Effects of Papaverine on Cell Separation in the Mammalian Kidney Papilla.* (28639)

LAWRENCE M. SLOTKOFF AND LAWRENCE S. LILIENFIELD
Cardiovascular Research Division, Departments of Medicine and of Physiology and Biophysics, Georgetown University Medical Center, Washington, D.C.

Direct micropuncture of the vasa recta of hamsters has demonstrated that the hematocrit of the blood perfusing the papilla is lower than that of the blood entering the renal artery(1). A possible explanation for this low red cell content can be deduced from the ingenious hypothesis suggested by Pappenheimer and Kinter(2,3) to explain the apparent separation of red cells and plasma within the mammalian kidney. It was suggested that in the intrarenal arteries the flow of blood is laminar and the cells therefore occupy the central stream. Under these conditions, vessels issuing at right angles to the interlobular arteries, such as the afferent arterioles, would draw off the peripheral plasma layer of the streaming blood. Because the first vessels to arise from the interlobular arteries are the juxtamedullary afferent arterioles, the juxtamedullary system would then be perfused by cell poor blood. Since the papilla is perfused via the vasa recta which arise from this system, it would be expected to contain blood having a very low hematocrit.

Obviously, this hypothesis has other implications. Since cell-poor blood continues to be skimmed off as the blood flows toward the outer cortex it would be expected that the red cell content of the blood would increase from inner to outer cortex. Studies in this laboratory(4) using labeled red cells and albumin failed to show any significant difference in the red cell content between these two zones although a very low red cell content in the papilla was noted.

In addition, if cell separation from plasma is a consequence of laminar flow, then at high velocity there would be expected a different separation than at low velocity since separation is a function of velocity. Lilienfield and Rose(5) using labeled red cells and albumin have shown that at high and low flow velocities the ratio of red cell to albumin transit times in the dog kidney remains unchanged. Since changes in velocity do not alter cell separation it becomes difficult to reconcile this with the plasma skimming theory.

An alternate hypothesis to explain the low papillary red cell content should be considered. There is anatomical evidence that, in the vasa recta, myogenic elements(6,7) exist which when constricted could offer an impedance to the passage of red cells into the papilla, and that proximal to these sphincters there are shunts which could draw off the cell rich blood. These shunting vessels do not reach the papillary zone, but rather loop back toward the outer medulla. Administration of a smooth muscle relaxant in this situation should dilate these sphincters, thus decreasing the impedance to the flow of red cells (Fig. 1). This would result in an increase in the red cell content of the papilla.

It had been observed in this laboratory and by others(8,9) that intraarterial administration of the alkaloid papaverine markedly lowers renal resistance and that its effect is prolonged for several minutes. Furthermore, intravenous papaverine affects arterial pressure only transiently (Fig. 2)(8). It was decided therefore to employ papaverine in this study as a muscle relaxant to test a myogenic sphincter hypothesis.

Methods. Ten normally hydrated mongrel

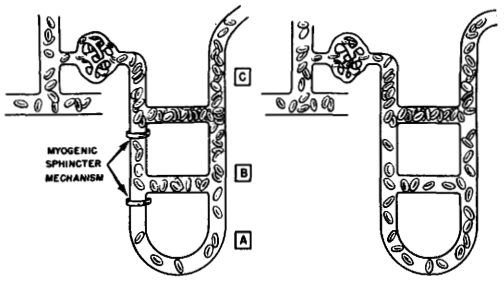

FIG. 1. (Left) Schematic representation of a possible sphincter mechanism for cell separation in a vas rectum of the mammalian kidney. (Right) Vas rectum with sphincter relaxed. See text.

* Supported in part by grants from USPHS.

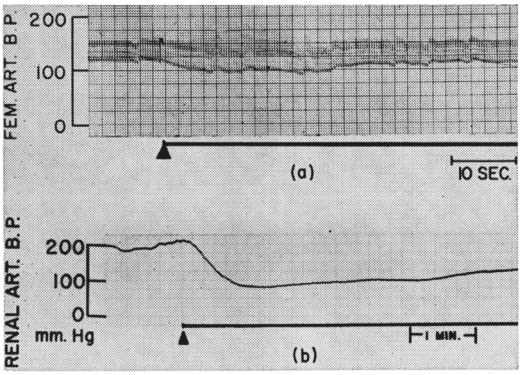

FIG. 2. (a) Effect of intravenously administered papaverine (40 mg/min) on peripheral arterial pressure. Papaverine infusion begun at ▲. See text. (b) Effects of intraarterially administered papaverine (40 mg/min.) on renal artery pressure. Renal artery perfused through constant infusion (Sigma-motor) pump. Mean pressures recorded. See text.

dogs weighing approximately 10 kg were anesthetized with sodium pentobarbital, 25 mg per kg. The abdomen was opened, and a loose tie placed around the pedicle of each kidney. The kidneys themselves were not handled during the procedure. One millicurie of I^{131} labeled human serum albumin and 3 millicuries of Cr^{51} labeled dog's own red cells were then injected intravenously. Papaverine-Cl in a solution of 5% glucose in water was administered by continuous drip at a rate of 40 mg per min into a femoral vein during the entire experiment, beginning just before injection of the radioactive blood. Approximately 30 minutes after injection of the radioactive material, an arterial blood sample was taken and both kidney pedicles were simultaneously ligated. The kidneys were removed and rapidly frozen in a dry ice-acetone mixture.

