Relation of Lymph to Distending Fluids of the Kidney.* (23792)

H. G. SWANN, A. A. ORMSBY, J. B. DELASHAW, AND W. W. THARP
Departments of Physiology and Biochemistry, University of Texas School of Medicine, Galveston

When the renal artery and vein are simultaneously clamped and then the vein cut and the organ allowed to drain, there flows out a fluid, to be designated as "kidney fluid," which differs considerably from systemic blood in composition. Its red cell content is only half that of blood(1,2). This fact has lead to the hypothesis that renal vascular blood, under the conditions of this experiment, is diluted with another fluid. The latter is designated as "red cell-diluting fluid," or, more simply, "diluting fluid." This term, along with the term "kidney fluid," will be frequently used in the present paper. "Kidney fluid" also differs from systemic blood in that its plasma is relatively high in K, Cl, PO_4 and urea, but low in glucose, inulin and protein(1). "Diluting fluid," it is therefore postulated, is not only red cell-free, but also differs from blood plasma considerably. Its origin is unknown. But we have postulated that it comes from a large interstitial compartment(1,3), basing the hypothesis in part on the similarities in composition of "diluting fluid" and renal lymph as reported by Sugarman, Friedman, Barrett and Addis(4) and Kaplan, Friedman and Kruger(5). A more extensive analysis of the composition of the various fluids is reported here: arterial blood, venous blood, renal lymph, urine, and the kidney's distending fluids. Because lymph is generally considered to resemble organ interstitial fluids closely(6), lymph analysis should give clues as to the nature of the kidney's postulated interstitial fluids. It is also known that as fluid drains from the functionally distended kidney, its content of red cells progressively decreases until the last portion to drain shows a red cell content of about 7%(1). It was thought that this sample should most closely resemble renal lymph in composition, if "diluting fluid" were indeed interstitial fluid, and hence this sample was analyzed separately from the major sample draining. Finally, quantitative measurements of the several plasma proteins in the various fluids were made, because this information might also furnish clues as to the nature of "diluting fluid."

Methods. Dogs were anesthetized with Pentothal,† 25 mg/kg, followed with barbital-sodium, 200 mg/kg. Capsular lymphatics served as a source of lymph; these, it is generally considered, drain primarily from the cortex, but in some dogs from the medulla as well(4,7,8,9). After exposing the kidney through a flank incision, the mass of tissue surrounding the posterior pole was tied, blocking lymph drainage at this point and causing capsular lymphatics to swell. Into one of these was inserted a fine, polyethylene catheter (O. D. 0.024 in.), and free drainage allowed. Lymph flow was slow, at a rate of 0.1-0.3 ml/hr (Table III). Since 0.4-0.8 ml was collected, this phase of the experiment took 2-4 hrs. During it, lymphatics of the anterior pole and of the hilus remained undisturbed. Also during this period, urine was collected from a catheter inserted into the homolateral ureter. No diuretics were administered. After the lymph sample was collected, the dog was suspended in the prone position and the kidney dissected free from all tissue except artery, vein and ureter. Subsequent samples were taken as previously described(1): first, blood samples from the carotid artery and from the renal vein while the organ was still functioning. Then hilar blood vessels were doubly clamped, the distended kidney removed and placed in a beaker, blood vessels cut, and the organ allowed to drain for 1 minute. In volume this sample comprises about 75% of the fluids which functionally distend the kidney; it will

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TABLE I. Composition of the Several Fluids.

