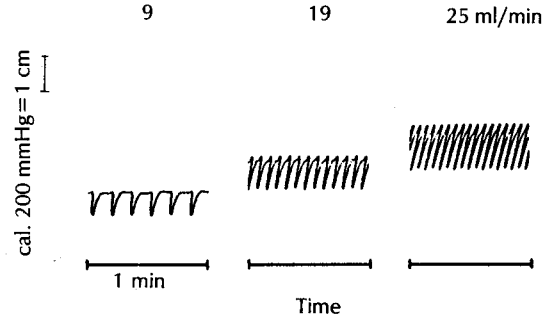


Fig. 1. *Recording of perfusion pressure vs. time, during the perfusion of renal arteries. The pressure tracings were steady in most cases.*

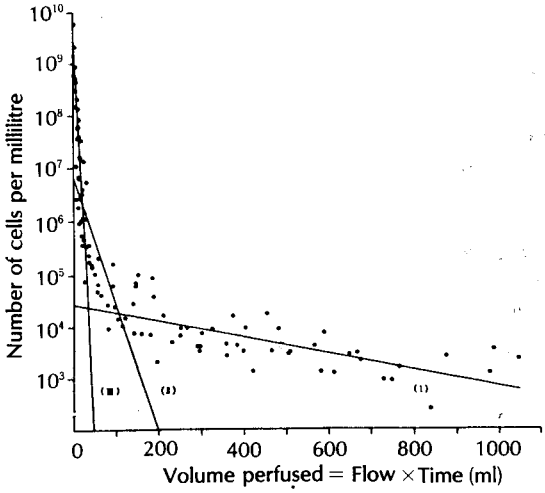


Fig. 2. *Red blood cell concentrations in the outflow obtained from 7 isolated kidneys. Red cell numbers are plotted against the cumulative volumes of fluid perfused with 9 ml/min on a semi-logarithmic scale. The washout curve may be expressed as the sum of three exponential components I, II and III.*

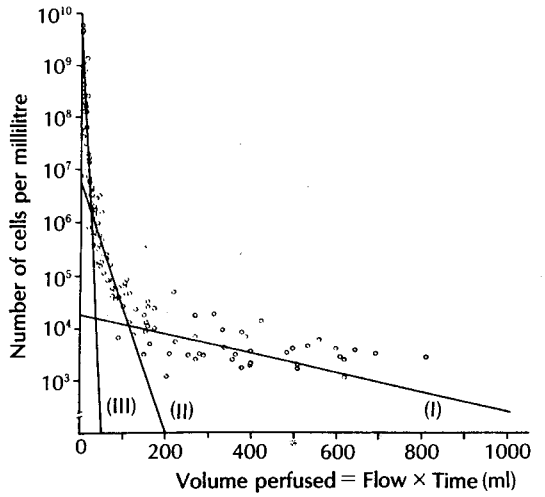


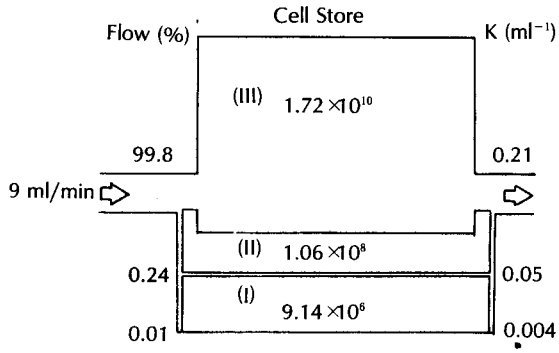
Fig. 3. *Red blood cell concentrations in the outflow obtained from 7 isolated kidneys. Red cell numbers are plotted against the cumulative volumes of fluid perfused with 19 ml/min on a semi-logarithmic scale.*

Urinalysis

Urine flow was measured U_{Cl} and U_{osm} were measured by means of Osmetter (Precision Osmometer Model 2007, Precision system Inc.) and Chloride Titrator (Americal Instrument Co.). The data are presented in Figs. 7 & 8.

