hibit haptoglobin passage but not as well as the chorionic membranes.

The IgA to IgG concentration ratio in maternal sera as compared with the same ratio in the dialyzates were different even though both substances have approximately the same molecular weight. These concentration ratios were the same as those previously obtained for maternal serum and its corresponding amniotic fluid(1).

The present findings indicate that the functions of the fetal membranes in protein transport across the placenta involve complicated selective mechanisms as well as simple filtration.

Summary. Maternal sera were dialyzed *in vitro* across individual or combined fetal membranes to elucidate the functions of the membranes in protein transport. Concentration of individual proteins in the dialyzates obtained, closely resembled the composition of amniotic fluid. Results of these *in vitro* studies indicate that the fetal membranes are able to select protein molecules for passage not only according to size, but also on the basis of their chemical structure.

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Effect of Released Urine Flow on Renal Tissue Creatinine, Urea and Na in Dogs.[*] (32215)

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Urea accumulates in renal tissue during stop-flow in dogs loaded with hypertonic sodium chloride(1). In these experiments stop-flow filtration is relatively high as denoted by creatinine accumulation in renal tissue(2). The tissue concentration of sodium did not change significantly during stop-flow in these experiments or any other experiment performed in osmotic diuresis.

Accumulation of urea in renal tissue suggests, as in the case of creatinine, that urea is trapped in certain portions of the renal tubule as the column of fluid moves along the nephron due to reabsorption of sodium chloride and water from the distal tubule and collecting duct. More explicit information of the side where urea accumulated during stop-flow (lumen, cell, interstitium) may be obtained by a comparative analysis of kidneys with obstructed ureters with kidneys in which the obstruction is released after similar stop-flow periods. If tissue accumulation of urea were due to intraluminal trapping during stop-flow, release of the obstruction should result in a prompt fall in the tissue concentration.

In the light of the above considerations it is the purpose of this paper: 1) to determine the effect of release of obstruction from the ureter on renal tissue concentrations of creatinine, urea and sodium chloride and 2) to use these data and those obtained during stop-

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flow to analyze the relative permeability of the loop of Henle and distal tubule to urea.

Methods. Following 24 hours of water and food deprivation, male and female mongrel dogs (16 to 20 kg) were anesthetized with Nembutal (30 mg/kg). Both ureters were catheterized. Creatinine and PAH were injected intramuscularly in amounts of 0.1 and 0.01 g/kg, respectively. Osmotic diuresis was induced by intravenous infusion of 1.0 osmolar solutions of mannitol or sodium chloride at a rate of 10 ml/min. The infusion fluid also contained 0.4 g/kg/l creatinine, 0.1 g/kg/l PAH and 0.025 g/kg/l KCl. The urine flow was maintained at a level of 5 ml/min or more per kidney for about 30 minutes preceding obstruction. Both ureters were obstructed for 50 minutes. Then, one kidney was removed without release of the obstruction, and the obstruction was removed from the other kidney. Following collection of urine for four to six minutes, the release-flow kidney was removed. The urine and the supernatant fluid from the boiled kidney homogenates were analyzed for PAH, creatinine, urea, sodium and chloride.

The double stop-flow procedure presented in this paper is a small variation of the single stop-flow procedure. A detailed exposition of the single stop-flow procedure and analytical techniques used have been reported elsewhere (3).

All p values mentioned in this paper were calculated using the paired variates method of the Student's t-test (C. R. C. Standard Mathematical Tables. The Chemical Rubber Co., Cleveland, Ohio. 13th Edition, 1964. p. 258), with the exception of p values of Table II.

Discussion of results. Effect of ureteral stop-flow on tissue concentration of urea and sodium. Fig. 1 presents data on urea in experiments reported previously(2). The tissue concentration of urea increased during stop-flow only in experiments with high levels of stop-flow filtration(2) ($NaHCO_3$, $NaNO_3$ and NaCl diuresis) ($p<0.05$); whereas the tissue concentration of urea did not increase in experiments with low levels of stop-flow filtration(2) ($Na_4Fe(CN)_6$ and Na_2SO_4 diuresis).

