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The Early Phase of Experimental Acute Renal Failure

II. Tubular Leakage and the Reliability of Glomerular Markers

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Summary. Experiments were designed to determine whether leakage of substances across the tubular epithelium, which are impermeant in the normal kidney, falsifies the measurement of glomerular filtration rate in acute renal failure. Permeability to those substances most commonly used for filtration rate determination, polyfructosan, inulin and ferrocyanide, was estimated by measuring their recoveries following perfusion through various nephron segments in haeme pigment, ischaemic and nephrotoxic models of actue renal failure. Late proximal recovery of ^{14}C ferrocyanide was only marginally decreased compared to controls, by a maximum of 6%. Distal recovery of polyfructosan, ^{14}C and ^{3}H inulin were depressed somewhat more, by a maximum of 11%. Urinary recovery of ^{14}C inulin was reduced by only 15% in kidneys showing severely restricted renal function. It is concluded that tubular leakage is not a feature of significance in the early phase of moderate acute renal failure, that ferrocyanide and inulin are reliable markers for the determination of nephron filtration rate and water reabsorption, and that the reduction in whole kidney inulin or polyfructosan clearance reflects primarily a reduction in glomerular filtration rate.

Key words: Acute renal failure – Tubular leakage – Proximal ferrocyanide recovery – Distal inulin recovery – Urinary inulin recovery.

INTRODUCTION

An investigation of the mechanisms responsible for the impairment of renal function in experimentally induced acute renal failure requires that glomerular filtration rate and tubular reabsorption can be reliably determined. Substances which are suitable for the measurement of filtration rate and tubular water absorption must remain in the tubular system after being freely filtered and must be neither reabsorbed nor secreted. One theory has suggested that in acute renal failure, the decrease in renal function is caused not by a reduction in renal blood flow and glomerular filtration rate but by a loss of filtered substances through an epithelium made friable and abnormally leaky by the damaging agent. The loss of a greater than normal percentage of filtered load across the tubular epithelium could account for the retention of nitrogenous substances normally excreted and give an erroneously low value for the glomerular filtration rate because of the non-quantitative excretion of marker substances such as inulin and creatinine.

The experimental evidence, which bears direct relevance to the leak hypothesis and does not rest upon subjective impressions, is of 3 types. Firstly, that the tubular fluid concentration of mannitol or creatinine relative to plasma is lower in acute renal failure than the corresponding value for inulin, indicating a loss from the tubule of impermeant low molecular weight substances. Although this was the case in uranium induced acute renal failure [12, 16], it could not be verified for mercuric chloride poisoning [7]. Secondly, that there is a decrease in single nephron inulin clearance measured at nephron puncture sites progressively more distal to the glomerulus, as if the quantity of inulin remaining in the tubule diminishes with passage along the nephron. This was reported for mercuric chloride induced renal failure [1] but was not confirmed subsequently in the same model [2, 3, 7] nor in dichromate [3] or ischaemia [20] induced renal failure. Thirdly, that a given quantity of intratubularly injected inulin could not be quantitatively recovered from the final urine and that portions of the non-recovered inulin may be found in the urine from the contralateral, non-injected kidney,

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implying leakage from the tubular system into the circulation. Extensive losses of microinjected inulin have been reported for mercuric chloride [19], uranyl nitrate [4, 18] and ischaemic [20, 6] renal failures but have not been found following 3 h partial ischaemia [5] after uranyl nitrate [8] or potassium dichromate [3] poisoning.

Even though critical scrutiny of the data presented in favour of leakage has revealed many inconsistencies [17] which cast doubt on the validity of the conclusions, the question concerning inulin leakage from the acute renal failure tubules and, hence, the reliability of filtration rate determination is far from settled. The present experiments were undertaken to determine whether, in a variety of models of acute renal failure in its early stage, tubular leakage was a predominant feature of the pathophysiology of this syndrome. Since for determination of single nephron filtration rate, either by micropuncture or with the Hanssen technique [11], and of whole kidney filtration rate, the substances most commonly employed are polyfructosan, inulin and sodium ferrocyanide, the recovery of these markers from various nephron segments was examined to determine with what confidence these substances could be employed to measure filtration rate at specific sites along the damaged nephron.