A thin transverse slice, approximately 5 mm in thickness, was cut from the frozen kidney with a band saw. Sample sections were then cut from the cortex, outer medulla, and papilla, care being taken not to include any major vessels. The sections were then weighed and assayed for radioactivity in a well-type scintillation counter. Aliquots of arterial blood samples were also analyzed for radioactivity.

During the entire experiment femoral artery blood pressure was monitored by means of a strain gauge transducer and direct-writing oscillograph.

At the end of the experiment urine was collected from the bladder and analyzed for radioactivity. Urine radioactivity varied from zero to less than 2% of the blood radioactivity. Nonprotein-bound I^{131} activity in tissue homogenates was less than 2% of total I^{131} activity in the tissues, as determined by trichloracetic acid precipitation. It is assumed therefore that the I^{131} counted in the tissue sections represents albumin content.

Determination of the amount of each of the 2 isotopes by differential decay was made by recounting after a 3-week interval. Concentration of labeled red cells in the tissues was expressed as the ratio of Cr^{51} in 100 g of tissue to the amount of Cr^{51} in one ml of red blood cells. Similarly, the concentration of labeled albumin was expressed as the ratio of I^{131} activity in 100 g of tissue to that of one ml of blood plasma.

Results. The mean red cell content in the papilla was 5.8 ml per 100 g, the medulla 10.2 ml per 100 g, and the cortex 5.6 ml per 100 g. The mean albumin content of the papilla was 28.6 ml. per 100 g of kidney tissue, of the outer medulla 27.0 ml per 100 g, and of the cortex 20.7 ml per 100 g (Table I).

During the experiment after tying and cutting the pedicles, the ligatures slipped off several kidneys before they were immersed in the dry ice-acetone mixture. This allowed the blood to drain out before freezing. These kidneys (0727 R, 0816 R and L) were not included in the statistical analysis of the data. It is of interest that the red cell volumes in these 3 cases are all many times lower than the group average. However the albumin contents in all zones are much less affected. It appears that the albumin is not confined to the same compartment as the red cells.

In one case (0818) the animal lost a large quantity of blood with a severe drop in blood pressure. This case was also excluded from the analysis. During the entire experiment arterial blood pressure was monitored. Except for a transient (30 sec-2 min) increase in pulse pressure there was little change in mean arterial pressure (Fig. 2).

Discussion. The striking difference between red cell and albumin content in all zones has no simple single explanation. Preliminary ob-

TABLE I. Red Cell and Albumin Contents of Dog Kidney.

Kidney No.		Red cell content ml/100 g tissue Cortex	Outer medulla	Papilla	Albumin content ml/100 g tissue Cortex	Outer medulla	Papilla
0713–61	R	5.10	13.4	6.4	20.2	25.2	28.1
	L	2.8	8.2	4.0	19.6	25.7	30.1
0714–61	R	7.2	11.0	7.7	‡59.3	‡59.8	‡57.0
	L	2.2	3.9	4.6	21.3	30.7	33.7
0720–61	R	3.7	10.3	6.6	16.5	30.0	22.4
	L	4.5	8.8	4.7	16.6	24.5	22.6
0721–61	R	6.1	8.8	9.2	20.1	23.5	24.9
	L	5.9	10.6	4.2	21.3	25.2	30.3
0726–61	R	6.7	7.5	3.3	18.1	22.0	20.2
	L	7.7	11.3	6.6	19.5	24.5	26.2
0727–61	R*	1.2	1.4	.75	43.2	53.9	50.1
	L	8.9	16.3	9.2	31.5	45.1	‡55.1
0816–61	R*	1.0	1.7	1.1	14.2	20.1	21.2
	L*	1.4	1.3	.9	16.6	21.8	18.8
0818–61	R†	6.4	5.7	2.2	24.5	28.5	24.0
	L†	3.0	4.7	1.6	21.8	27.7	26.5
0822–61	R	4.3	6.7	4.2	17.7	24.5	35.7
	L	4.5	10.2	6.8	18.2	29.1	33.4
0823–61	R	8.0	11.4	4.6	24.6	25.6	33.4
	L	7.2	11.3	4.7	24.3	23.1	30.8
Mean		5.6	10.2	5.8	20.7	27.0	28.6
S.D.		2.0	2.9	1.9	3.9	5.8	5.0
S.E.		.36	.54	.35	1.1	1.6	1.4