The tissue concentration of sodium did not change significantly during stop-flow in any of these experiments.

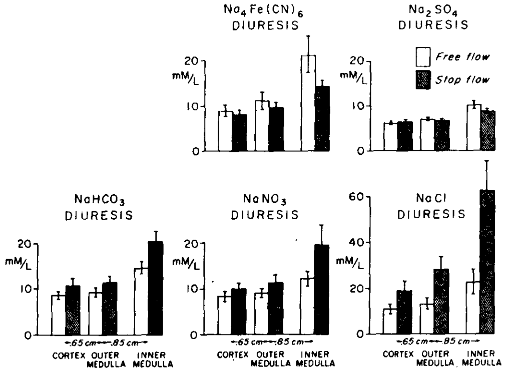

FIG. 1. Renal tissue concentrations of urea during single stop-flow experiments (2) in $Na_4Fe(CN)_6$ diuresis ($N = 5$); in Na_2SO_4 diuresis ($N = 5$); in $NaHCO_3$ diuresis ($N = 7$); in Na NO_3 diuresis ($N = 5$); and in NaCl diuresis ($N = 6$). Empty bars represent concentrations from the kidneys removed with free-flow of urine. Hatched bars represent mean concentrations from the kidneys removed during ureteral stop-flow. Vertical lines represent standard error of mean.

Effect of release urine flow on tissue and urine concentrations of creatinine, urea and sodium. Table I presents data of 9 experiments using the double stop-flow technique. Four experiments were done in mannitol diuresis, and 5 in sodium chloride diuresis. Mean urine flows were 13.0 (SE of mean ±2.2) and 7.2 (SE of mean ±0.9) ml per minute per kidney, in mannitol and in NaCl diuresis respectively. The plasma concentrations of creatinine, urea, and sodium did not change by more than ten percent throughout each experiment.

Creatinine. In mannitol diuresis, the postobstructive concentration of creatinine in renal tissue did not change significantly, despite the fact that a small but significant increase was observed during stop-flow(3). But the mean concentration of creatinine in the last urine collected, following release of obstruction, was significantly higher than in the free-flow urine, ($p<0.05$), which indicates that insufficient time lapsed during the postobstructive period to return the tissue concentration to free-flow levels. *In sodium chloride diuresis,* release of obstruction produced a marked decrease in the tissue concentration of creatinine ($p<0.05$). This finding is apparently due to the

TABLE I. Effect of Release of Obstruction on Renal Tissue Concentrations.

	Cortex		Outer medulla		Inner medulla	
	St.F.	R.F.	St.F.	R.F.	St.F.	R.F.
	millimols/liter					
			Creatinine			
Mannitol diuresis	5.2 ± .6	4.2 ± .2	4.5 ± .5	3.7 ± .1	4.9 ± .4	4.6 ± .2
NaCl diuresis	17.1 ± 1.4	8.4 ± 1.5	22.9 ± 1.3	6.8 ± .9	25.1 ± .7	7.7 ± .1
			Urea			
Mannitol diuresis	8.3 ± .9	8.4 ± 1.1	8.9 ± 1.0	9.1 ± 1.2	10.4 ± 1.7	12.8 ± 1.6
NaCl diuresis	13.6 ± 2.0	9.0 ± .7	22.5 ± 4.4	10.8 ± .9	44.8 ±10.4	28.1 ± 4.5
			Sodium			
Mannitol diuresis	64.6 ± 2.0	66.9 ± 2.3	79.1 ± 5.2	95.2 ± 3.5	80.3 ± 5.0	94.1 ± 2.6
NaCl diuresis	103.5 ± 3.1	100.6 ± 2.2	127.4 ± 5.4	152.1 ± 3.3	148.2 ± 5.6	159.5 ± 6.4

St.F. = stop-flow kidneys; R.F. = release-flow kidneys. Values are means ± SE of means from 4 dogs in mannitol diuresis and five in sodium chloride diuresis, expressed in mM/l total tissue water.

washout of the accumulated creatinine in the lumen of the nephron during stop-flow(2).