	A*	B*	C*	D	E	F	G	H
	Renal venous blood	Lymph	Urine	Artery Vein	Kidney fluid I Vein	Kidney fluid II Vein	Kidney fluid II Lymph	Diluting fluid Lymph
Hematocrit	38			39/38	22/38	7/38		
Plasma: Na	148	140	132	.97	.99	.98	1.03	1.03
K	4.5	5.9	183	1.04	1.22	2.26	1.73	1.86
Cl	120	129	64	1.05	1.07	1.10	1.01	1.00
PO_4 as P	4.7	5.3	322	1.13	1.81	1.76	1.56	1.66
Glucose	109	28	0	1.00	.84	.23	.90	.57
Urea	33	46	2050	1.00	1.60	1.95	1.40	1.47
Protein	6.4	3.2		1.05	.69	.50	1.02	.98

* The units employed in these columns are as follows: Na, K and Cl, in meq/l; PO_4 as P, glucose and urea, in mg %; protein, in g %.

be designated as Kidney Fluid I. The kidney was then transferred to a second beaker and allowed to drain for 15 minutes more. This last sample was about 3 ml in volume; it is designated as Kidney Fluid II. Heparin was used as anticoagulant. Routinely, microhematocrits were taken. Plasmas of large samples (arterial and venous bloods and Kidney Fluid I) and urine were analyzed for Na, K, Cl, PO_4 (as P), glucose, urea and protein by methods previously described(1). In the case of small samples (plasma of Kidney Fluid II and lymph), Na and K were measured by flame photometry, and Cl, glucose, urea and protein by the micromethods of Natelson(10). Inorganic phosphate was determined by the improved quinaldine-red method of Soyenkoff(11). Plasma protein patterns were obtained by paper electrophoresis, stained with bromphenol blue, and the intensity of the spots recorded by means of a densitometer (Photovolt Model 501A). Areas under the curves, measured by planimeter, were considered proportional to the amount of protein. Three fractions were measured: (1) albumin and α-1 globulin. (2) β-globulin and α-2 globulin and (3) γ-globulin.

Results. In Table I are shown, in Columns A, B, and C, averaged results for five dogs for renal venous blood, lymph and urine respectively. In subsequent columns are shown ratios of the contents of various substances in various fluids; from these ratios the raw data may readily be deduced. The table confirms a number of conclusions already drawn in a previous report(1): 1) as blood flows through the kidney, it changes only minimally in composition (Column D); 2) the first three-quarters of fluid to drain from the kidney (Kidney Fluid I) has a hematocrit less than that of blood (Column E) and the last quarter relatively few red cells (Column F); 3) plasma of "kidney fluid" is richer than blood plasma in K, Cl, PO_4 and urea, but poorer in glucose and protein (Columns E and F). The table also presents in Column G the composition of the last quarter of "kidney fluid" to drain (Kidney Fluid II) relative to that of lymph. This part of the kidney's distending fluid has the same content as lymph of Na, Cl, glucose, and protein, somewhat more urea and PO_4, and clearly more K. In Column H of the table are shown ratios of calculated content of various substances in "diluting fluid" to those in lymph. Methods used for the calculation have been previously described (1). Data for Kidney Fluid II and for renal venous blood were used. The ratios found are

TABLE II. Plasma Proteins in the Several Fluids.

	Artery	Renal vein	Kidney fluid I	Kidney fluid II	Lymph
Total protein, g %	6.7	6.4	4.4	3.2	3.2
Albumin and α-1 globulin, %	30	30	28	29	36
β-globulin and α-2 globulin, "	42	44	43	42	42
γ-globulin, "	28	26	29	29	22

TABLE III. Data on 5 Dogs.

Lymph flow, ml/hr	Protein: Lymph, g %	Protein: Lymph / Vein	Protein: Lymph / Kidney fluid II	K, meq/l: Lymph	K, meq/l: Kidney fluid II	K, meq/l: Urine	Cl, meq/l: Lymph	Cl, meq/l: Kidney fluid II	Cl, meq/l: Urine
.09	2.9	.56	.97	7.2	9.5	119	125	129	64
.31	2.7	.40	1.00	4.5	10.1	232	131	132	98
.11	2.2	.34	.82	6.3	11.4	198	136	134	89
.25	5.8	.83	1.07	5.8	9.6	136	132	138	38
.35	2.5	.38	1.04	5.9	10.6	228	122	123	30
Avg									
.22	3.2	.50	.98	5.9	10.2	183	129	131	64

in general close to those of Column G.