METHODS

Preparation of the Animals and Experimental Models

The surgical preparation of the animals for micropuncture has been described in detail elsewhere [15]. In brief, in Sprague-Dawley rats under Inactin anaesthesia, the trachea, 1 jugular vein and 1 femoral artery were cannulated with polyethylene catheter. Arterial blood pressure was monitored throughout, and each animal received a continuous infusion of 0.9% NaCl alone, or containing 10% polyfructosan (Inutest, Laevosan), at a rate of 0.4 ml/h/100 g BW, via the jugular vein. The dorsal surface of the kidney was exposed through a flank incision and the ureter cannulated for the collection of urine. Renal polyfructosan clearance was determined as described previously [15].

The experiments were performed on 7 groups of animals ranging in weight from 166–302 g. One group represented normal renal function and served as control. One group demonstrated haeme pigment induced renal failure, investigated 17–24 h after injecting 0.75 g/kg methaemoglobin intravenously, following 30–40 h dehydration. Two groups illustrated ischaemic damage, examined 1–4 h after clamping the renal artery for 45 or 60 min. Three groups represented nephrotoxic renal failure, observed 24–33 h following the administration of 15 mg/kg uranyl nitrate or within 3–12 h after 4.7 or 6.0 mg/kg mercuric chloride subcutaneously. The exact method of production of these experimental models has been reported [15].

Micropuncture Technique

Micropuncture was performed by 2 operators, who simultaneously viewed the surface of the kidney through a Leitz double microscope at a magnification of ×160. Tubular segments were identified by injecting a bolus of 0.5 or 1.0% lissamine green solution from 8 μ OD micropipettes into randomly selected proximal tubules with the aid of a mercury manometer. Quantitative collection of tubular fluid was performed using 9 μ OD micropipettes, filled with Sudan-black stained paraffin oil, and were only accepted if the 4–8 tubular diameter oil block remained stable throughout the collection period. Glomerular markers were injected as described previously [10] from 5–7 μ OD micropipettes containing the test substance dissolved in bicarbonate Ringer's solution and coloured with either 0.5% lissamine green (Chroma, Stuttgart) or 0.1% FD and C green (Keystone, Chicago). The perfusion pipette was placed in the early or mid proximal tubule at least two convolutions distal to the lissamine green pipette, which was left *in situ*. To prevent the perfusate entering the more proximal lissamine green pipette, the mercury manometer was adjusted so that a gentle stream of lissamine green flowed throughout the perfusion and collection periods. After the end of perfusion, collection was continued for at least twice the passage time between the lissamine green and collection pipettes.

The experimental series are summarized in Table 1 and consisted of the following:

Series 1. To determine whether polyfructosan, when analysed with the anthrone photometric method, is suitable for the measurement of renal function in damaged kidneys, distal, late and early proximal fluid samples were collected from normal and 45 min ischaemic kidneys of animals which had received no polyfructosan and were compared to standard polyfructosan solutions by chemical analysis. In addition, the recoveries of polyfructosan and ^{14}C inulin from the distal tubule of normal and 45 min ischaemic kidneys were determined and compared.

Series 2. To investigate to what extent the proximal tubule was leaky to a low molecular weight glomerular marker in relatively severely damaged methaemoglobin, ischaemic and nephrotoxic models of acute renal failure, the recovery of ^{14}C labelled sodium ferrocyanide from the late proximal tubule was determined.

Series 3. To assess in the same models of acute renal failure the degree to which a higher molecular weight substance, inulin, was retained during its passage through the proximal tubule and loop of Henle, the recovery of ^{3}H inulin from the distal tubule was examined.

