NATURE OF FLUIDS WHICH FUNCTIONALLY DISTEND THE KIDNEY*

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After the renal artery is occluded, there drains out of the renal vein a volume of fluid equal to 13 to 30 per cent of the functionally distended kidney. A preliminary analysis of this fluid showed that it differed considerably from blood: it contained only about half the red cells that a simultaneously drawn sample of arterial blood contained and its plasma contained more K and more Cl, but the same amount of Na, as did blood plasma (1). The source of this fluid was puzzling; it was suggested that a more comprehensive analysis might reveal its origin. In the present study such analyses have been carried out, along with simultaneous analyses of systemic arterial blood, of renal venous blood (just before the occlusion), and of urine. The analyses made included: hematocrit value, red cell count, Na, K, Ca, Cl, PO_4, urea, plasma protein, plasma albumin, glucose, and, in some cases, inulin, diodrast, and freezing point depressions. Because it was known from the preliminary study that the fluid draining from the functionally distended kidney was peculiar in composition, it was thought to be of interest to investigate the fluid draining from another functionally distended organ. The spleen was chosen for this purpose. It has also been found that the fluid draining from the kidney after arterial occlusion changes in composition as successive fractions flow out. This fact prompted an analysis of the successive fractions.

Methods

Dogs were anesthetized with sodium pentobarbital and suspended in the standing position. Their kidneys were then exposed through a flank incision and all capsular circulation, when present, was excluded by appropriate ties. The main renal artery and vein and the ureter were exposed but denervation was avoided. The gonadal branch of the left renal vein was tied. The ureter was cannulated with a 20 cm. piece of plastic tubing so that urine could be collected.

In the first experiment to be reported, four animals were injected with inulin and diodrast:

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they were first primed with 2 ml./kg. of 5.7 per cent diodrast and 1 ml./kg. of 0.8 per cent inulin. Immediately following this, a slow infusion was started of 0.1 ml./kg. min. of 5.7 per cent diodrast and the same volume of 0.4 per cent inulin. In all experiments except the first, saline in equal volumes was used for both priming and infusing solutions. These injections, continued all through the experiment, resulted in urine flows of about 1 ml. per 3 minutes (see data); this result is usually considered to be an oliguria in dogs, but actually it is at about this rate that they produce urine normally. About 10 minutes after the priming injections, the collection of urine was started.

About 15 minutes later, blood samples were taken. To obtain the renal venous sample, a long 18 gauge syringe needle was placed, usually through an incision in the contralateral flank of the dog, in the left renal vein: it was run into the vena cava and up the vein until, by inspection, its point was in the hilus. Then a 15 ml. blood sample was drawn at a rate of about ½ ml. per second. Next an arterial sample was taken from the carotid artery. Finally the renal artery and vein at the pedicle were doubly clamped with hemostats, the vessels cut between the clamps, the kidney removed and placed in a beaker, and the renal vein cut. All the last maneuvers were done with dispatch: about a minute elapsed from the drawing of the venous sample until drainage of the kidney was started. Drainage is fairly rapid: 80 per cent in the 1st minute and 90 per cent by the end of the 2nd minute. The last 10 per cent flows out slowly, being completed in 5 to 15 minutes. In the present experiment the organ was allowed to drain for about 15 minutes, the beaker being whirled occasionally to mix in anticoagulant. (As shown previously, the fluid flowing out comes exclusively from the renal vein and none, if they are also cut, from ureter or artery (1).) Care was taken to keep all urine out of the fluid collected in the beaker. Heparin was used as anticoagulant in all cases. Centrifugation of the samples was started about 15 minutes after they were drawn.

In this experiment, the quantity of diodrast and inulin left behind in the tissue was also ascertained. After blood drainage was complete, the urinary inulin and diodrast left in the pelvis were carefully flushed out: water was gently injected several times up the ureter and into the pelvis, and then allowed to drain. Then the renal capsule was stripped off and the tissue homogenized in a Waring blendor with water. An aliquot of the homogenate was then analyzed for inulin and diodrast.

The experiments on the spleen were conducted in essentially the same way as those on the kidney. The vessels of the splenic pedicle in four dogs were isolated so that a splenic venous sample could readily be drawn. Carotid arterial blood furnished an arterial sample. Finally, the vessels of the spleen were clamped with hemostats, the organ removed and put in a beaker, and the vein cut. Drainage of contained fluid was permitted to proceed for 15 minutes.

When, as in the second and third experiments, successive fractions of fluid from the kidney were desired, the procedure was essentially the same. The arterial sample was collected first; then the vein was cannulated with the needle. The artery and vein were occluded with a rubber-guarded hemostat which closed off the artery completely and closed off the vein except for the cannulating needle. The fluid draining from the needle was then collected in successive 3 ml. samples. Drainage is slower in this case: 50 per cent at 2 minutes and 90 per cent at 6 minutes after the artery was clamped.