Table II indicates the relative proportions of plasma proteins in the several fluids. Both lymph and Kidney Fluid II had the same quantity of protein, *i.e.* one-half that of plasma. In both samples of "kidney fluid" and in arterial and venous plasma, the fractional distribution of protein between the 3 moieties was the same. Lymph had, proportionately, slightly more albumin and slightly less gamma-globulin than did plasma.

Table III shows for each of 5 dogs rates of lymph flow and their individual protein contents. This is of interest because of the report of Sugarman *et al.*(4) that protein content of renal lymph varies inversely with its rate of flow. In our experience, as the table shows, this was not apparent: lymph protein content was about the same for all dogs, in spite of flow variation. The table also shows ratios of lymph protein content to venous plasma content and to Kidney Fluid II content: lymph has half the quantity of protein that venous plasma has, but almost exactly the same quantity of protein as Kidney Fluid II.

In a previous paper, it was reported that "kidney fluid" was not related to urine in any consistent way(1). The same conclusion may be drawn for renal lymph (with confirmation for "kidney fluid") from Columns B and C of Table I: lymph, for example, is high in its Cl content whereas urine is low. And urine has a very high K, PO_4 and urea content, whereas lymph content of the 3 is only slightly higher than that of blood plasma. In the previous paper it was also pointed out that even though different dogs were forming urines of considerably different composition, this did not affect the composition of "kidney fluid." The same conclusion applies to kidney lymph; illustrative support for K and Cl is given in Table III. Although each dog was excreting the 2 ions at different rates, this individual variability in urinary content is not matched at all by variability in lymph content or Kidney Fluid II content. Instead, both of the latter fluids show a quite constant amount of the 2 ions.

Discussion. Our analysis of renal lymph confirms that of Sugarman *et al.*(4) and Kaplan *et al.*(5) with respect to its urea and sugar content: higher than plasma for urea and lower for sugar. But it differs from that of Sugarman *et al.* in that we found the protein content of lymph to be quite constant at about 3.2 g % (Table III). We also failed to observe the inverse relation of protein content to lymph flow reported by Sugarman *et al.* However, in our experiment only very slow lymph flows were involved; at these low flows, Sugarman *et al.* also found the lymph protein content to be about 3 g %.

Kidney lymph, from this analysis, is fairly similar to cervical lymph(12): higher than blood plasma in its content of K, Cl and PO_4 but lower in its content of protein. The two, in fact, have the same content of protein: half that of plasma. However, it is thought that renal lymph is formed in a fashion far different from cervical lymph. We postulate that renal lymph and the "diluting fluid" have a common origin: they both are a mixture of plasma and tubular resorbate. The theory was originally anticipated by Ludwig(13) and support for it has been obtained by Sugarman *et al.*(4), Kaplan *et al.*(5), and Foldi

and Romhanyi(8).

Before considering this hypothesis, two others, both of which we think erroneous, will be discussed. We have previously argued(1) that "diluting fluid" does not originate in any way from tubular urine because: 1) its composition is conspicuously different from that of the tubular contents; 2) its composition is quite constant relative to blood and does not vary from dog to dog, as does that of urine; and 3) its content of protein remains at about 3 g % as successive fractions drain, whereas this would not occur if successive fractions contained more and more tubular urine. All of these arguments can equally well be applied against the theory that "diluting fluid" is proximal tubular urine which, under the conditions of the experiment, reenters the vascular system by passing backwards through the glomerular endothelium. In addition, if such reentry occurred, "diluting fluid" should be very rich in inulin and diodrast, because proximal tubular urine is also (presumably) very rich in them. But this is known not to be so (1). Finally, the hypothesis that "diluting fluid" originates in part from cell juices, due perhaps to anoxia, has also been discussed and tentatively dismissed(1).