Series 4. To determine the loss of inulin along the entire tubular system of milder ischaemic or mercuric induced acute renal failure, the serial recovery of ^{14}C inulin from the ureteral urine in four 15 min periods was measured.

Quantification Procedure

For each experiment, a freshly made solution was used both as a standard and as perfusion solution. Aliquots of this solution were introduced into the tip of paraffin oil filled perfusion pipettes and sealed with Sudan black stained castor oil. Three randomly selected perfusion pipettes were reserved and their contents analysed at the same time as the tubular fluid or ureteral urine samples. The average contents of the three perfusion pipettes was taken as 100% and the content of the collected sample was expressed relative to this value.

The chemical determination of polyfructosan was performed on 5 nl aliquots of both standard solutions and collected samples using the anthrone photometric method [13]. Radioactive determinations of ^{14}C and ^{3}H were made on the entire standard or collected sample, which was added quantitatively to a liquid scintillation fluid, either a modified Bray's solution or Aquasol, and counted for 10 or 20 min in a Packard tri-carb.

Table 1. The 4 experimental series, depicting the number and type of experimental models examined, the volume and concentration of the test substance injected, the perfusion and collection sites and the mean perfusion time ± S.D

Series	Experimental model	Animals (*n*)	Perfusion substance	Concentration	Perfusion volume (nl)	Perfusion time (min)	Perfusion site	Collection site
1.	Normal kidneys	4	–	–	–	–	–	distal, late and early prox
	45 min ischaemia	6	–	–	–	–	–	
	normal kidneys	6	polyfructosan	200 mg%	48–66	3.46 ± 1.19	early-mid prox	distal
	45 min ischaemia	9				5.25 ± 1.84		
	normal kidneys	4	^{14}C inulin	5 μCi/ml	29	2.73 ± 0.93	early-mid prox	distal
	45 min ischaemia	4				3.07 ± 1.89		
2.	Normal kidneys	4	^{14}C ferrocyanide	10–36 μCi/ml	29	1.53 ± 0.74	early prox	late prox
	0.75 g/kg methaemoglobin	3				1.61 ± 0.78		
	60 min ischaemia	4				2.60 ± 1.68		
	15 mg/kg uranyl nitrate	3				1.71 ± 0.80		
	6 mg/kg mercuric chloride	4				2.78 ± 1.15		
3.	Normal kidneys	5	^{3}H inulin	10–24 μCi/ml	23	1.32 ± 0.35	early-mid prox	distal
	0.75 g/kg methaemoglobin	4				1.83 ± 1.33		
	60 min ischaemia	4				1.41 ± 0.56		
	15 mg/kg uranyl nitrate	4				2.08 ± 1.38		
	6 mg/kg mercuric chloride	4				1.88 ± 0.86		
4.	Normal kidneys	6	^{14}C inulin	5–10 μCi/ml	56–58	3.73 ± 1.08	mid-late prox	ureteral catheter
	45 and 60 min ischaemia	10				4.88 ± 2.02		
	4.7 mg/kg mercuric chloride	7				3.74 ± 1.80		

Statistical Analysis

The experimental data were pooled in each group and expressed as mean ± S.D. Statistical evaluation of the results was performed with the Student's *t* test for unpaired data when the variance ratio of the 2 populations proved this to be applicable. Otherwise, the Wilcoxon test for unrelated samples was employed. The data were considered to have reached statistical significance when the 2-tailed probability was equal to or less than 0.05.

RESULTS

The Appearance of the Kidneys and Their Function

The experimental models selected were all devoid of collapsed tubules, and in all nephrons there was a spontaneous flow of tubular fluid. Occasional distal tubular casts were to be seen in the 0.75 g/kg methaemoglobin kidneys, and uniformly dilated tubules was a characteristic of the 60 min ischaemic and 6.0 mg/kg mercuric chloride models.