Not included in average.
* Ligature slipped off before freezing.
† Severe blood loss and shock.
‡ Values statistically too far outside group range.

servations made in this laboratory and elsewhere(2,4,5) imply that in the cortex, at least, much of the albumin is located extravascularly. On the other hand the hematocrit of the vasa recta blood as determined after direct micropuncture in hamsters is considerably lower than that of the blood entering or leaving the kidney(1). It appears then that in the papilla much of the great difference between the observed red cell and albumin content is due to the low hematocrit of the perfusing blood.

To interpret the effects of papaverine on the intrarenal separation of red cells it is necessary to compare these results with those previously reported from this laboratory in a study employing an identical technique save for the administration of this drug(4) (Table II).

The only area of the kidney where significant differences between the two groups are noted is in the papilla. In this zone there is a red cell content 2.3 ml per 100 g higher in the dogs given papaverine than in those without, with a p value of $<.05$. Furthermore the albumin content is 9.9 ml per 100 g lower in the papaverine treated dogs with a p value of $<.001$.

No significant change in total papillary blood volume is expected on the basis of the

TABLE II. Comparison of the Red Cell and Albumin Contents of the Group of Dogs Given Papaverine and a Control Group.

	Red cell content ml/100 g kidney Papaverine	Control	Difference	Albumin content ml/100 g kidney Papaverine	Control	Difference
Cortex	5.6	5.5	.1	20.7	22.8	2.1
Outer medulla	10.2	10.4	.2	27.0	30.7	3.7
Papilla	5.8	3.5	*2.3	28.6	38.5	†9.9

* $p < .05$. † $p < .001$.

present hypothesis. However, only a gross estimation of the blood volume is possible in this study. The red cell and albumin contents may not simply be summed. Firstly, it is most likely that a considerable quantity of the measured albumin occupies an extravascular compartment. Secondly, the red cell and albumin contents are calculated by relating the radioactivity contained in 100 g of tissue to one ml of peripheral blood. This figure represents, therefore, ml of peripheral red cells of plasma/100 g of renal tissue. If there is dilution or concentration of the plasma albumin or change in size of the red cells intrarenally, then one ml of peripheral plasma or red cells would not occupy a volume of one ml in the kidney. For example, after papaverine an increase of 2.3 ml of red cells and a decrease of 9.9 ml albumin was observed. It would appear that total blood volume decreased. However, if the albumin in the papilla is 2- or 3-fold concentrated, as is likely(10), then this 9.9 ml of albumin occupies a compartment of 3.3-5.0 ml in the papilla. If there was no significant alteration in red cell size then total blood volume would not have changed appreciably. Thus it is likely that under the influence of papaverine there was an increase in red cell content of the papilla without a significant change in blood volume. As expected there was no change in red cell content or total blood volume in the outer medulla or cortex after administration of papaverine.

Since there has been no direct investigation of the fluid dynamics of the mammalian renal circulation or of an analog model it is impossible to predict hematocrit changes after papaverine administration in the various zones of the kidney on the basis of a plasma skimming hypothesis. Information concerning the behavior of the red cells, the efficiency of central streaming, boundary layer stability, velocity, and vascular elasticity of the intrarenal circulation must be awaited. It is possible that the observed changes are compatible with plasma skimming but, as previously discussed, there are other factors which cast doubt upon the existence of plasma skimming in the mammalian kidney.

The proposed hypothesis finds support in our present knowledge of renal anatomy. The presence of contractile elements in the walls of the descending limbs of the vasa recta has been frequently described(7,11,12). Furthermore, Trueta(12) and others(6) have shown that the vasa recta loop back at different levels so that not all of these vessels reach the tip of the papilla.

What end this cell separation serves is a matter of conjecture. Thaysen and Lassen (13), and Kramer and Deetjen(14) have suggested that most of the oxygen consumed by the kidney is utilized in the metabolic processes involved in the active reabsorption of sodium. This process takes place primarily in the outer medulla and cortex. Richterich (15) had shown that the papilla of mammalian kidney has practically no oxygen uptake. He has also shown that the content of cytochrome oxidase and other oxidative enzymes is extremely low in the papilla as opposed to cortex. Therefore there is very little need for oxygen-carrying red cells in the papilla. This may be viewed as a reason for or a consequence of a low papillary red cell content. Furthermore, the extremely high osmolality of the papillary region would seem to be an unwholesome milieu for red cells that are highly sensitive to osmotic changes. Perhaps the sphincters protect the red cells by impeding their entrance into the papilla where they are not needed.