The samples were analyzed as follows: an aliquot of the whole blood was used for the determination of the hematocrit value, red cell count, glucose, and diodrast. The remainder was centrifuged and its plasma analyzed for the other compounds. The following methods were used: Hematocrit value by means of Wintrobe tubes; red cell count by conventional hemocytometry; Na, K, and Ca by the flame photometric method of Kingsley and Schaffert (2); Cl by the method of Schales and Schales (3); inorganic phosphate by the method of Fiske and SubbaRow (4); urea by the method of Ormsby (5) as modified by Kawerau (6); total protein and albumin by colorimetric biuret with the method of Weichselbaum (7); glucose

(whole blood) by the method of Nelson (8) and Somogyi (9); inulin by the method of Roe, Epstein, and Goldstein (10); and diodrast (whole blood) by the method of Alpert (11).

RESULTS

The results of the first experiment, in which four dogs were tested after injecting inulin and diodrast, are shown in Tables I through IV. Table I gives the volume of the fluid draining from the kidney. The ratio of this volume

TABLE I

Fluid Draining from Kidney, etc.

No.	Fluid draining	Weight of drained kidney	Ml. draining per 100 gm.	Diluting fluid volume: I	Urine flow
	ml.	*gm.*			*ml./min.*
1	10.5	33.0	24.2	0.35	0.07
2	17.5	48.4	26.6	0.92	0.67
3	10.0	26.0	27.8	0.70	0.21
4	20.0	40.8	32.9	0.64	0.35
Averages.....	14.5	37.1	27.9	0.62	0.33

TABLE II

Formed Elements of Samples

No.	Hematocrit reading			Red cell count			Mean corpuscular volume		
	Artery	Renal vein	Kidney fluid	Artery	Renal vein	Kidney fluid	Artery	Renal vein	Kidney fluid
	per cent	*per cent*	*per cent*	*millions/ cmm.*	*millions/ cmm.*	*millions/ cmm.*	μ^3	μ^3	μ^3
1	42.3	43.7	32.4	6.4	5.7	4.4	65	77	78
2	34.6	34.9	18.2	5.4	5.7	2.5	64	61	73
3	45.0	46.5	27.4	7.1	6.8	4.5	63	68	61
4	49.3	49.5	30.1	7.2	7.1	4.5	69	70	67
Averages.....	42.8	43.7	27.0	6.5	6.3	4.0	65	69	70

times 100 to the volume (or weight) of the drained kidney plus the volume of fluid draining gives the figures of column 4: the volume draining per 100 gm. of functionally distended kidney. For this series of 4 dogs, it averages 27.9 ml. Combining the data of this paper, a previous one (1) and other unpublished data, the average for 22 dog kidneys is 26.2 ml. ($\sigma = 6.6$) per 100 gm. of functionally distended kidney. This grand average will be used in subsequent computations. The table also shows the urine flows observed in the 4 dogs: about 1 ml. in 3 minutes. The factor I will be discussed below.

Table II presents the data on hematocrit and red cell counts of the blood

samples and of the fluid draining from the kidney. The latter will be designated henceforth as "kidney fluid." The hematocrit values of arterial and renal venous blood are essentially the same, as can be deduced from the data of Van Slyke, *et al.* (12). The same is true of the red cell counts. In explanation, the quantity of urinary water removed from the blood in its passage through the kidney is so small compared with the quantity of blood flowing through the kidney—0.3 ml. per minute compared with approximately 150 ml. per minute—that it is undetectable with the technics of the hematocrit reading or red cell count or plasma protein measurement (see Table IV). The table also shows the mean corpuscular volume of each red cell: it does not change as the blood passes through the kidney.

The hematocrit and red cell counts of the kidney fluid, as Table II shows, are about two-thirds those observed in arterial or renal venous blood. This dilution of red cells indicates that blood entering the kidney mixes with a fluid of lower hematocrit count. We shall assume that the kidney fluid is a mixture of vascular blood and a cell-free fluid which dilutes the blood. On this basis we shall compute the relative proportions of blood and "diluting fluid" yielded by the isolated kidney. The latter's relative volume may be calculated thus:—

$$I = \frac{H_V - H_K}{H_K}, \tag{1}$$

in which H_V is the hematocrit of renal venous blood and H_K the hematocrit of kidney fluid. The ratio I is dimensionless, but in effect it is the volume of diluting fluid which mixes with 1 ml. of vascular blood after the artery is clamped.