The polyfructosan clearances, urine flow rates and urine to plasma polyfructosan concentration ratios, measured only in those animals in which the ureteral urine was not used for recovery determinations, are summarized in Table 2. The polyfructosan clearance

Table 2. The polyfructosan clearance, Cp, urine flow rate, *V*, and urine to plasma polyfructosan concentration ratio, U/Pp, obtained in the experimental models. The data are given as mean ± S.D., * denotes statistically significant difference from the control values in normal kidneys. The data for 45 min ischaemic and 4.7 mg/kg mercuric chloride kidneys were measured in another experimental series, reported previously [15]

Experimental model	Dose	*n*	Cp ml/min/100 g	*V* μl/min/100 g	U/Pp
Normal kidneys	Control	5	0.39 ± 0.06	2.67 ± 1.28	151 ± 112
Methaemoglobin	0.75 g/kg	4	0.25* ± 0.04	1.55 ± 0.85	183 ± 58
Post-ischaemia	45 min	10	0.13* ± 0.08	2.60 ± 1.89	49* ± 37
	60 min	4	0.07* ± 0.08	2.60 ± 1.30	24* ± 16
Uranyl nitrate	15 mg/kg	4	0.28* ± 0.02	5.94* ± 5.06	69* ± 31
Mercuric chloride	4.7 mg/kg	8	0.21* ± 0.04	1.20* ± 0.27	180 ± 63
	6.0 mg/kg	4	0.15* ± 0.05	1.69 ± 1.13	118 ± 60

was significantly depressed compared to the controls in all models of acute renal failure; urine flow rate was significantly reduced only in 4.7 mg/kg mercuric chloride kidneys and was increased in 15 mg/kg uranyl nitrate kidneys; the urine to plasma polyfructosan ratio was significantly decreased in 45 and 60 min ischaemic and 15 mg/kg uranyl nitrate kidneys.

Series 1

Tubular fluid samples from the distal, late proximal and early proximal tubules in normal kidneys, which had received no polyfructosan, were obtained from 16 nephrons. In at least half the samples from each nephron segment, the apparent polyfructosan concentration was only 3 mg% or less, the mean values being 8.3 ± 12.9, 9.0 ± 15.7 and 3.8 ± 8.8 mg% in distal, late and early proximal samples, respectively, which were not significantly different from each other or zero. Tubular fluid samples from the three tubular segments in 45 min ischaemic kidneys, also having received no polyfructosan, were obtained from 25 nephrons. In only a third of the samples was the apparent polyfructosan concentration 5 mg% or less and the mean values of 18.2 ± 20.0, 26.7 ± 26.3 and 19.9 ± 27.4, in distal, late and early proximal samples, respectively, were not statistically different from one another but were all statistically different from zero. Thus, the possibility arises that when employing polyfructosan test solution with a concentration of only 200 mg%, this artifact causes the recovery to be overestimated. This question was clarified by comparing the recovery of polyfructosan and ^{14}C inulin from the distal tubule of normal and 45 min ischaemic kidneys. The results are depicted in Figure 1. For both normal and 45 min ischaemic kidneys the recovery of glomerular markers was not complete, the statistically significant reduction in polyfructosan recovery of 8% comparing well with the not significant reduction in ^{14}C inulin recovery of 7%.