Summary. The low red cell content of the renal papilla can be explained by the plasma skimming hypothesis of Pappenheimer and Kinter. However, previous studies raise serious doubt as to the existence of this mechanism in the mammalian kidney. An alternate explanation is here offered. It is proposed that the descending limbs of the vasa recta contain myogenic sphincters which impede the passage of red cells into the papilla under normal conditions. The cell rich blood proximal to the sphincters is shunted back toward the outer medulla. Papaverine was administered intravenously in order to relax these sphincters. A rise in red cell content in the papilla and no change in the outer medulla and cortex was observed. These findings are compatible with the proposed hypothesis.

The authors wish to extend their sincere gratitude to Mrs. Jean Young and Mr. Thomas Doyle for invaluable assistance.

1. Ullrich, K. J., Kramer, K., Boylan, J. W., *Progs. Cardiovas. Dis.,* 1961, v3, 395.

2. Pappenheimer, J. R., Kinter, W. B., *Am. J. Physiol.,* 1956, v185, 377.

3. Kinter, W. B., Pappenheimer, J. R., *ibid.,* 1956, v185, 399.

4. Lilienfield, L. S., Rose, J. C., Lassen, N. A., *Circulation Res.,* 1958, v6, 810.

5. Lilienfield, L. S., Rose, J. C., *J. Clin. Invest.,* 1958, v37, 1106.

6. von Möllendorff, W., *Handbuch der mikroskopischen Anatomie des Menschen,* Springer, Berlin, 1930.

7. Longley, J. B., Banfield, W. G., Brindlye, D. C., *J. Biophys. and Biochem. Cytol.,* 1960, v7, 103.

8. Darby, T. D., Sprouse, J. H., Walton, R. P., *J. Pharm. and Exp. Therap.,* 1958, v122, 386.

9. Thurau, K., Kramer, K., *Pflüger's Arch.,* 1959, v269, 77.

10. Lassiter, W. E., Gottschalk, C. W., Mylle, M., *Clin. Res.,* 1962, v10, 66.

11. Smith, H. W., *The Kidney,* Oxford University Press, New York, 1955.

12. Trueta, J., Barclay, A. E., Daniel, P. M., Franklin, K. J., Pritchard, M. M. L., *Studies on the Renal Circulation,* Oxford, 1947.

13. Thaysen, J. H., Lassen, N. A., Munck, O., *Proc. 1st int. congr. nephrol.,* Geneve/Evian. 692, 1961.

14. Kramer, K., Deetjen, P., *ibid.,* 687.

15. Richterich, R., *Heart, Kidney and Electrolytes,* Grune and Stratton, New York, 1962.

Received July 15, 1963. P.S.E.B.M., 1963, v114.

Induction of Graft-Versus-Host Reaction in Newborn Mice by Injection of Newborn or Adult Homologous Thymus Cells.* (28640)

M. W. COHEN, G. J. THORBECKE, G. M. HOCHWALD AND E. B. JACOBSON

Departments of Pathology and Neurology, New York University School of Medicine, New York City

Homologous adult thymus cells have been shown capable of inducing runting in newborn animals(1) and secondary disease in irradiated adult mice(2). Although these experiments indicated that thymocytes were quantitatively less effective than other lymphoid cells in producing these effects, spleen cells from animals thymectomized at birth were relatively ineffective(3).

While newborn spleen and blood cells are indicated to be incapable of producing graft-versus-host (G.V.H.) reaction(4), no studies with the newborn thymus have been reported. In the present studies with mice, no obvious quantitative differences could be found between the effectiveness of newborn and adult thymus cells in producing G.V.H. reactions.

Materials and methods. Animals: Newborn A/Hestor mice (Darwin Farms) injected within 24 hours of birth were used as recipients. Newborn (0 to 3 days old) and adult C57B1/6 mice of both sexes were used as donors.

* Supported by USPHS grant.

Cell Suspensions: The organs of the donor mice, thymus, spleen, or liver, were teased in medium 199, the cells counted, and 0.1 ml aliquots injected intraperitoneally into the experimental animals. As controls in some experiments, a portion of the thymus cell suspension was disrupted before injection either by freezing and thawing or by sonic oscillation (15 minutes, 9 kilocycles, Raytheon magneto striction oscillator). The latter treatment was estimated to disrupt at least 95% of the cells.

Spleen Index Calculation: The determination of G.V.H. reaction was by Simonsen's method of spleen assay(4). The spleen weight/body weight ratio was determined at the age of 8-10 days. Spleen indices were calculated by dividing the relative spleen weight for each experimental animal by the means of the relative spleen weights for litter-mate (non-injected) controls. Since the average spleen index of 65 control animals was 1.00 $\pm$ 0.11 S. D., animals with indices $\geqq$ 1.3 were considered to show a G.V.H. reaction.

Results. The results (Table I) show