YALE JOURNAL OF BIOLOGY AND MEDICINE 45, 217–223 (1972)

Some Artifacts in Measuring Single Nephron Glomerular Filtration Rate

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Received for publication 19 November 1971

The existence of many artifacts in measuring single nephron glomerular filtration rate (SNGFR) by micropuncture technique has been pointed out by several investigators. One of the more important sources of error is "retrograde contamination." This artifact was initially suggested by Rector and co-workers in 1966 under conditions of partial ureteral obstruction(1). Subsequently Brenner and co-workers(2) in their study of the effect of furosemide on proximal reabsorption, claimed to have evidence for retrograde contamination when a short oil block was used. It is assumed that during sampling a pressure gradient is created between the two ends of the oil column used to block the tubule distal to the puncture site. Such a gradient, greater in conditions of high intratubular pressure (such as partial ureteral obstruction or furosemide diuresis) would be sufficient to force the distal tubular fluid between the tubular wall and the oil column. Such retrograde flow will spuriously raise the inulin concentration and the volume of the collected sample with consequent erroneously high values of TF/P inulin ratio and SNGFR. In view of these considerations it has become conventional to discard unexpectedly high values of these parameters. This would seem to be an unjustified selection of data since no direct demonstration of retrograde contamination has yet been reported.

We have recently obtained evidence, by direct demonstration, that retrograde contamination does not take place in either hydropenia or saline diuresis when mineral oil is used to block the proximal tubule and a conventional collection technique is employed. Neither did we find objective evidence for such an artifact when a high pressure gradient was deliberately created across the oil block during the collection, even in the presence of partial ureteral obstruction.

Two original methods have been used: the split-droplet technique and the distal perfusion technique. The split-droplet technique (Fig. 1) involves intra-

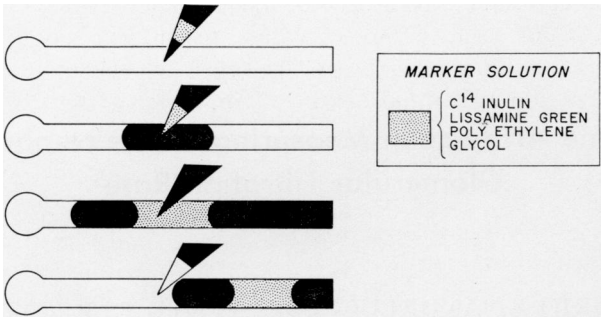

FIG. 1. Schematic drawing of split-droplet technique to detect retrograde contamination.

tubular injection of an oil droplet, splitting of the oil column with a droplet of "marker" solution, and tubular fluid collection in the same pipet when the proximal oil droplet is distal to the puncture site. The C^{14} inulin in the marker (about 200 cpm/nl) is necessary to reproduce the usual experimental condition; the lissamine green (5% solution) is useful for visualizing the marker solution and gives by itself a visual test for eventual retrograde contamination; the polyethylene glycol (PEG, MW 4000; 5% solution) is necessary to block reabsorption of the marker droplet. This technique was used in three hydropenic rats (nine tubules) and three rats undergoing saline diuresis (21 tubules) that were receiving no radioactive inulin intravenously. In each collection the marker droplet remained clearly visible in the tubular lumen throughout the 2-min collection period. The constant presence of the marker clearly indicates that it was not reabsorbed, not washed downstream by incomplete collection of tubular fluid, not aspirated back into the pipet by back contamination. Also, no evidence of retrograde contamination was detected by analyzing the collected fluid for C^{14} inulin (Fig. 2).

The radioactivity in the collected fluid was approximately the same as in "blank" pipets, i.e., pipets in which the marker solution was aspirated and then

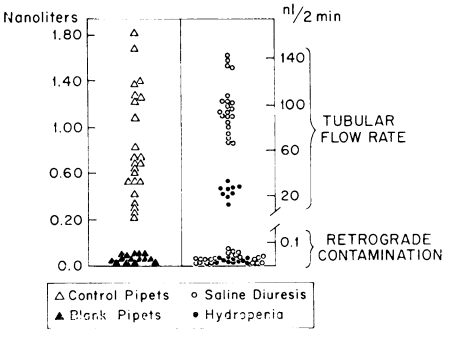

FIG. 2. Contribution of retrograde contamination to the collected tubular fluid samples, in experiments utilizing the split-droplet technique. "Control" pipets: pipets in which the "marker" solution was aspirated and then counted. "Blank" pipets: pipets in which the "marker" solution was aspirated and then expelled.

expelled. Total collected tubular fluid ranged from 20 to 140 nl; retrograde contamination, if any, contributed less than 0.05 nl. The length of the oil block in these experiments ranged between 3–10 tubule diameters.