In Table I are shown the values for I computed from the hematocrit values given in Table II. It was found that on the average it is 0.62, *i.e.* 0.62 ml. of diluting fluid was mixed with 1 ml. of blood flowing through the kidney to make up the composite kidney fluid. Combining these data with others in order to obtain the average for a large group, the mean I for 22 kidneys is 0.80 ($\sigma = 0.33$). Another useful statement is that of the percentage of kidney fluid which is diluting fluid; it is given by the ratio: $\frac{100\ I}{1 + I}$. On the average, the percentage is 44: almost half the kidney fluid is diluting fluid. Table II also shows that the mean corpuscular volume of the red cells is the same in kidney fluid as in renal blood. The low hematocrit readings of the kidney fluid, therefore, cannot be ascribed to a decrease in red cell size.

The data for the individual dogs for the remaining analyses are shown in Table III and the averages in Table IV. In arterial and renal venous blood, the concentrations of all substances, inulin and diodrast excluded, were essentially the same, again reflecting the relatively slow rate of urine secretion

compared with the profuse renal blood flow. For example, if the values of Table IV are used and if renal blood flow is assumed to be 150 ml. per minute, the kidney was presented with 33 mg. per minute of urea. It excreted (at a urine flow of ⅓ ml. per minute) 0.9 mg. per minute—a quantity of urea not

TABLE III

Composition of Blood and Kidney Fluid

Dog No.	Analysis	Artery	Renal vein	Kidney fluid	Urine	Analysis	Artery	Renal vein	Kidney fluid	Urine
1	Na, *m.eq./*	154	156	154	30	Protein,	4.6	4.6	2.8	3+
2	*liter*	154	138		70	*gm. per*	4.8	5.2	2.5	Tr.
3		154	134	154	86	*cent*	4.7	4.9	3.5	Tr.
4		151	165	161	225		5.6	5.3	3.2	Tr.
1	K, *m.eq./*	5.2	5.0	6.6	140	Albumin,	3.1	3.1	1.8	
2	*liter*	7.1	5.2	7.9	86	*gm. per*	2.7	2.7	1.5	
3		4.7	5.2	5.1	84	*cent*	3.7	3.8	2.5	
4		4.7	4.8	5.8	94		2.8	2.4	2.2	
1	Ca, *m.eq./*	5.3	5.2	5.2	5.5	Glucose,	115	119	81	Neg.
2	*liter*	6.0	5.6	5.7	4.9	*mg. per*	67	59	32	Neg.
3		4.7	5.0	4.9	4.4	*cent*	61	69	35	Neg.
4		5.0	5.2	5.1	5.0		62	68	100	Neg.
1	Cl, *m.eq./*	120	120	135	34	Inulin, *mg.*	17.2	12.0	13.6	3,950
2	*liter*	124	117	132	25	*per cent*	12.8	6.8	8.8	400
3		127	126	144	87		20.6	15.0	14.5	1,160
4		124	116	136	238		11.8	10.7	12.0	1,080
1	PO_4 asP,	6.6	4.3	12.1		Diodrast,	5.4	1.7	2.9	7,400
2	*mg. per*	4.0	3.9	7.3	30.2	*mg. per*	3.3	.9	1.9	1,200
3	*cent*	4.0	3.7	6.1	86.8	*cent*	11.4	8.1	7.3	3,040
4		6.2	5.4	8.8	95.4		6.5	3.4	5.5	2,130
1	Urea, *mg*	8	7	38	120					
2	*per cent*	11	20	28	355					
3		27	24	30	265					
4		47	43	58	300					

reliably detectable by current analytical methods. It is only when one comes to inulin and diodrast that there is an obvious arteriovenous difference. However, the kidney fluid differs clearly from venous or arterial blood: it is the same in content for Na and Ca, higher for K, Cl, PO_4, and urea, and lower for total protein, albumin, and glucose. Also the kidney fluid bears little resemblance to urine that is being simultaneously formed: the latter is relatively high (as in K or urea content) in some constituents, but low (as in Cl) in other

constituents, without evidently influencing the content of kidney fluid in these same substances.

The same conclusion is emphasized by the data of individual dogs in Table III. Dog 4, for example, was excreting a urine concentrated in Na, Cl, and PO_4, while dog 2 was excreting a urine relatively low in them. But, for each of the dogs, the content of the kidney fluids in these same substances was essentially the same. The conclusion is clear: the kidney fluid is quite constant in composition, and apparently independent of the varying composition of the urine. The two fluids appear to be unrelated.

