Volume reabsorption by the Loop of Henle: A micropuncture study

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Summary. The observed extension of glomerulo-tubular balance beyond the proximal tubule is thought to be due either to flow dependent reabsorption by non-accessible proximal segments and pars recta, or to osmotic volume flow out of the descending limb of the loop of Henle.

Although the general features of the countercurrent system are well known[2,3], a number of functional details are still controversial[4,5]. Equilibration of osmotic pressures along the descending limb of Henle's loop (DHL) is mediated by water abstraction in certain animals[6], by solute entry[7] or by a combination of both processes[8] in others. The importance of these aspects on the action of the loop in volume reabsorption can be assessed by micropuncture experiments requiring sampling from the renal cortex only. The present experiments were undertaken to estimate the volume reabsorbed by the loop of Henle (HL) with respect to changes in glomerular filtration rate (GFR), proximal transport and delivery of filtrate beyond the last proximal convolution.

Methods. 45 Sprague-Dawley rats were prepared for micropuncture with the technique previously described[9-13].

Free-flow experiments: the last proximal and the 1st distal convolutions of 48 superficial nephrons were identified and mapped. Free flow total collections were obtained, as previously reported[13], at the early distal and, immediately afterwards, the end proximal sites.

Microperfusion experiments: 31 nephrons were identified and perfused in vivo with a technique and apparatus that have been described in detail elsewhere[9,11]. Total collections of perfusate were made at the early distal and, subsequently, at the last proximal convolutions: perfusion rates were always calculated in vivo[11,13]. An artificial perfusate was used in 8, an ultrafiltrate of rat plasma in 23 tubules; 3 of the former and 9 of the latter group were perfused directly from the last accessible convolution at 2 different perfusion rates. Delivery to the loop was given by collection rate (CR) or directly by perfusion rate (PR). HL reabsorption was computed in these nephrons as PR − CR during microperfusion, by the formula:

$$Jv = NFR \cdot Pin \cdot \left(\frac{1}{TFp} - \frac{1}{TFd}\right),$$

during free-flow, where in = inulin concentration in plasma (P), distal (d), or proximal (p), tubular fluid (TF); NFR indicates nephron filtration rate, Jv the absolute rate of reabsorption in nl · min^{-1}. The reabsorptions were calculated from the average value of NFR or PR measured at distal and proximal collection sites. The volume marker was chemical inulin for the free flow experiments, radioactive ^{14}C-labeled-inulin (in the carboxyl group) and ^{131}I-iothalamate in the microperfusion experiments. These different markers had previously been found equivalent in our lab[9,11,13]. Details of chemical and radioactive measurements, maintenance infusion of inulin and fluids, and saline loading have been published elsewhere[9,13,14]. The data were analyzed statistically, means and SD calculated; significance of differences was assessed by paired and unpaired t-tests; regression analysis was performed by least square methods.

Results and discussion. The results obtained are reported in the table. The average reabsorption rate by the HL was 9.9 ± 4.5 nl · min^{-1} when measured by free-flow total collection, for an average delivery rate of 16.8 ± 6.9 nl · min^{-1}. The influence of delivery on resorption was established by the regression analysis reported in figure 1: Jv was a linear function of delivery rate to the HL. The slope indicates that the reabsorption was 59% of the volume delivered during hydropenia. The slope obtained during saline loading indicates that a lower fraction of the volume delivered is reclaimed between the 2 puncture sites with respect to non volume expanded animals. The average resorption measured during saline was not decreased, because of the higher delivery. The results obtained with microperfusion are shown in the table. There was no difference between perfusion with ultrafiltrate and artificial solution. The average Jv measured by microperfusion was less than that obtained during free flow, averaging 6.9 ± 3.7 nl · min: however, the average rate of delivery or perfusion was lower. Therefore the data were analyzed as for the free flow samples and plotted on figure 1: the slope obtained was similar to that of saline loading. However, in the present study the HL includes non-superficial segments of the late proximal tubule, where flow-dependent reabsorption could have occurred, since we have shown that it is dependent upon intratubular flow rate[11,12] in the proximal convolution. Nevertheless, the pars recta exhibits a low, passive rate of reabsorption, which responds to stimuli other than those important in earlier segments[15,16]. Moreover, when HL was perfused directly from the last loop at 2 different rates, no significant change in resorption occurred. These data, shown in figure 2, argue against a proximal mediation of the effects reported in figure 1. They indicate that the composition of the perfusate may be critical for the occurrence of flow dependence along HL,