Data Analysis

Since an exponential term mathematically represents washout from a single compartment (mixed-



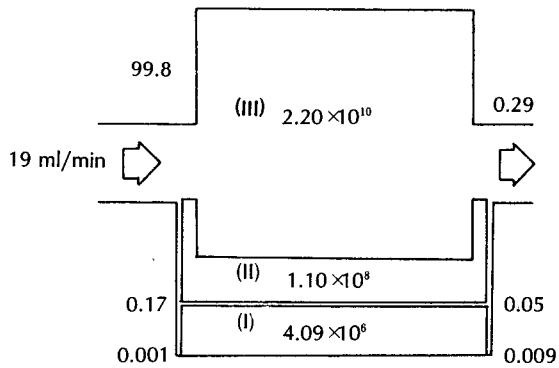


Fig. 4. *Three compartment, simple biophysical models of circulation of RBCs through kidney determined from analysis of washout curves obtained during perfusion with 9 ml/min (above) and 19 ml/min (below). Flow (%), flow to a compartment (% of total kidney inflow); cell store, RBC content in a compartment; k, rate constant for washout of compartment (ml^{-1}). I, fast compartment; II, intermediate compartment; III, slow compartment.*

flow vessel), the cellular washout curves were fitted to an equation consisting of a series of n exponential terms. The red cell counts (cells/ml) determined at 1 ml intervals during the first perfusing were plotted on a semilogarithmic scale as a function of the volume of perfusate collected (ml) from the renal vein. Since the curves were nonlinear, more than one exponential term was needed to describe the cellular washout ($n>1$) adequately. Therefore, the washout data for each group were fitted to a

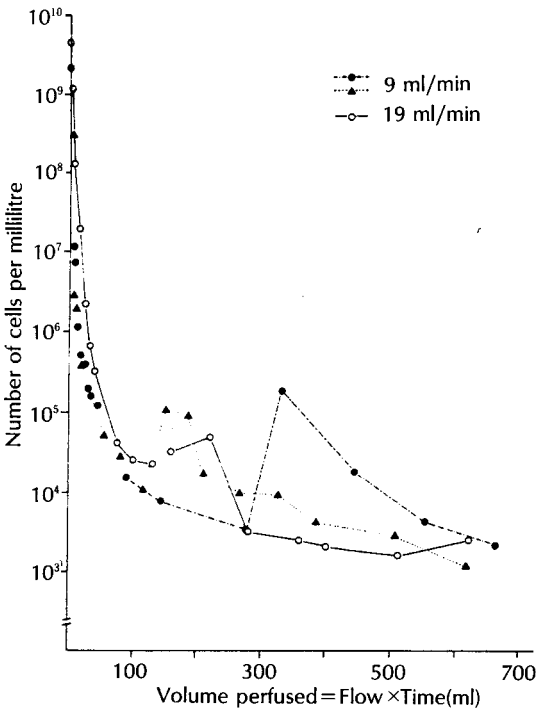


Fig. 5. *Red blood cell concentrations in the outflow obtained from 3 isolated kidneys (group I, group II). Red cell numbers are plotted against the cumulative volumes of fluid perfused on a semi-logarithmic scale.*

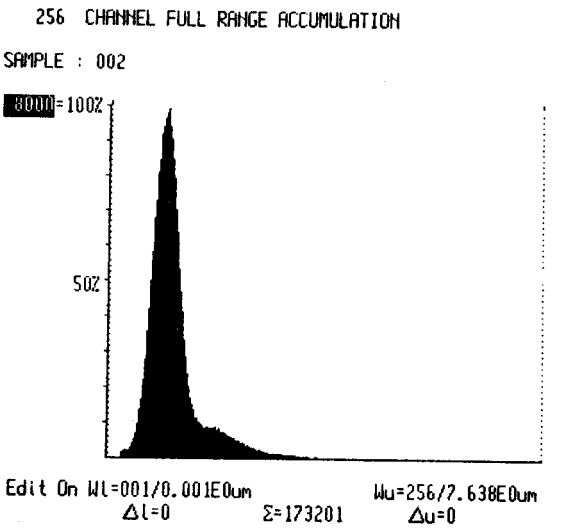


Fig. 6. *Typical RBC volume distribution curve of the outflow. It is comprised of RBCs and some small amount of platelets and WBCs. Y axis indicates number of cells recorded as frequency of pulses and on X aixs, the size of pulses eg. as cell volume or cell diameter, is shown. The peak points is approximately 80 to 100 μm^3 for RBCs and the right side hump represents larger cells such as white blood cells.*