TABLE IV

Average Composition of Blood, Kidney Fluid, and Splenic Fluid

Analysis*	Kidney				Spleen		
	Artery	Renal vein	Kidney fluid	Urine	Artery	Splenic vein	Splenic fluid
Hematocrit	42.8	43.7	27.0		40.6	41.8	56.4
Na	154	149	156	103	147	150	147
K	5.4	5.0	6.4	101	5.1	4.7	5.5
Ca	5.3	5.3	5.3	5.0			
Cl	124	120	137	96	116	113	128
PO_4	5.2	4.3	8.6	70.8	5.1	5.1	5.3
Urea	23	24	39	260	24	22	25
Protein	4.9	5.0	3.0		5.4	5.2	6.0
Albumin	3.1	3.0	2.0				
Glucose	76	79	47	0	117	75	85
Inulin	14.6	12.6	11.8	1,650			
Diodrast (whole blood)	6.7	3.3	4.4	3,440			

* The same units were used as in Table III.

The renal tissues' contents of inulin and diodrast were not presented in Table III. They were, respectively, for the 4 dogs, in mg./100 gm. tissue: 31, 22, 42, and 28 for inulin and 37, 35, 45, and 37 for diodrast. Averages are, respectively, 31 and 38 mg. The tissue content was only a very small fraction (one-thirtieth or less) of the simultaneous urinary content. The implication of these data will be discussed below.

Table IV also shows the average quantities of the various blood constituents in the fluid draining from the spleen after its artery had been clamped. The fluid was rich in red cells, confirming Allen and Reeve (13) and reflecting the storage function of the organ. Its plasma resembled arterial and splenic venous plasma closely in all constituents except Cl. The experiment was unsatisfactory in one important aspect: the 15 minute period alloted for drainage of the functionally distended spleen was by no means sufficient for complete drainage of the organ. Rather, fluid continued to drain for 30 to 60

minutes, all the while becoming richer in red cells. For this reason, the experiment does not give a complete analysis of the fluids which functionally distend the spleen. But it does show that the fluid distending the spleen is,

TABLE V

Composition of Individual Samples of Kidney Fluid

Analysis*	Artery	Renal vein	Kidney fluid, successive 3 ml. samples						Urine
			2	3	4	5	6	7	
Dog 5									
Hematocrit....	40.5	38.1	29.2	19.9	13.5	9.1	7.7	6.3	
Na.....	142	148	148	144	138	142	160	139	220
K.....	6.3	3.3		11.6	16.0	13.7	22.8		84
Cl.....	118	111	123	126	132	134	142	144	426
PO_4.....	6.7	5.9	6.9	8.1	9.5	9.7	10.3	10.8	31
Urea.....	25	12	14	21		22	29	32	1,100
Protein.....	5.3	6.4	4.6	3.1	3.1	3.1	2.8	3.1	Tr
Glucose.....	104	88	76	70	73	62	65	70	0
Freezing point depression, °C.....	0.612	0.611	0.627	0.652	0.662	0.692	0.684	0.702	
m.osM/liter..	330	330	338	351	360	372	368	378	
Dog 6									
Hematocrit....	35.4	34.0	17.9	9.1	7.1	3.8			
Na.....	134	148	139	138	136	144			208
K.....	2.8	3.3	4.9	7.6	6.0	8.7			151
Cl.....	108	107	118	124	125	129			133
PO_4.....	5.2	4.5	7.4	7.8	7.7	8.0			156
Urea.....	28	23	24	29	30	33			840
Protein.....	6.7	7.2	5.1	3.8		3.1			++
Glucose.....	75	64	54	47	44	14			0
Freezing point depression, °C.....	0.657	0.604	0.601	0.608	0.631	0.669			
m.osM/liter .	353	324	323	326	339	360			

* Units as in Table III, except for glucose which is in mg./100 ml. plasma.

except for its red cell content, close to systemic blood in composition. By contrast, the kidney fluid differs considerably from blood.

When the successive fractions of fluid draining from the kidney were isolated and analyzed separately, it was found, as shown in Table V for two dogs, that the hematocrit value progressively decreased as fluid drained out. (In the table, the numbers of the kidney fluid samples refer to the serial 3 ml. samples. No. 1, not shown, was discarded because it was contaminated with

heparin. Its hematocrit value was the same as that of renal venous blood.) In these two dogs, the last 3 ml. of fluid to drain from the kidney contained only about 5 per cent red cells. Table VI shows a similar experiment on 4 dogs, the only difference being that the samples analyzed were the middle third (fraction II) and the last third (fraction III) of the draining fluid. Freezing point depressions of the three types of plasma were also measured in these experiments. (The Na content of the plasma in the four dogs of Table VI was not measured.) The two tables show small and slow progressive changes, except for Na, in the constituents of the kidney fluid as it drains out:

TABLE VI

Successive Samples of Kidney Fluid and Diluting Fluid

Analysis*	Artery	Renal vein	Kidney fluid		Diluting fluid		Ratio: Dil. fraction III / Venous blood	Urine
			Sample II	Sample III	Sample II	Sample III		
Hematocrit...	46.5	47.2	25.0	14.5				
I.....					0.88	2.24		
$100\,I/(1+I)$..					47	69		
Na‡.....	138	148	139	145	134	144	1.0	214
K.....	5.5	5.5	6.7	8.0	7.4	8.6	1.5	
Cl.....	120	118	126	135	135	143	1.2	161
PO_4.....	6.1	5.9	8.8	10.4	10.5	11.5	2.0	360
Urea.....	32	32	39	51	47	59	1.8	1,780
Protein.....	6.5	6.2	3.0	2.8	1.1	2.0	0.3	Tr
Albumin.....	3.3	2.9	1.3	1.6	0.3	1.3	0.4	
Glucose.....	118	129	82	68	53	54	0.4	0
m.osM/liter...	346	323	357	366	378	376	1.2	

* Units as in Table III, except for glucose which is in mg./100 ml. plasma.

‡ Calculated from dogs 5 and 6 of Table V.

the content of K, Cl, PO_4, and urea slowly rises with each successive sample and the protein and glucose content slowly falls. Its freezing point also slowly falls, showing an increase in osmolarity.

Two questions arise concerning the diluting fluid (*i.e.* the red cell–free fluid which is mixed with blood in the kidney): what is its composition and does it change as the process of drainage proceeds? With the assumption that the red cells measure the amount of blood present, we can calculate the composition of the remaining diluting fluid by difference. To do this, we have used two formulas, the first allowing for distribution of some blood constituents equally between red cells and plasma and the second allowing for distribution of others only in plasma. In the first group are urea and Cl, in the second Na, K, PO_4, protein, albumin, and glucose.

The basis for calculating the composition of diluting fluid lies in the defini-

tion that it is mixed in a certain volume with renal vascular blood, the two together making up "kidney fluid." I represents the relative volume of diluting fluid. Let X represent the volume of blood; then $I + X$ represents the volume of kidney fluid. Then the concentration of a given substance in diluting fluid, S_D, times its relative volume, plus the concentration in renal blood, S_V, times its volume equals the concentration in kidney fluid, S_K, times its volume, or

$$S_D(I) + S_V(X) = S_K(I + X)$$

and

$$S_D = S_K + \frac{I}{X}(S_K - S_V). \tag{2}$$

Renal blood may be taken to have the composition of either arterial or venous blood, since they are essentially the same. We have chosen that of renal venous blood, which is S_V. The ratio I/X remains to be defined. It will depend upon the permeability of the red cells for the substance. Considering first the case in which the substance enters the red cells freely, the volume of blood, X, is given by definition in Equation 1: it is 1. By the same equation, the diluting fluid's volume is given by I. Equation 3 now becomes

$$S_D = S_K + \frac{1}{I}(S_K - S_V). \tag{3}[1]$$

This formula was used for calculating the concentrations in diluting fluid of urea and Cl (and osmolarity), which are given in Table VI. Both substances penetrate red cells freely (14–16). The calculation for Cl content must be considered only an approximation, however: it is assumed to be equally distributed between plasma and red cells, which is only approximately true. Also the pH of the fluids was not known (16), diffusion of CO_2 from the samples was not prevented, and their O_2 content was not guarded, all of which influence the cellular Cl content. Hence, the results of the calculation should be accepted with reserve. The calculated content, however, does match well that of dogs 5 and 6 in Table V, in which the diluting fluid was only slightly contaminated with blood.

Many substances in the diluting fluid do not, however, penetrate red cells readily: Na, K, PO_4, protein, and albumin. In the case of the first three ions, penetration has been demonstrated but it is felt to be too slow to occur, in any appreciable magnitude, in the 15 minutes which elapsed between the

[1] It would be more accurate to calculate the concentrations of these substances in plasma and red cell water. This has been done, but the results were unrewarding and will not be presented here. Such refinements of calculation are rendered superfluous both by the natural variation in these biological data and the limitations of our analytical technics.

drawing of samples and their centrifugation (17, 18). The volume of diluting fluid for such substances, since by definition it contains no red cells, will be given by I as in Equation 4. Blood contains red cells, the quantity being given by its hematocrit value, H/100 or 0.01 H. This is to be subtracted from the total volume of blood, or 1. Hence Equation 2 becomes:

$$S_D = S_K + \frac{1 - 0.01\,H}{I}(S_K - S_V). \qquad (4)^2$$

This formula was used to calculate the content of the diluting fluid in Na, K, PO_4, plasma protein, and albumin, the results being shown in Table VI.