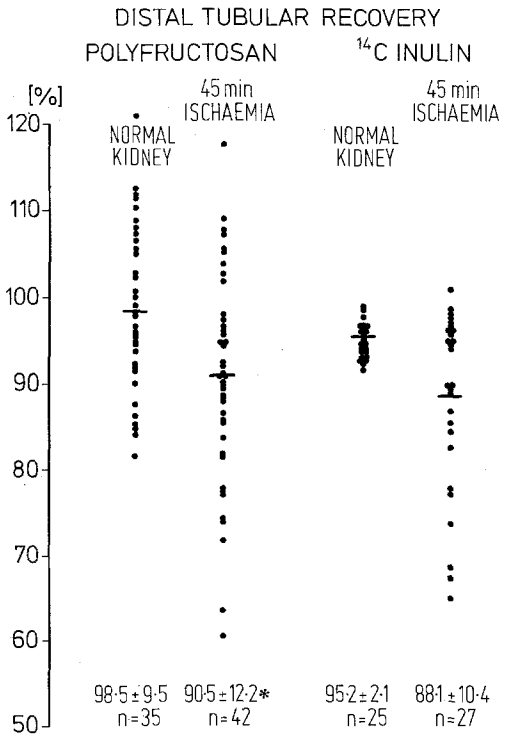

Fig. 1. The polyfructosan and ^{14}C inulin recoveries from the distal tubules of normal and 45 min ischaemic kidneys. The data are expressed as mean ± S.D., * indicates statistically significant difference from normal kidney recovery

Series 2

The recoveries of ^{14}C sodium ferrocyanide from the late proximal tubule of models of more severe acute renal failure are illustrated in Figure 2. The depressions in recovery by 3%, 6% and 5% for 0.75 g/kg methaemoglobin, 60 min ischaemia and 6.0 mg/kg mercuric chloride kidneys, respectively, were statistically significant but that of 2% for 15 mg/kg uranyl nitrate kidneys was not.

Series 3

The recoveries of ^{3}H inulin from the distal tubule after its passage through the proximal tubule and loop of Henle, in more severely damaged models of acute renal failure, are depicted in Figure 3. The decreases in recovery by 3%, 11%, 10% and 5% in 7.5 g/kg methaemoglobin, 60 min ischaemia, 15 mg/kg uranyl nitrate and 6.0 mg/kg mercuric chloride kidneys, respectively, were statistically significant.

Series 4

The recoveries of ^{14}C inulin from the final urine after passage through the entire nephron, in relatively mild forms of acute renal failure, are shown in Figure 4. The reduced total recovery of 15% for 45 and 60 min ischaemic kidneys (where recoveries were similar, permitting the data to be pooled) was statistically significant, whereas that of 2% for 4.7 mg/kg mercuric chloride kidneys was not. In the normal and mercuric chloride kidneys, most inulin was recovered in the first 15 min collection period. For the ischaemic kidneys, however, inulin recovery was perceptibly slower, occuring predominantly in the first 2 or in the second period in half the cases. In all models, inulin excretion was virtually zero by the fourth period.

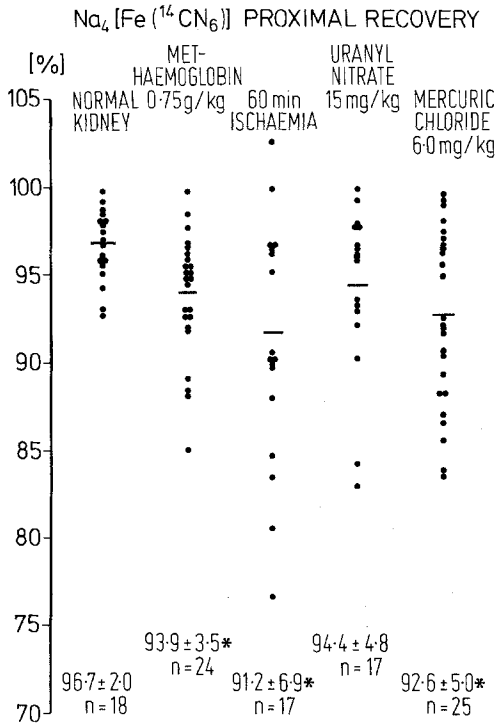

Fig. 2. The ferrocyanide recoveries from the late proximal tubule of more severely damaged methaemoglobin, ischaemic and nephrotoxic models of acute renal failure. The data are given as mean ± S.D., * denotes statistically significant difference from normal kidney recovery