Table 2. Analysis of red cell washout from the renal vessels of isolated rabbit kidneys perfused with 9 ml/min

Parameter	Component			Total
	I	II	III	
Intercept C_0 (cells, ml^{-1})	$2.58 \times 10^4 \pm 5.31 \times 10^3$	$6.67 \times 10^6 \pm 3.25 \times 10^6$	$3.49 \times 10^9 \pm 6.05 \times 10^8$	$3.49 \times 10^9 \pm 6.53 \times 10^8$
Rate const. k (ml^{-1})	$3.86 \times 10^{-3} \pm 2.85 \times 10^{-4}$	$4.76 \times 10^{-2} \pm 9.63 \times 10^{-3}$	$2.08 \times 10^{-1} \pm 2.68 \times 10^{-2}$	
% of total flow	<0.01	0.244±0.12	99.8±0.011	100
Cell stroe C_0/k (cells)	$9.14 \times 10^6 \pm 2.66 \times 10^6$	$1.06 \times 10^8 \pm 5.96 \times 10^7$	$1.72 \times 10^{10} \pm 2.45 \times 10^9$	$1.73 \times 10^{10} \pm 2.42 \pm 10^9$
% of total RBC	0.069±0.026	0.79±0.52	99.1±0.54	100

Values are the mean±SE of 7 experiments.

multiexponential curve and then the parameters for each term in the series were obtained by subtracting the contribution of each exponential term from the curves with a curve-peeling technique (Jacquez 1972).

Mathematically, the linear equation, $\log y = \alpha V + \log\beta$ fits the straight line in Figs. 2 & 3, where y represents the cellular concentrations and V, the volume perfused (flow x time), α the slope, and β the intercept respectively. Since the slope is negative, this linear equation is rewritten in a form of $y = \beta e^{-\alpha V}$ where β is the initial concentration of the compartment and α is the slope. Thus we could estimate V_{half} from the intercept and rate constant k ($0.693/V_{half}$). From this analysis we are able to estimate both the total number of red cells from the renal system and the pattern of their distribution in the vasculature. The total red cell store (C_n) of a compartment is the area under each straight line in Figs. 2 & 3 and is obtained by evaluating the integration of the exponential function from zero to infinity;

$C_n = \int_0^\infty \beta e^{-\alpha V} dV = \beta/\alpha$

Table 2. summarizes the results of the compartmental analysis. All the values (mean ± SE) were tested with t test at the 95% confidence level.

RESULTS

The infusion pressures shown in Fig. 1 resulted

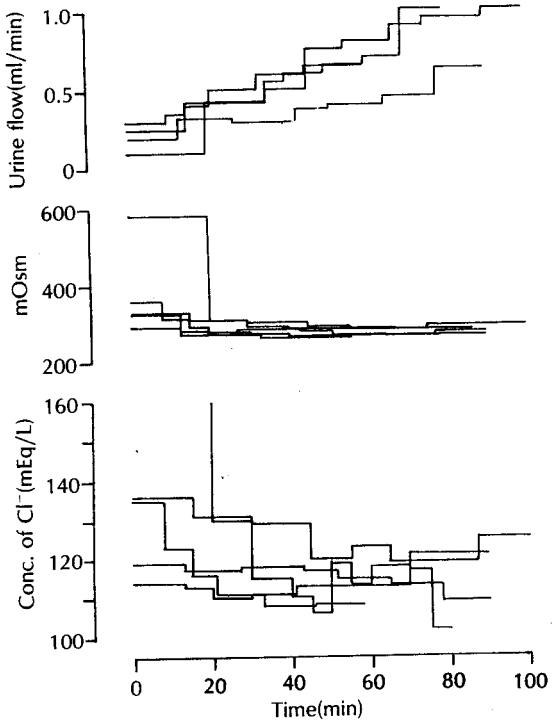


Fig. 7. *Time course of urine flow, osmolality and concentration of chloride in urine excreted by left and right kidneys after infusion into the renal artery of Krebs-Henseleit soluation with 9 ml/min.*