The case of glucose is complex and obscure. It does not penetrate the dog's red cells (15, 19), although they hold it in some fashion (20), perhaps by adsorption. The situation is undoubtedly further complicated by the kidney's ability to manufacture sugar (21). For this study, in any event, we employed Equation 4 to calculate the diluting fluid's glucose, assuming that the sugar does not penetrate red cells and that the adsorption (?) coefficient does not change. That this is approximately correct is again shown by the data of dogs 5 and 6 in Table V.

The data in Table VI show (from the ratio $100\ I/(1 + I)$) that the diluting fluid comprised about half of fraction II (the middle third to drain) and two-thirds of fraction III (the last third to drain). As the kidney fluid flowed out, it had less and less blood and correspondingly more and more diluting fluid. The last portion of the diluting fluid had in it somewhat more of all substances, Na and glucose excepted, than the middle third draining. The magnitude of the change is considered small, however; it is especially to be noted that although the urinary content of several substances, *e.g.* urea or PO_4, was very high, the kidney fluid, even in its last portions, did not begin to approach the urinary concentrations. The data of Table V also support this conclusion; it is particularly in the last samples to flow out, in which the amount of blood was small but diluting fluid large, that the relatively small changes in composition of the diluting fluid may be observed. The diluting fluid is definitely hypertonic to both venous and arterial blood plasma, the excess of molarity being 20 to 50 m.osM/liter. The high concentration of the diluting fluid in Cl appears importantly involved in this hypertonicity.

The analyses for albumin were poor, with considerable irregularity in the data. However, the over-all import of the data is clear with respect to the total protein concentration: in both dogs 5 and 6 of Table V and the 4 dogs of Table VI, the protein of the diluting fluid remained fairly constant. Some preliminary electrophoretic analyses of the proteins were also made, particularly of the last samples to drain, in which plasma from blood contributed, because

[2] This is fundamentally the same equation as that used in the preliminary report (1), except that venous blood concentrations have been substituted for arterial blood concentrations.

of its small volume, only a minimal amount of protein to the total being analyzed. They showed plentiful quantities of globulin to be present, in roughly the same proportion to albumin as was found in arterial or renal venous blood. Also, the last samples to drain (*e.g.* as in dogs 5 and 6 of Table V), which are very low in blood content, were noted to clot readily, thus indicating the presence of fibrinogen. Apparently the proteins of the diluting fluid are not far different from those in blood plasma.

DISCUSSION

The assumption was made above in Results that renal vascular blood was diluted with a red cell-free fluid to make up the composite "kidney fluid." Justification for the assumption is to be found in the analyses of arterial and renal venous blood. Their compositions are essentially the same; hence, it is unlikely that the blood composition changes abruptly in any part of the renal vasculature, in its anatomical sense, as it traverses the kidney. The kidney fluid must therefore be composed of vascular blood plus another fluid. The latter is designated "diluting fluid"; we must search for its origin in some region other than the renal blood vessels themselves.

Summarizing the characteristics of the fluid which normally distends the kidney, it is the same as blood in Na and Ca content, high in K, Cl, PO_4, urea, and molarity, but low in protein, albumin, glucose, and red cells. The composition of the fluid which, under the conditions of the experiment, apparently mixes with vascular blood, has been deduced. It is summarized in column 8 of Table VI by a group of ratios referring its composition to that of renal vascular blood. The fluid flowing from the isolated kidney changes progressively in composition until finally the last portion to drain is about 90 per cent diluting fluid. This fluid does not appear to be influenced by the urinary constituents in any consistent way.

These data all lead to the hypothesis that the diluting fluid—it will be recalled that it is large in volume since it furnishes about half of the fluid which functionally distends the kidney—is actually renal interstitial fluid (or lymph). We shall first show that our original hypotheses about its origin were wrong. We shall then discuss the evidence that the fluid is interstitial fluid.

We had previously guessed, first, that the diluting fluid must in some way be related to the urine; second, that it might be tubular urine; and third, that the plasma proteins, by their osmotic pressure, "draw" the urine back into the vascular system so that it drains out of the vein under the conditions of the experiment (1). The present data disprove all these guesses. In the first place the urine in all these animals was very high in K, PO_4, urea, inulin, and diodrast, *e.g.* about 50 times more urea than in the blood or kidney fluid. If any of these urinary substances entered the diluting fluid, even in small quantities, it would have shown up in the analyses and changed strongly the ratios

reported here. But this did not occur; therefore, the composition of the urine does not appear to influence the diluting fluid. The same phenomenon is particularly clear from the data on individual dogs (Table III): although urinary constituents vary greatly from dog to dog, the composition of the kidney fluid does not.