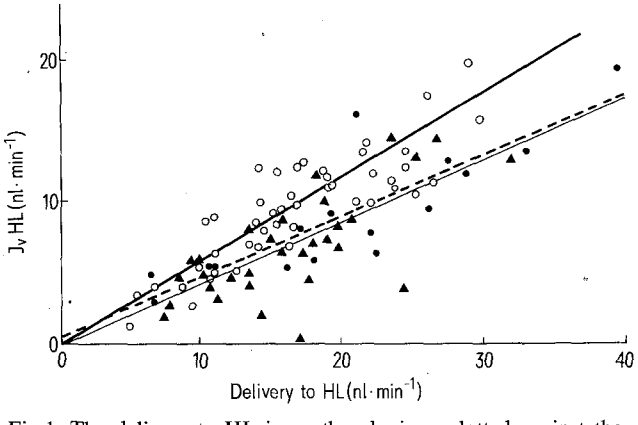

Fig. 1. The delivery to HL is on the abscissa, plotted against the calculated rate of reabsorption. Open circles indicate the 48 measurements obtained by free-flow total collections during hydropenia: the heavy line is the calculated regression line. The equation is: $Y = -0.13 + 0.59$ X; N = 48; R = 0.87; F = 169.1; $p < 0.001$. Closed circles refer to data obtained during saline loading. The equation is: $Y = 0.56 + 0.42$ X; N = 16; R = 0.83; F = 34.9; $p < 0.01$ (dashed line). The triangles indicate experiments by microperfusion. The equation (continuous light line) is: $Y = -0.13 + 0.43$ X; N = 31; R = 0.70; F = 29.6; $p < 0.01$. Regression lines in hydropenia and saline loading were significantly different by covariance analysis.

	NFR nl · min^{-1}	Fractional reabsorption Distal	Proximal	Delivery to HL nl · min^{-1}	HL reabsorption Absolute nl · min^{-1}	As a fraction of delivery	As a fraction of NFR	N
Free flow								
Saline infusion	30.8± 13.6	0.64± 0.12	0.32± 0.16	20.4± 9.2	9.1± 4.6	0.46± 0.14	0.32± 0.15	16
Paired difference		0.32± 0.15						
Paired t-test		8.53						
Hydropenia	40.0± 11.9	0.80± 0.08	0.52± 0.15	16.8± 6.9	9.9± 4.5	0.58± 0.13	0.28± 0.12	48
Paired difference		0.28± 0.12						
Paired t-test		15.94						
Microperfusion								
Ultrafiltrate of plasma	20.5± 6.7	0.56± 0.16	0.23± 0.14	15.8± 6.3	6.8± 3.9	0.43± 0.17	0.33± 0.16	23
Paired difference		0.33± 0.16						
Paired t-test		10.22						
Artificial perfusate	20.4± 4.6	0.47± 0.06	0.13± 0.09	17.8± 4.7	7.2± 3.2	0.38± 0.07	0.34± 0.09	8
Paired difference		0.34± 0.09						
Paired t-test		10.97						
Perfusion of the same loops at 2 different rates	25.7± 6.8	← High PR →			6.3± 5.2			12
	15.7± 6.1	← Low PR →			6.8± 4.9			
Combined	20.5± 6.2	0.54± 0.15	0.21± 0.14	16.4± 5.9	6.9± 3.7	0.42± 0.15	0.34± 0.14	31

The table reports data obtained by free-flow micropuncture and microperfusion. NFR indicates nephron filtration rate: in microperfusion experiments the in vivo-measured perfusion rates are also reported under this heading. HL = Henle's Loop; N = number of observations; R = correlation coefficient. The combined microperfusion data were obtained by adding the results obtained with the ultrafiltrate with those of the artificial perfusate, which were not different. The NFR is lower during saline infusion than during hydropenia: this was intentionally produced in several instances by aortic constriction in order to obtain a wide range of deliveries to the HL.