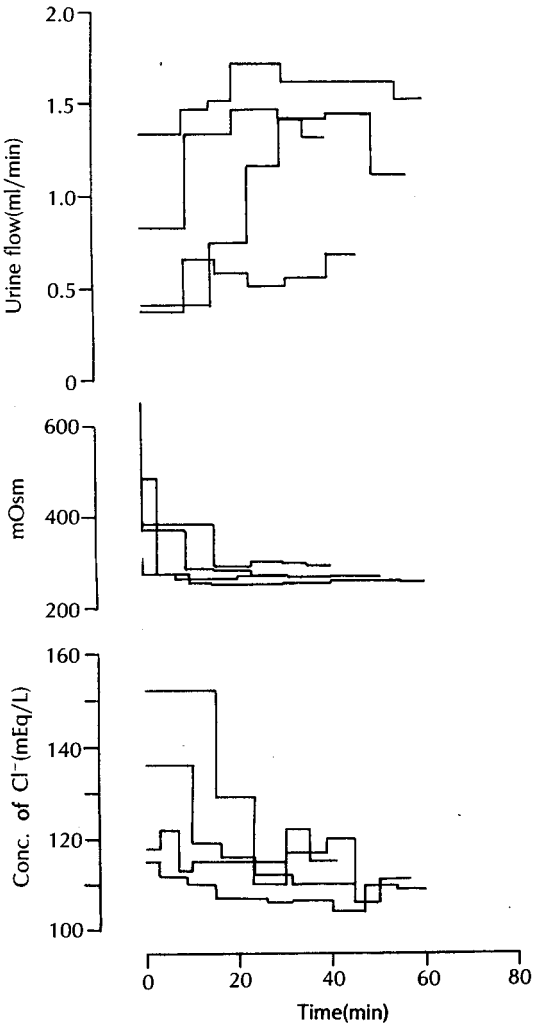


Fig. 8. *Time course of urine flow and osmolality, concentration of chloride in urine excreted by left and right kidneys after infusion into the renal artery of Krebs-Henseleit solution with 19 ml/min.*

from the serial combination of resistances in the renal vasculature and cannulae without kidneys, of which we subtracted to compute the real intrarenal pressures. The pressures thus obtained ranged from 80 mmHg (9 ml/min pefusion) to 200 mmHg (19 /mi/min perfusion).

The initial cell counts of outflow before the perfusion started were same as the RBCs in the venous blood and were usually within a range of 10^{10} to 10^{9} cells/ml. It is difficult to collect fractionated samples

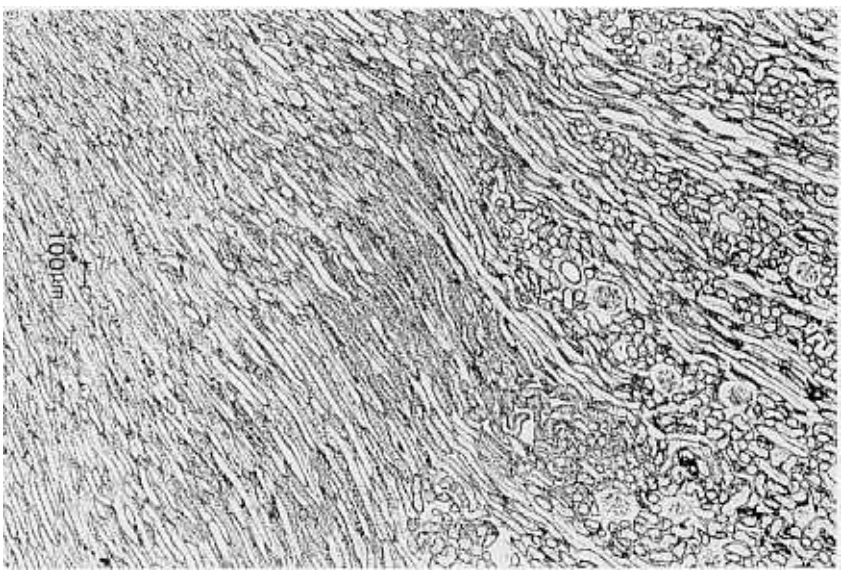


Fig. 9. Histological section showing juxtamedullary glomeruli and border of inner medulla of rabbit kidney. (Gomoris trichrome stain × 40)