Urea. The change in tissue and urine concentrations of urea follows a parallel pattern to that of creatinine. Thus, no significant change in tissue concentration was observed in the postobstructive kidney in mannitol diuresis; and a marked decrease was observed in sodium chloride diuresis ($p<0.05$).

Sodium. The tissue concentration of sodium increased significantly in inner and outer medulla in mannitol diuresis ($p<0.05$); and it increased consistently in both outer and inner medulla in NaCl diuresis although the increase was significant only in outer medulla ($p<0.01$).

The changes in tissue and urine concentration of chloride, induced by release of the obstruction, paralleled those of sodium in all experiments. No significant changes in the tissue water content were observed following release of the obstruction.

Fig. 2 shows the concentration pattern of creatinine, PAH, urea and sodium observed in the urine samples collected following release of obstruction. As one would expect(4), the concentrations of sodium decreased and those of creatinine increased over the free-flow levels. The concentrations of urea followed a parallel pattern to those of creatinine although the urine to tissue concentration ratios of urea decreased slightly when corrected for the same ratios of creatinine, indicating(4) that some urea was reabsorbed during the period of obstruction. The concentrations of p-aminohippurate (PAH) also increased as expected. The concentration peaks of PAH and creatinine were closer in these experiments with 50 minutes of stop-flow than in experiments with shorter stop-flow periods. Apparently the amount of PAH carried towards the distal nephron (site of creatinine concentration peak) in long stop-flow periods exceeded the limited increase in concentration that apparently takes place in the proximal tubule.

To obtain a clue as to the relative site of accumulation of urea (lumen, cell, interstitium) in sodium chloride loaded dogs, the following parameter is evaluated:

Postobstructive Tissue Residuum (PTR). PTR = PEOK − PW, where PEOK (plasma extraction in obstructed kidneys) is calculated from data obtained from single stop-flow experiments(2) and PW is calculated from data obtanied from double stop-flow experiments. PEOK = $(C_{StF} - C_{FF})/P$, where C_{StF} is the tissue concentration of the substance studied in stop-flow kidney (single stop-flow experiments); and C_{FF} is its concentration in the free-flow kidney; and P is the mean plasma concentration during the period of obstruction. PW (postobstructive

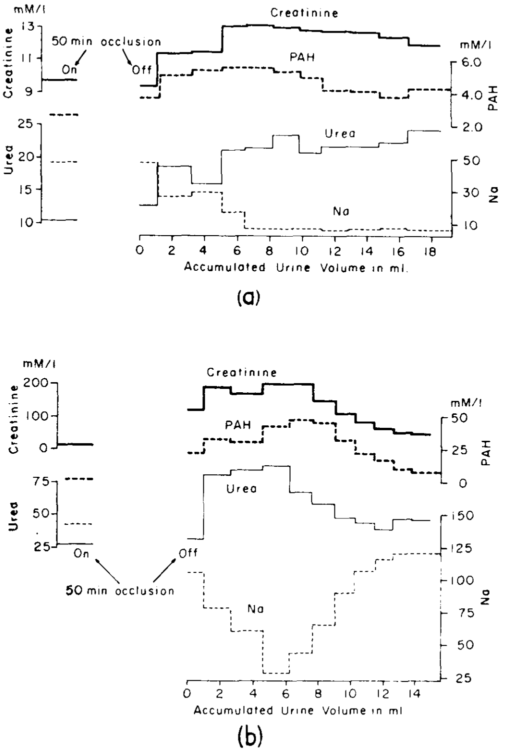

FIG. 2. Postobstructive urine concentration pattern. (a) One dog in mannitol diuresis. Free flow of urine = 13.0 ml/min/kidney. Control creatinine U/P ratios = 3.21. (b) One dog in NaCl diuresis. Free flow of urine = 9.4 ml/min/kidney. Control creatinine U/P ratio = 4.64. Plasma concentrations remained constant throughout both experiments.

washout) = $(C_{StF} - C_{RF})/P$, where C_{StF} is the tissue concentration of the substance studied in the stop-flow kidney (double stop-flow experiments); C_{RF} is this tissue concentration in the release flow kidney; and P is its mean plasma concentration.