Instead, we postulate that "diluting fluid" and renal lymph are both a mixture of plasma and tubular resorbate. It is thought that plasma penetrates the peritubular capillary endothelium with utmost freedom (see below), and then in the interstitial space it is admixed with a large volume of tubular resorbate which is in transit toward capillary lumina. At the instant, in the present experiment, when hilar blood vessels are clamped, the interstitial space is ballooned out with resorbate and blood plasma; these fluids then, under pressure from the elastic distended kidney, are pushed into the most accessible channel: they run easily back through the porous endothelium and thence out of the renal venous system mixed in turn with vascular blood. In summary, the sources of "kidney fluid" are postulated to be these: 1) vascular blood 2) plasma filtered from blood, and 3) tubular resorbate. The last 2 of these 3 comprise what we designate as "diluting fluid." A preliminary estimate of the volume of tubular resorbate may be made from the protein content of "diluting fluid": it is roughly the same as that of the filtered plasma, and the 2 together comprise about half of the "kidney fluid."

Furthermore, if one makes the ancillary hypothesis, justification for which will be presented below, that the resorbate involved comes primarily from the proximal tubules, one can explain in part the unusual composition of "kidney fluid." Proximal tubular resorbate might be expected to have, in comparison with blood plasma, the following composition: the same amount of Na, more K (14), and little or no protein. Such a fluid as this, mixed with vascular plasma, resembles the observed "kidney fluid" and lymph fairly closely. However, the theory fails to account for the concentration of three substances in "kidney fluid": urea, Cl and sugar. One would expect both urea and Cl, particularly the latter(15), to be low in resorbate and hence low in "kidney fluid." One would also expect that the sugar content of proximal resorbate, and therefore of "kidney fluid," would be about the same as that of blood, but this is not so. The kidney's ability to manufacture sugar complicates the matter greatly (16).

In support of the hypothesis that the resorbate comes primarily from the proximal convolutions of the nephron, it is known that proximal resorbate is much greater in volume than that from the distal convolutions, being, in obligative resorption, some 4 times greater than resorption from other regions(17). *Pari passu*, the volume of the proximal convolutions, as calculated from dimensions given by Maximov and Blood(18) for human kidneys, is some 8 times greater than that of the distal convolutions. Finally, the composition of proximal resorbate is generally thought to be constant, and not to vary as does urine composition, a characteristic shared by "kidney fluid."

The presence of protein molecules in "kidney fluid" and renal lymph in the same proportions as in blood plasma indicates, following Grotte's recent hypothesis(19), that very large "leaks" must be present in the peritubular capillary endothelium. These are thought to be much bigger than the pores in

which molecular seiving of different sizes of protein molecules might occur. Grotte was able to give a preliminary estimate of the size of "leaks" in leg and liver capillaries: they must be greater in diameter than 232 Ångstroms, but smaller than 700. In peritubular capillaries holes of this size are also apparently present: Pease(20) finds numerous pores in the thin endothelium from 400 to 500 Å wide. Using Pease' microphotographs, we estimate (with due caution) that the peritubular capillary wall has roughly 40 to 120 "leaks" per square micron of surface, with a combined area of 5 to 25% of wall area. It is through this porous membrane—indeed, it is more like a collander—that plasma is postulated to run so freely. There appears, thus, to be good morphological data in support of the experiments and hypotheses here reported.

These deductions fit well into our original hypothesis concerning the peculiar renal circulation(1). As we now view it, peritubular vascular blood runs in channels bounded by a membrane containing numerous large leaks some 500Å in diameter. These prevent passage of red cells into interstitial fluid, but they allow all plasma constituents to enter with complete freedom. Thus a specialized plasma circulation to the tubular portions of the nephron is furnished. Plasma, simultaneously diluted with incoming resorbate, is thought eventually to run into a profuse system of microcanaliculi which penetrate deep into each cell and which are separated from cell constituents only by delicate invaginated plasma membranes(21,1).‡ A morphological intimacy like this is, indeed, almost a prerequisite for the completeness of the kidney's extraction from blood plasma of substances like p-amino-hippuric acid.