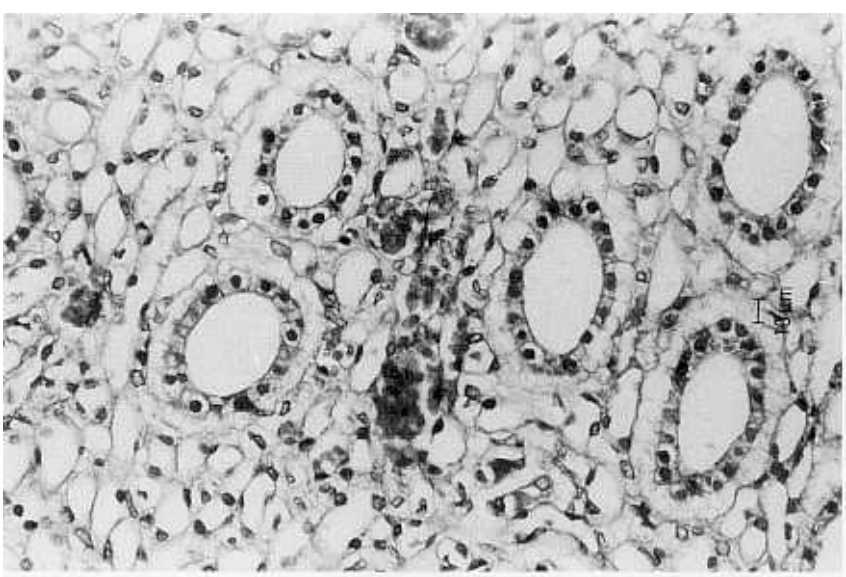


Fig. 10. Renal tubular epithelial cells and vascular cells. (Gomoris trichrome stain × 400)

Table 3. Analysis of red cell washout from the renal vessels of isolated rabbit kidneys perfused with 19 ml/min

Parameter	Component			Total
	I	II	III	
Intercept C_0 (cells, ml^{-1})	$1.88 \times 10^4 \pm 3.23 \times 10^3$	$5.92 \pm 10^6 \pm 1.91 \times 10^6$	$4.44 \times 10^9 \pm 7.70 \times 10^8$	$4.45 \times 10^9 \pm 7.70 \times 10^8$
Rate const. k (ml^{-1})	$8.63 \times 10^{-3} \pm 3.02 \times 10^{-3}$	$4.86 \times 10^{-2} \pm 7.73 \times 10^{-3}$	$2.93 \times 10^{-1} \pm 7.57 \times 10^{-2}$	
% of total flow	<0.001	0.172±0.06	99.8±0.06	100
Cell stroe C_0/k (cells)	$4.09 \times 10^6 \pm 9.87 \times 10^5$	$1.10 \times 10^8 \pm 2.95 \times 10^7$	$2.20 \times 10^{10} \pm 3.97 \times 10^9$	$2.21 \times 10^{10} \pm 4.00 \times 10^9$
% of total RBC	0.025±0.007	0.68±0.16	99.3±0.21	100

Values are the mean±SE of 9 experiments.

in the early stage of perfusion where the cell counts decreased very rapidly especially when the flow rate was increased to 19 ml/min. Therefore, we used smaller volumes of samples more frequently at 19 ml/min than at 9 ml/min.

Figs. 2 (9 ml/min) and 3 (19 ml/min) show the overall changes of cell counts against volumes perfused. Essentially after 100 ml perfusion, the trends of slowly decreasing cell counts are exactly the same between 2 groups. The solid lines are means of each individual analysis representing the whole group of different flow rates. The only difference between group I (Fig. 2) and group II (Fig. 3) is that the cell counts in the early stage (compartment III) for group II are higher than those in group I and the cell counts in the later perfusion (compartment I) of group I are higher than those of group II. This tendency is reflected in the compartmental analysis of Fig. 4.

Even though the difference between group I (9 ml/min) and II (19 ml/min) may not be statistically significant (not $p>0.05$, but $0.1>p>0.05$), group I has more cell stores (doubled: 9.14×10^6) in the slow compartment (I) than group II (4.09×10^6). However in the fast compartment (III) the group II has more cell stores (2.20×10^{10}) than group I(1.72×10^{10}).