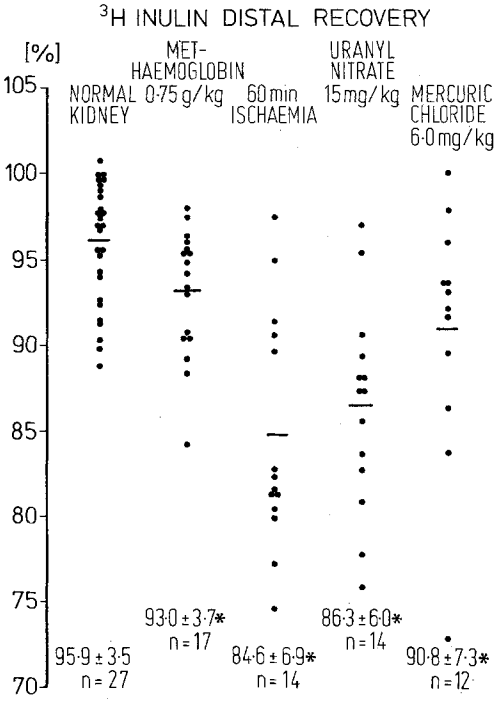

Fig. 3. The ^{3}H inulin recoveries from the distal tubule of more severely damaged methaemoglobin, ischaemic and nephrotoxic models of acute renal failure. The data are expressed as mean ± S.D., * indicates statistically significant difference from normal kidney recovery

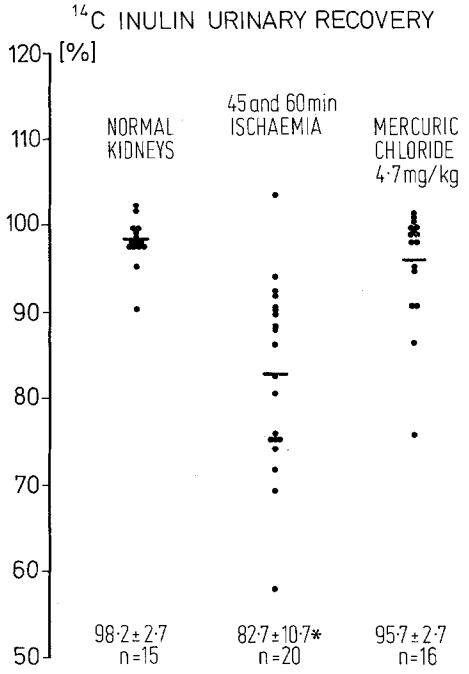

Fig. 4. The ^{14}C inulin recoveries from the final urine of milder forms of ischaemic and nephrotoxic models of acute renal failure. The data are given as mean ± S.D., * denotes statistically significant difference from normal kidney recovery

DISCUSSION

The analysis performed on fluid samples obtained from 45 min ischaemic nephrons demonstrates that some chromogen is present in the tubular fluid of these damaged kidneys which may lead to overestimations of polyfructosan concentration when using an anthrone photometric analysis. Despite the good agreement between chemical and radiochemical determinations of distal recovery in 45 min ischaemic kidneys, chemical analysis is not suitable for estimating low concentrations of polyfructosan or inulin in the tubular fluid of damaged kidneys, and when measuring recovery or single nephron filtration rate, preference should be given to the more reliable radiochemical methods. However, since the chromogen concentration remained constant along the nephron, the magnitude of the artifact must decrease as the concentration of polyfructosan or inulin increases in the tubule. Thus, in final urine, where polyfructosan or inulin concentrations are very high, this error should be negligible.

With regard to the proximal tubular recovery of the smaller substance, ferrocyanide, significant reductions were apparent. However, since most of the models examined have elevated intratubular pressures [15], which has been demonstrated to increase the

permeability of smaller, usually non-permeant molecules in the normal kidney [14], this phenomenon may be unrelated to the acute renal failure. Nevertheless, the losses experienced were negligible and do not preclude the use of this substance for the determination of single nephron filtration rate from the proximal tubule in these models of acute renal failure.