Furthermore, because the diluting fluid has in it 1 to 3 gm. per cent protein, whereas tubular urine has only minimal quantities, the diluting fluid cannot be tubular urine. Finally, the plasma proteins apparently do not act to draw the diluting fluid back into the blood. If they did so, they would be progressively diluted as the fluid drains out of the kidney. But as shown, the diluting fluid has a relatively constant composition in successive fractions with respect to its protein content. This is particularly clear in the data of Table V.

The data on inulin and diodrast also strongly support the conclusion that the diluting fluid is not tubular urine. It was originally reasoned that the average tubular content of the two compounds must be high relative to blood. Furthermore, because urine is so high—100 to 1000 times more of the compounds than in blood—the difference must be conspicuous. Moreover, the volume of the diluting fluid was known to be large. Hence, if the diluting fluid originated from the tubules, large quantities of the two compounds should be found in one of two places after the kidney was drained. They should be found in the diluting fluid; or else, if they stayed inside the tubular lumina, they should be found in the tissue itself. Upon test, however, neither inulin nor diodrast was found to be conspicuously elevated in either the kidney fluid or the renal tissue:—kidney fluid had about the same quantity as blood and the tissue 3 to 10 times more. The latter, though elevated, is far less than could be expected from a consideration of the high content of urine in the two compounds and the large volume of diluting fluid. The data show, then, that the original hypothesis is clearly wrong: the kidney fluid is definitely not a mixture of blood and urine. The data also suggest—but only very crudely—that the volume of the tubular lumina must be relatively small.

The facts favoring the hypothesis that the diluting fluid is renal interstitial fluid (or lymph) are these: other analyses of kidney lymph have been made and they show a composition similar to that reported here for diluting fluid. The data are best grasped by comparing the two ratios: lymph content to blood plasma content and diluting fluid content to plasma content:

	Lymph/plasma	Diluting fluid/plasma
Protein (22)	0.3	0.3
Urea (22)	1.4	1.8
Glucose (23)	0.9	0.4
Inulin (23)	0.7	0.5*

* Calculated with Equation 4 from data of Table IV, using arterial blood, as did Kaplan, Friedman, and Kruger (23).

The figures agree well, except for glucose, and this changes in the same direction in each case. For all four constituents, kidney lymph and diluting fluid have approximately the same composition. Lymphs in general (*e.g.* thoracic and cervical) are also known to be high in Cl and in osmolarity compared with plasma (24). Although there are no figures available for kidney lymph with respect to these two variables, our data show the same trend: high Cl and high osmolarity in the diluting fluid. Furthermore, the volume of the diluting fluid is consonant with the known volume of interstitial fluids: some 15 per cent of a tissue's weight (25). All the chemical evidence, in summary, points to the "diluting fluid" as being interstitial fluid.

Another source of diluting fluid which has not yet been considered is the cells themselves: it might come primarily from the intracellular compartment. If this were true, then the last portion of the kidney fluid to drain should be rich in the solutes of cell juice. This is partly true, for the last samples to drain contain more K and PO_4 than blood plasma (Tables V and VI). But the increase is small: only approximately twice the quantity in plasma, whereas one might expect, if cell juice were really included, the increase to be ten- to twentyfold. Furthermore, if cell juices entered, the Cl content of the kidney fluid should decrease and not increase as it was observed to do. For these reasons, in our opinion, intracellular fluid is not importantly involved. The same sort of arguments may be used against the thesis that anoxia causes the release of intracellular fluid. Also, since the kidneys have mostly drained (90 per cent, see Methods) by 3 minutes after the artery is clamped, the period of anoxia is short. Furthermore, the period of anoxia was deliberately lengthened in the experiment first reported (1) without conspicuously influencing the composition of the kidney fluid.

The recent reports of Allen and Reeve (13) and Pappenheimer and Kinter (26) can be readily explained from this analysis. Both groups tagged the plasma proteins, the one with Evans blue dye and the other with radioactive iodine, and found in the functional kidney an amount of protein which was disproportionately large compared with the kidney's red cell content. They concluded that the kidney's "functional hematocrit" value is low, that is, that the blood inside the kidney is relatively poor in red cells. The present work shows why there is an apparent excess of plasma proteins: they are present not only in the vascular compartment but also in the interstitial compartment. The latter is large in volume; it contains about 2 gm. per cent protein but no red cells. The total protein, then, in the functioning kidney appears to be disproportionately large. The same explanation, it is probable, will account for the low "functional hematocrit" reading of the liver (13).