We studied three individual cases in Fig. 5 which showed heterogeneous decrease in cell counts. During the perfusion the kidneys showed patchy and heterogenous clear washout patterns from the surface of the kidneys. However, the cell counts after 500 ml perfusion were similar and decreased continuously with the same k (kinetic rate decay constants in Table 2). When we analyzed a case of 9 ml/min in the Fig. 5 it added another exponential decay of cell counts after 350 ml perfusion. Thus the whole washout curve showed double exponential decays not simultaneously, but following one after another.

The data resulted from the means of each showing in Fig. 2, 3 and 4 must be the sums and means of respresenting the washout of 3 RBCs compartments at the same time and there should be RBCs remaining in the kidney even after 500 ml perfusion. Therefore we have examined the kidney tissues histologically looking for the RBCs after certain amounts of perfusion presuming that the remaining RBCs at that time of perfusion must represent the slow compartment (I). The histological findings in Figs. 9 and 10 are showing well cleared cortical vessels and some remaining RBCs in the medullary capillaries. In Fig. 10, many RBCs are still found in vasa recta implying that these cells may come out of the kidney later with further perfusion.

While perfusing the kidneys, we have collected uring from the ureter cannula. The urine flow, osmotic pressures and Cl^- concentrations are presented in Fig. 7 (group I) and 8 (group II) respectively. Since urine formation is the final objectives of the renal function, we compared the results between group I and II while perfusing the kidneys with cell-free Krebs-Henseleit solution.

There are individual differences among subjects, when they were standardized by minutes. The urine flow increased greatly in group II after 10 min perfusion while group I showed gradual increases during 100 min perfusion with lesser degree. Osmotic pressures remained isotonic after 20 min perfusion in both groups, but later turned out to be a little lower (280 mOsm/L) than the infusate (295 mOsm/L). Chloride concentration tended to be

(105~120 mEq/L) in urine than in the perfusate (100 mEq/L).

DISCUSSION

From the minipump, we could simulate pulsatile flow into the renal artery as shown in Fig. 1. It is expected to have increased pulses and pressures when flow rate is increased. Because of small internal diameter, cannulation itself had extra-renal resistance and was accounted for.

When the perfusate was not filtered properly through millipore filter paper (0.22 μm), the pressure profile was not stable but rather incremental depending on the degree of blockade at the microcirculation level due to particles. Our pressure recordings during one or two hour period did not show any variations, which indicates the steady RBC clearance from the organ. However in the cases of different RBC concentrations as shown in Fig. 5, the pressure changes corresponded with increase or decrease in RBC concentration.

Our main objective of this study is to apply RBC washout method for renal transplantation: the kidney is free of RBCs from microcirculation before being transplanted into patients. It is obvious from Table 2 and 3 that almost 99% of RBC can be washed out of kidney with only 2 ml (k_{III}=0.21 & 0.29) of perfusate in both groups. However, 1% or less (0.069% and 0.025%) of RBCs, are slowly coming out with $V_{1/2}$ (290 ml, adjusted from our raw data). At least 600 (2×290) ml of perfusate was required to clear RBCs from the slow compartment of rabbit's kidneys (11.5 gm and 12.1 gm). In the case of human adult kidneys (250~300 gm), it may require 50~60 (2×25 or 30) ml of pefusate to clear 99% of RBCs in the renal vasculatures.

We have also confirmed the cellular particles in two ways. The RBCs are the major cellular components and present in high concentration level as shown in the distribution curve in Fig. 6. The cellular components are mostly comprised of RBCs and some small amount of platelets and WBCs. The slides made from the drops of venous outflow after long perfusion periods contained rabbit's RBCs. The rabbit's RBCs were smaller (61 μm^3) than human's (87 μm^3) (William 1956) and the concentration was same as the initial cell concentrations (5×10^9 cells/ml) in Figs. 2 & 3.

Our compartmental models of both groups (9 ml/min, 19 min) shown in Fig. 4 are unique and very explicit in terms of cell stores, flows, and k distributions in the renal microcirculation. The flow distribution is mainly through the fast compartments (III), and is independent of variations in flow rate. Therefore, cell stores are confined in the fast compartment over 99% in both groups.