PTR is that portion of the increase in tissue concentration in the stop-flow kidney that remains above the free-flow level following release of obstruction and is expressed in units of ml of plasma per ml tissue water.

Table II presents the calculated PTR values for creatinine and urea in eleven dogs loaded with sodium chloride. Six experiments were performed using the single stop-flow procedure(2) and 5 with double stop-flow procedure. The PTR values of both substances were not only small but statistically not significant. These findings strongly suggest that urea, as creatinine, accumulated in the lumen of the nephron during stop-flow and was washed out by the fresh filtrate following release of obstruction.

Evidence for restricted permeability of the tubule to urea. Data on renal tissue urea presented herein can be explained on the basis of the following hypothesis: 1) that urea is reabsorbed from the proximal convoluted tubules (cortex) and collecting ducts (medulla); and 2) that the loops of Henle (medulla) and the distal convoluted tubules (cortex) offer a restricted permeability to urea. Reabsorption of urea from the proximal convoluted tubule and from the collecting duct would account for the decrease in the U/P ratios of urea in the postobstructive samples when corrected for the U/P ratios of creatinine. Restricted permeability of the loops of Henle and distal tubules to urea would account for the data on urea that paralleled the results obtained with creatinine: 1) increase in tissue

TABLE II. Postobstructive Tissue Residuum (PTR) in NaCl Diuresis.

	Creatinine			Urea		
	PEOK	− PW	= PTR	PEOK	− PW	= PTR
	ml of plasma/ml of total tissue water					
Inner medulla	7.33	5.04	2.29	6.90	5.21	1.69
	±1.15	±.49	$p > .10$	±.82	±1.26	$p > .20$
Outer medulla	5.97	4.76	1.21	2.71	3.56	−.85
	±1.48	±.73	$p > .40$	±.47	±.82	$p > .30$
Cortex	4.22	2.53	1.69	1.46	1.40	.06
	±.76	±.44	$p > .05$	±.22	±.36	$p > .80$

PEOK = plasma extraction in obstructed kidneys; PW = postobstructive washout; PTR = postobstructive tissue residuum (see text). Values are mean ± SE of means from 6 single stop-flow experiments (2) and 5 double stop-flow experiments. p values express the probability that the PTR values are equal to zero calculated by the unpaired variates method.

concentration of urea during stop-flow in the 3 regions of the kidney in dogs loaded with monovalent salts of sodium; in these experiments large amounts of filtrate are carried out along the nephron as depicted by the relatively high accumulation of creatinine all over the kidney(2); 2) increase in concentration of urea in the postobstructive urine samples; and 3) decrease in tissue urea in the release-flow kidney observed in sodium chloride diuresis.

Reabsorption of urea from the proximal convoluted tubule has been repeatedly advocated(5-7) as well as from the collecting duct(8-10).

The hypothesis that the loop of Henle and distal tubule offer a restricted permeability to urea does not conflict with the prevailing view that urea, reabsorbed from the collecting ducts, is transferred into the descending limb of the loop of Henle(5,11,12) or into the ascending limb of the loop(9) if one considers the possibility that entrance of urea into the loops is not accomplished by simple diffusion but perhaps by active secretion. As a matter of fact, in the references cited above, the authors felt that the possibility of active transport could not be disregarded.

Support of the hypothesis of restricted permeability to urea in loops and distal tubules may be obtained from micropuncture experiments in rats. Lassiter *et al*(6) found a lower efflux of urea from distal than from proximal tubules in antidiuresis and in saline diuresis. Gottschalk *et al*(13) found a high recovery of urea in the urine following its microinjection in the distal tubule in saline diuresis. Carrasquer *et al*(14) using the micropuncture technique, found an increase in the intraluminal fluid to plasma (TF/P) ratios of H^3 inulin and C^{14} urea in the distal tubules of rats loaded with NaCl, by obstruction of the ureter. The TF/P ratios of H^3 inulin and C^{14} urea returned to free-flow levels as soon as the obstruction was released. Capek *et al*(15) have found the permeability of the proximal tubule to urea to be 20 times that of the distal tubule.

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