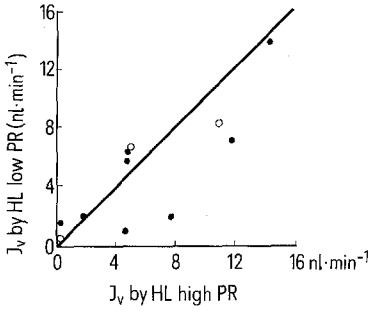

Fig. 2. The reabsorption measured at high perfusion rates (PR) is plotted against the paired measurements obtained on the same loops of Henle (HL) at lower rates of perfusion. The identity line is traced. The paired measurements are significantly correlated ($R = 0.81$; $N = 12$; $p < 0.01$), but not significantly different. Open circles refer to artificial perfusate, closed circles to ultrafiltrate of rat plasma.

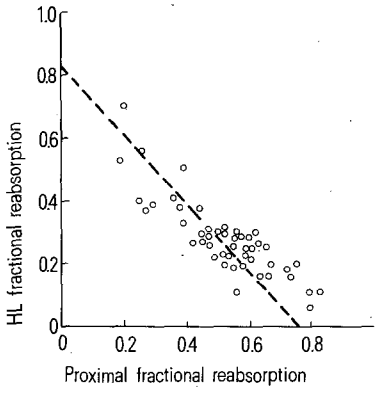

Fig. 3. The fractional reabsorption measured by free-flow micropuncture at the end of the proximal tubule is on the abscissa, plotted against that of the loop of Henle. There is a significant inverse correlation between the 2 variables. The regression equation is: $Y = 0.83 - 1.09\,X$; $N = 48$; $R = 0.85$; $p < 0.001$.

since the phenomenon was present during perfusions which included proximal segments, that presumably altered the composition of the perfusate. Therefore it seems reasonable to assume that at least a significant portion of the Jv measured was due to HL. The fall in fractional reabsorption of HL during saline loading, being similar to a well known proximal response to volume expansion[9,10], could also have occurred along the pars recta. Nevertheless, if osmotic pressure equilibration along the DHL occurs mainly by water abstraction[6], the volume of water reabsorbed must represent a fixed fraction of flow into the system, and should be directly proportional to the average interstitial osmotic pressure.

Since saline diuresis is accompanied by decreased urine and medullary osmolality[17], fractional reabsorption along HL should be lower during volume expansion. A fractional reabsorption of 40% would require a maximum interstitial osmolality of 1.6 times that of plasma, not an unreasonable value to be reached by the bend of the short loops[18]. This would indicate equilibration by water abstraction only, while true higher osmolalities would then suggest simultaneous entry of solutes along the DHL.

The major result of the present study is that an important piece of information can be added to the knowledge on proximal reabsorption: the amount of fluid reaching the earliest distal convolution accessible on the renal surface is directly related either to the rate of filtration or artificial perfusion with a fluid similar to end proximal urine. Therefore fractional reabsorption by HL and proximal tubule are inversely related (figure 3) since the former represents a constant fraction of delivery: when this falls with increasing proximal resorption, HL volume transfer becomes a lower fraction of NFR. Thus, the phenomenon of glomerular tubular balance is fully expressed up to the early distal tubule, although it represents mainly a proximal mechanism. By consequence the average fraction of filtrate reabsorbed up to the distal sampling site is 80%, and remains relatively constant with changing filtration. During saline loading, the fraction reabsorbed is less than during hydropenia, but the relationship with NFR still holds true. This extension of glomerulo-tubular balance beyond the proximal tubule is accomplished by the average

reabsorption of 28% of GFR and 58% of volume delivery between the sampling sites, independent of flow rate, though sensible to inhibition by volume expansion. Whether this is accomplished by gradient-driven water abstraction along the DHL or by flow-dependent reabsorption along the inaccessible portion of the proximal tubule and pars recta, cannot be established by the present data.