This theory of renal circulation is to be contrasted with that of Pappenheimer and Kinter, who suggest that plasma is twice skimmed off from red cells, first at inner afferent arterioles (*i.e.* those closer to the medulla), and second at the start of the peritubular capillary bed(22). The volume of the plasma-rich channels is thought to be larger than that of red cell channels. The data on composition of "kidney fluid," particularly in protein, oppose this hypothesis. We consider that plasma skimming, in the conventional sense of the term, does not occur in the discrete vascular system of the kidney. Rather, in peritubular capillaries, the porous endothelium "seines out" red cells and allows a large volume of plasma to enter the interstitial space and to come into utmost intimacy with tubular cells.

Summary. Renal lymph from dogs was analyzed, along with the fluids which naturally distend the kidney, arterial and renal venous blood, and urine. Lymph resembled the last quarter of the kidney's drained fluid in its content of Na, Cl, glucose and protein, but had less urea, K and PO_4. Both fluids had half the protein content of blood plasma, but the same fractional distribution, measured by paper electrophoresis, of albumin and α-1 globulin, β-globulin and α-2 globulin, and γ-globulin. Both fluids were also observed to have a constant composition in the face of varying urinary composition. The hypothesis is developed that fluids which functionally distend the kidney and which drain out of the vein when the artery is clamped originate from two sources: vascular blood and a large interstitial compartment. It is further postulated that blood plasma enters the interstitial compartment with utmost freedom. This compartment in turn is functionally distended with plasma plus resorbate in transit from tubules to the vascular system.

‡ The theory assumes that the basement membrane presents no barrier to passage of solvents or solutes.

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Microcirculatory Changes in the Liver of Choline-Deficient Rats.*† (23793)

A. M. RAPPAPORT, M. H. KNISELY, J. H. RIDOUT AND CHARLES H. BEST

Department of Physiology, Banting and Best Dep't of Medical Research, University of Toronto, Canada, and Department of Anatomy, Medical College of S. Carolina, Charleston, S. C.

The promptness with which lesions occur in zone 3 of the "liver acinus" of a rat even after a short period of choline deficiency(8) made us think that a circulatory factor must be playing its part in this disorder. By "liver acinus" we mean the structural and functional hepatic unit(1-2), the concept of which is based on the dependence of a microscopic amount of hepatic parenchyma on the terminal afferent branches bringing in materials for metabolism and on the associated terminal bile ductule carrying away the secretory product, the bile. Zones can be outlined in the acinus, areas of distinct enzymatic patterns(3), close to, or remote from the source of supply of oxygen and nutrients, *i.e.*, the terminal afferent vascular branches. One can easily see (Fig. 1) that it is zone 3 of the acinus farthest from the terminal portal and hepatic arterial branches that shows the fatty change first. The following method was adopted to observe the early microcirculatory changes in choline-deficient livers.

Method. Fifteen weanling rats (male and female) weighing between 60-80 g were fed a severely choline-deficient diet for periods ranging from 12 hours to 7 days. The diet contained 20% mixed proteins, was low in methionine and supplemented with cystine to make the organic sulfur adequate. The intrahepatic circulation was studied *in vivo* in Dr. Knisely's laboratory using his transillumination technic with the fused quartz rod(4). A technic was developed to immobilize the liver and to observe its circulation under physiologic conditions in laparotomized rats. A polyethylene apron (Fig. 2) cut to the width and length of the abdominal cavity is slipped with its folded upper margin beneath the liver. This apron has a fine hole in the lower midline through which is passed a polyethylene tubing flanged at one end. The tubing is then connected with a container of warm Ringer solution. Inflow and pressure of the solution are adjusted by a micrometer screw. This device made it possible to control the pressure gradient between abdominal and thoracic cavity. The pressure in the latter was raised by the insufflation of O_2 under 40-50 mm Hg *via* a tracheal cannula in order to immobilize diaphragm and liver(5). The insufflation pressure was controlled by a needle valve. However, the increase in insufflation pressure required to immobilize these structures was 1/6

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