The distal perfusion technique involves: puncture of the tubule with a pipet containing the marker solution (C^{14} inulin, lissamine green and PEG); measurement of the free-flow intratubular pressure by the Landis technique; puncture of the same tubule with a second collecting pipet containing mineral oil at a point proximal to the first pipet; injection of mineral oil and tubular fluid collection into the collecting pipet at the same time that the tubule distal to the oil block is perfused with marker solution through the first pipet (Fig. 3). Thirteen tubules in two rats undergoing saline diuresis were studied by this technique. The rats were infused with H^3 inulin to permit measurement of SNGFR. Table 1 summarizes the results. Despite relatively short oil blocks and favorable pressure gradient for retrograde flow toward the collecting pipet no evidence of retrograde contamination could be detected even in the tubules numbers 3 and 13 in which both TF/P inulin–H^3 ratio and especially SNGFR were higher than usual.

A possible drawback to the methods described is the presence of PEG in the marker solution. The PEG, in fact, might make the marker more viscous than the normal tubular fluid, thus preventing any slipping around the oil drop. Therefore eight additional proximal tubules were studied both in hydropenia (four tubules) and partial ureteral obstruction (four tubules) utilizing the distal-perfusion technique without PEG in the marker solution; in these experiments the rat was not infused with radioactive inulin to prevent any interference in measuring labeled inulin originating from retrograde contamination. The results are summarized in Table 2. It is evident that even without PEG no retrograde contamination took place despite oil blocks as short as two tubule diameters.

These results suggest that the seal between tubular wall and mineral oil block is tight enough to prevent retrograde contamination despite steep pressure gradients across the oil column. This is probably true for castor oil as well, since it is

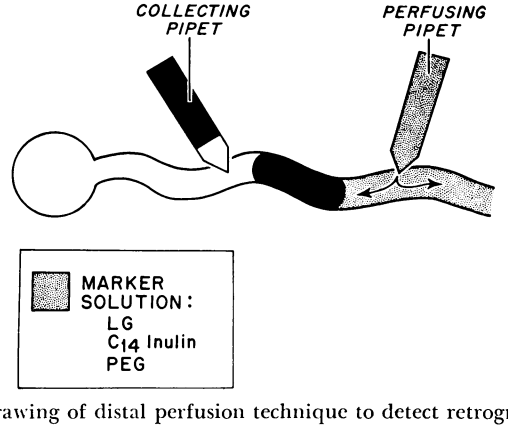

FIG. 3. Schematic drawing of distal perfusion technique to detect retrograde contamination.

more viscous than mineral oil. This may not, however, be the case with Kel-F oil used by Brenner and co-workers(2). Results of their furosemide studies(2) certainly suggest retrograde contamination. Kel-F oil is much less viscous than mineral oil and it is possible that it does not form a tight seal with the tubular wall.

Although our studies suggest that retrograde contamination is not an important artifact in measuring SNGFR, they do not solve the problem of the sporadic spuriously high values of TF/P inulin ratio and/or SNGFR. It is possible that in the process of puncture a fistula is created between the punctured proximal convolution and an underlying tubule. If the fistulous tract connects with a proximal tubule, SNGFR will be falsely high, but TF/P inulin may appear normal. If the fistulous tract connects with an underlying distal tubule both may be increased. This is a potential source of error difficult to be demonstrated during an ordinary collection. It may, however, be seen during a microperfusion with solution containing lissamine green. It should be noted that such artifact is, in our opinion, much more common, for anatomical reasons, when the ventral rather than the dorsal surface of the kidney is punctured. The rat peritoneum in fact covers only the ventral surface of the kidney and is not removed for micropuncture. Therefore, when puncturing the ventral surface of the kidney, even with a well-sharpened pipet, it is not unusual, especially in hydropenic rats, to observe marked dimpling of the capsule by the pipet tip. Suddenly the resistance imposed

TABLE 1

STUDIES ON RETROGRADE CONTAMINATION DURING SALINE DIURESIS WITH THE DISTAL-PERFUSION TECHNIQUE IN THE PROXIMAL TUBULES. MARKER CONTAINING PEG 5%

Rat no.	Tubule no.	TF/P_{In}	Nephron gfr nl/min	Perfusion pressure cm H_2O	I.T.P.[a] cm H_2O	Block size T.D.[b]	C^{14} inulin cpm	RGC[d] nl
I	1	1.45	37.2	55		3	6.3	0.020
	2	1.75	48.8	69		5	3.4	0.010
	3	2.03	(87.0)[c]	66		2	0.6	0.003
	4	1.46	37.2	44		3	2.2	0.010
	5	1.61	31.1	66		2	2.9	0.010
Mean		1.66	38.5	60	45	3	3.1	0.011
II	6	1.63	—	44		2	10.4	0.050
	7	1.33	—	44		4	0.0	0.000
	8	1.04	62.9	55		8	0.0	0.000
	9	1.73	61.4	44		3	1.7	0.008
	10	1.44	56.9	51		4	0.9	0.004
	11	1.43	48.2	55		4	0.6	0.000
	12	1.22	50.5	29		4	0.0	0.000
	13	2.80	(162.0)[c]	55		3	22.8	0.110
Mean		1.58	55.9	47	29	4	4.6	0.022

[a] I.T.P. = Free flow intratubular pressure.