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Culture of presumptive epithelial cells from jejunal mucosa of axenic rats[1]

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Summary. Strains of presumptive epithelial and fibroblast cells were prepared from the jejunal mucosa of axenic rats. Cells were cultured on collagen gels, in highly enriched media supplemented with homologous sera and hormones, and were maintained for more than 7 weeks.

Very few successful attempts have been reported[2,3] for a true, dividing monolayer culture of mammalian intestinal cells, although several laboratories have been able to maintain suspensions of enterocytes[4-8] for varying lengths of time. The problem of culturing the epithelial cells of the jejunum is complicated by the presence of luminal bacteria, fungi and other microorganisms, which contaminate, overtake and overwhelm the initial culture in the absence of large doses of antibiotic and antimycotic agents. The major difficulty, however, would seem to lie in the damaged cell membranes, which cannot be readily avoided during the isolation of the cells for subsequent culture[9], and which presumably contributes to the poor attachment of these cells to the substratum. The only successful culture of intestinal epithelial cells has been obtained via explants from rat duodenum[3], which is relatively free of microorganisms[10]. Lichtenberger et al.[3] were able to promote the growth of the epithelial cells in preference to fibroblasts by treating the cultures with pentagastrin.

This report describes the use of germ-free rats for the successful culture of presumptive epithelial cells from jejunal explants, as a proliferative monolayer on collagen gel in the presence of pentagastrin. Several experimental criteria in support of the epithelial phenotype are presented and discussed.

Materials and methods. Jejuna were excised under sterile conditions from germ-free rats (male, 42-day-old, strain CD axenic, Charles River, Boston, USA), flushed and everted. Thin rings (1 mm thick) were sectioned from small segments and then further cut into 1-mm^3 pieces. These were placed in 5 ml of media in plastic tissue culture dishes (60 mm, Falcon Plastics, Culver City, USA) which pre-

Summary of growth characteristics of selected strains of intestinal jejunal cells

Strain	Original medium	Original supplements	Strain morphology	Stimulation of growth[a,b] Pentagastrin	Insulin	MEM D-valine treatment
Ia	Medium 199	Pentagastrin, cortisol	Epithelial	+	−	R[c]
Ib	Medium 199	Pentagastrin, cortisol	Epithelial	+	−	R
II	Medium 199	Pentagastrin, cortisol	Fibroblast	−	+	S[d]
III	Waymouth MD 705/1	None	Fibroblast	−		S
IV	Waymouth MD 705/1	Pentagastrin	Fibroblast	−		S
V	Waymouth MD 705/1	Pentagastrin	Fibroblast	−		S
VI	CMRL 1066	None	Fibroblast	−		S
VII	CMRL 1066	None	Fibroblast	−		S
VIII	CMRL 1066	None	Fibroblast	−		S
IXa	CMRL 1066	Pentagastrin	Epithelial	+	−	R
IXb	CMRL 1066	Pentagastrin	Fibroblast	−	+	S

[a] Tests performed between 2 to 4 weeks after strains established from original colonies. [b] Effect measured by significant increase in both diameter and number of colonies/plate compared with observations recorded on appropriate controls. [c] R = resistant (cell survived 4 weeks exposure to MEM D-valine medium + 10% dialysed fetal calf serum). Pentagastrin was provided throughout this treatment. [d] S = sensitive (cells became unattached and degenerated after exposure to MEM D-valine medium + 10% dialysed fetal calf serum). Pentagastrin was provided throughout this treatment.