The difference between two groups can be regarded as the division between 3 ml (1.72×10^{10} in group I) and 4 ml (2.20×10^{10} cells in group II) of the whole blood cells. This difference cannot explain the significance of the functions unless, the urine flow was regarded as the major function of the kidney. Our data presented in Figs. 7 & 8 implies that the higher flow rate of pefusion (Fig. 8) generates more urine formation than the lower flow rate (Fig. 7). The U_{osm} in the middle of Figs. 7 & 8 is not variable during the slow compartment perfusion (20 min to 60 min). But the lower osmotic pressure (280 mOsm/L) than infusate (295 mOsm/L) implies that ADH (antidiuetic hormone) may be necessary at the last part of perfusion to make urine more concentrated (Thuran 1964; Gussis *et al.* 1979). This mechanism of the urine formation during RBCs washout experiments reguires further studies in the experimental designs to contemplate possible hypotheses.

Our histological pictures (Figs. 9, 10) show that the renal tubular epithelial cells and the vascular cells can survive to give enough RBCs at the last stage of perfusion even after 1 hour (group II) or 2 hours (group I). This picture also proves the kidney tissues are functioning to yield urine formation, absorption and secretion during perfusion.

Nontheless, the urine flow is found to be dependent upon blood flow or infusate flow through the renal arteries. During washout RBCs different compartments may affect the urine formation, and concentrating ability with many unknown mechanisms which we can not elucidate at the present time.

REFERENCES

Aukland K: Methods for measuring renal blood flow. *Annu Rev Physiol 42: 543-555, 1980*

Bankir L, Farman N, Gunfeld JP, De Latour EH, Funck-Bretano JL: Radioactive microsphere distribution and single glomerula blood flow in rabbits and rats: Erraneous findings with 15 um microspheres. *Kidney Int 15: 125-133, 1979*

Beeuwker R: The vascular organization of the kidney. *Annu Rev Physiol 42: 531-542, 1980*

Groom AC, Song SH, Campling B: Clearance of red blood cells from the vascular hed of skeletal muscle with paticular reference to reticulocytes. *Microvasc Res 6: 51, 1973*

Gussis GL, Jamison RL, Roberton CR: Determination of erythrocyte velocities in the mamalian inner renal medullar by a vido velocity-traking system, *Microv Res 18: 370-383, 1979*

Jacquez JA: *Compartmental analysis in biology and medicine.* Elsevier Publishing Co. Amsterdam, New York, *1972, 103-104*

Jones DB: The complementary role of scanning electron microscopy in renal pathological diagnosis. *Scan Electron Microsc 1: 323-332, 1983*

Karberg L, Kallstog O, Ojteg G, Wolgast M: Renal medullary blood flow studied with 86-Rb extraction method. Methological consideration. *Acta Physiol Scand 115: 11-18, 1982*

Katz MA, Blantz RC, Rector FC, Seldin DW: Measurement of intrarenal blood flow. I. Analysis of microsphere method. *Am J Physiol 220: 1903-1913, 1971.*

Kritz W: Structural organization of the renal medulla: Comparative and functional aspect. *Am J Physiol 241: R3-R16, 1981*

McNay JL, Abe Y: Pressure dependent heterogeneity of renal cortical blood flow in dogs. *Circ Res 27: 571-587, 1970*

Pallone TL, Channing RR, Jamison RL: Renal medullary microcirculation. *Physiol Review 70: 885-920, 1990*

Pappenheimer JR, Kinter WB, Hematocrit ratio of blood within mammalian kidney and into significance for renal hemodynamics. *Am J Physiol 185: 377-390, 1956*

Pallone TL, Robertson CR, Jamison RL, Renal medullary microcirculation. *Physiol Rev 70: 885-920, 1990*

Song SH, Groom AC, Storage of blood cells in spleen of the cat. *Am J Physiol 220: 779-784, 1971*

Song SH: Kinetics of the red cell washout from coronary vessles of isolated, feline hearts. *Can J Physiol Pharmacol 53: 755-762, 1975*

Thurau K: Renal hemodynamics. *Am J Med 36: 698-719, 1964*

William SS: *Handbook of biological data.* WADC Technical Report. *1956, 275*