The difference in composition between renal vascular blood (the word "vascular" is used in its anatomical sense of arteries, capillaries and veins) and interstitial fluid shows that a barrier of some sort must partially separate the two fluids. In such circumstances, the interstitial fluid would be a diffusate

(ultrafiltrate?) of blood and a fluid modified by metabolic activities. Thus, the barrier blocks red cell passage and hinders free passage of plasma protein, thus leading to the formation of the red cell–free and protein-poor interstitial fluid. The high content of interstitial fluid in K and PO_4 perhaps reflects its intimacy with cell juice, which contains large amounts of the two ions. Physiologically, the fluid is much more closely related to plasma, however, than it is to cell juice. Hence we prefer to call it "interstitial fluid."

The hypothesis that the diluting fluid is interstitial fluid explains another phenomenon observed in this experiment. The interstitial fluid must lie outside the capillaries. Under the conditions of the experiment, after the arterial occlusion, it must return to the vascular bed and then drain out of the renal vein. But because it is trapped outside the capillary barrier, it would drain out more slowly than the blood drains out of its vascular channel. The red cell content of kidney fluid, then, falls in successive fractions as interstitial fluid enters the renal vasculature and slowly runs out the renal veins.

A morphological basis for this concept is suggested by Pease's recent reports. He finds that when the renal cells are functional, and not collapsed as they are at the customary necropsy, the plasma membranes are invaginated into the body of the cell to create a system of minute channels "ballooned out" with fluid. Pease calls them "cisterns" (27–29). The space is not intracellular in the sense that the channels do not open into the cell juice. And it is not interstitial in the sense that it does not lie between cells. Rather, it is apparently a special cavity beyond the porous capillary endothelium (also discovered by Pease (29)) and beyond each cell's basement membrane. It is these "cisterns," we suspect, which furnish the space from which a fraction of the interstitial fluid comes. The invaginated plasma membranes would permit a truly extraordinary intimacy of interstitial fluid with the cells.

In a recent review (25), Manery inclines to the belief that muscle interstitial fluid is so small in volume that it plays only a minor role in circulation of metabolites. However, in the kidney the fluid appears to be a critical mediator between vascular blood and cells. Its volume in this organ appears very large relative to capillary volume. If about 5 per cent of the blood in the organ lies in the capillaries, just as in other systemic capillary beds (30, 31), and 14 per cent of the functional kidney is blood, the capillary volume would be only 0.7 per cent of the renal volume. But the interstitial fluid's volume appears to be 13 per cent of the renal volume, or some 20 times greater than capillary volume. Apparently a small stream, the capillary circulation, feeds a very large lake, the interstitial fluid. If much of the plasma circulates outside of the endothelial walls, the arrangement would be advantageous: it would, in effect, make a "capillary bed" for the plasma some twenty times bigger than it is for the red cells of the parenchyma.

Pappenheimer and Kinter (26) suggested, from a study of the low "func-

tional hematocrit" value of the kidney, that the red cells of influent blood are separated from plasma inside the kidney. The present work supports this concept with one modification: it is a protein-poor plasma which is thought to circulate freely through the separate and voluminous interstitial channel (see reference 32). The hypothesis further suggests that the functional circulation of the kidney is far less a "closed system" than is usually supposed. Rather, it is an "open system," except for the blood's formed elements, to be compared functionally with the open system of invertebrates: a celomic fluid bathing each individual cell.

SUMMARY AND CONCLUSIONS

The nature of the fluid draining from the kidney, after its artery was occluded, was investigated. Samples of systemic arterial blood, renal venous blood and urine were also analyzed. It was found that the fluid draining from the kidney after occlusion is a mixture of vascular blood and another fluid designated as "diluting fluid," each contributing half to the composite mixture. In volume the mixture is 26 per cent of the functionally distended kidney. With the assumption that the renal extracellular fluid can be considered a simple mixture of blood plasma and a cell-free fluid, the composition of the "diluting fluid" was deduced from the known compositions of vascular blood and total fluid draining. The ratios of its content in a given substance to that in systemic (or renal venous) plasma are: for Na and Ca, 1.0; K, 1.5; Cl, 1.2; PO_4, 2.0; urea, 1.8; plasma protein, 0.3; albumin, 0.4; glucose, 0.4, and osmolarity, 1.2. The fluid bears little or no relation to urine, especially since the urine varied considerably between individual dogs whereas the "diluting fluid" was relatively constant in composition. It was also found that the hematocrit of the fluid draining after arterial occlusion progressively decreased as it flowed out, until the last portion contained only 5 per cent red cells.

It is concluded that since renal lymph has approximately the same composition in protein, urea, glucose, and inulin as does "diluting fluid," the latter is, in all probability, renal interstitial fluid. Under the conditions of the experiment, it drains out of the kidney slowly relative to blood drainage. It is large in volume, particularly when compared with the capillaries that nourish it. Its high protein content explains the observation that the kidney is apparently naturally distended with a fluid disproportionately rich in plasma protein.

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