[b] T.D. = Length of oil block in tubular diameters.

[c] Values in parentheses are not included in the calculated mean.

[d] RGC = Retrograde contamination.

TABLE 2

STUDIES ON RETROGRADE CONTAMINATION, DURING HYDROPENIA AND PARTIAL (25–30 CM H_2O) URETERAL OBSTRUCTION WITH THE DISTAL PERFUSION TECHNIQUE IN THE PROXIMAL TUBULES. MARKER WITHOUT PEG

Condition	Tubule no.	Volume of collected tubular fluid nl	Collection time min	I.T.P.[a] cm H_2O	Perfusion pressure cm H_2O	Block size T.D.[b]	C^{14} inulin cpm	RGC[c] nl	RGC[c] % of collected tubular fluid
Hydropenia	1	20.4	3	17.6	20.1	2	0.0	0.0	0.0
	2	63.4	4	17.6	34.1	4	0.0	0.0	0.0
	3	24.4	2	21.6	34.3	4	0.56	0.008	0.03
	4	17.5	2	16.5	16.9	3	0.0	0.0	0.0
Partial	5	22.7	3	33.4	34.7	4	0.0	0.0	0.0
ureteral	6	85.6	9	28.5	33.8	2	1.56	0.02	0.02
obstruction	7	19.8	3	34.3	35.5	2	0.0	0.0	0.0
	8	11.1	3	29.6	33.8	5	0.31	0.004	0.04

[a] I.T.P. = free flow intratubular pressure.

[b] T.D. = length of oil block in tubular diameters.

[c] RGC = retrograde contamination.

by the capsule is overcome and the pipet tip penetrates several layers deep into the kidney. If the pipet tip is then withdrawn and positioned into a superficial lumen, a fistulous tract between tubules is created. Similar fistulous tracts may be generated during long collections when respiratory or arterial movements of the kidney or both are not prevented in micropuncture preparation.

Another possible explanation of spuriously high values for TF/P inulin ratio and SNGFR may be the retrograde collection; i.e., the erroneous collection of tubular fluid distal to the oil block. In our experience this is not unusual in conditions of low-flow rate such as partial ureteral obstruction or, more frequently, in case of collections from distal tubules.

We know that the collection procedure implies intratubular injection of an oil drop and a transitory suction of tubular fluid to overcome the resistance of the pipet tip. The injection of oil will spread the oil down and upstream (Fig. 4). When the flow rate is low, it is not unusual for the operator to apply the initial suction immediately, without waiting until the oil column has moved distal to the pipet tip. It is erroneously assumed that the proximal part of the oil column will be aspirated back into the collecting pipet followed by the tubular fluid from the proximal tubule. That is not necessarily true. Sometimes, in fact, if the suction is too strong and the pipet tip too big with the bore toward the collecting duct, it will be the distal part of the oil column that is aspirated into the collecting pipet. The remaining column will block the fluid coming from the glomerulus, allowing the collection of fluid from the collecting duct and from the distal tubule of other nephrons through their distal connections. Obviously the TF/P inulin will be enormously elevated thus accounting for the huge values so frequently obtained from distal collections and usually interpreted as retrograde contamination. Actually it is a retrograde collection.

We have sometimes seen that at the end of the collection, the withdrawal of the collecting pipet from the distal tubule was followed by a movement and disappearance of the oil block in a direction expected to be toward the glomerulus; the measured TF/P inulin ratio in these cases was tremendously high. These were also the instances in which continuous or at least frequent aspiration was necessary to keep the collection. We could also reproduce such an artifact. Results of microdissection experiments carried out in order to determine the direction of the tubular flow after collection from distal tubules support this view.

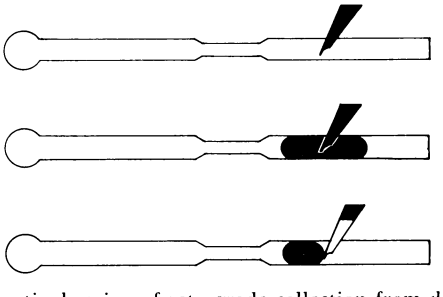

FIG. 4. Schematic drawing of retrograde collection from distal tubules.

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