Concerning the distal tubular recovery of the larger molecule, inulin, all models examined showed a significantly reduced recovery. However, excluding perhaps the 60 min ischaemia and 15 mg/kg uranyl nitrate models, these losses are not of sufficient magnitude to exclude inulin from being a suitable marker for the determination of single nephron reabsorption or filtration rate from a proximal or distal puncture site.

For the urinary inulin recovery, a reduction could not be detected in the 4.7 mg/kg mercuric chloride kidneys, although in the ischaemic models (8 at 45 min and 2 at 60 min), significant losses were apparent, suggesting that whole kidney inulin clearance is somewhat underestimated in ischaemic models of acute renal failure.

Although no other information is available concerning the reliability of ferrocyanide or inulin in the various nephron segments in experimental models of acute renal failure, some determinations of inulin recovery after passage along the whole nephron have been made in some experimental models. The results of such investigations are conflicting and can be divided into those which show complete or nearly complete recovery of microinjected ^{3}H inulin and those which indicate substantial losses. The discrepancies between those data showing nearly complete recovery and those demonstrating extensively reduced recovery of microinjected inulin in acute renal failure are difficult to resolve. One explanation may be that the kidneys which experience nearly no losses are less damaged than those where recovery is incomplete. In the present study, for the 45 and 60 min ischaemic models and the 4.7 mg/kg mercuric chloride model, and in other investigations for the 3 h partial ischaemia [5] and uranyl nitrate [8] models, where losses were absent or moderate, whole kidney inulin clearances were 31%, 50%, 49% and 55% of control, respectively. For the few hours following 60 min ischaemia [20], 5 mg/kg [18] or 25 mg/kg [4] uranyl nitrate, where losses were substantial, whole kidney inulin clearances were lower, at 14%, 3% and 21%, respectively, of control.

The reduced ipsilateral recovery of microinjected inulin has been interpreted by all those who have found it to be indicative of a tubular leakiness. However, the failure to recover inulin need not indicate leakage across the tubular epithelium, for the non-excreted portion may be sequestered within the tubular system. Were, for instance, glomerular filtration rate to be severely reduced, tubular fluid flow might be insufficient to carry the microinjected inulin along the nephron and into the ureteral catheter. Alternatively, should the tubules contain casts and debris, as suggested for the 60 min ischaemic model [20], the passage of inulin would be impeded by the obstruction, thus restricting its excretion. Since, for all microinjection studies where the ispsilateral recovery was depressed in acute renal failure, with the exception of one [19], the total inulin recovered from both kidneys was far from complete, the possibility that some inulin is retained in the ipsilateral kidney deserves serious attention.

The real evidence supporting tubular leakiness is the demonstration of inulin excretion by the contralateral, non-injected kidney. In 2 of the investigations purporting to have demonstrated tubular leak, despite considerable restriction of whole kidney inulin clearance and ipsilateral inulin recovery, contralateral inulin excretion was absent [18] or marginal [4]. In another study, where contralateral excretion was verified [19], in 3 out of 6 of the microinjections, contralateral excretion exceeded 72%, indicating that decapsulation of the kidney, together with the large micropipettes and long injection periods employed, may have resulted in leakage at the puncture site. Similarly, in another investigation confirming contralateral excretion [20], the greater contralateral than ipsilateral inulin excretion in 10 instances out of 18, may suggest that the very high intratubular pressures in these kidneys caused inulin escape from the puncture site. The evidence indicates that contralateral excretion of inulin need not accompany even the most reduced ipsilateral recovery and thus the possibility that inelasticity of the damaged tubular epithelium and elevated intratubular pressure instigates an artifactual loss of inulin must be considered.

The role that insufficient tubular fluid flow and loss of inulin at the puncture site may have played in reducing inulin recovery cannot be determined retrospectively. However, in the present experiments, these factors can be excluded. Only nephrons with demonstrable superficial tubules were selected for the urinary recovery experiments, thus precluding the existence of any mechanical blockage in the loop of Henle. Adequate tubular flow was guaranteed, since existing tubular flow was enhanced by adjusting the upstream lissamine green pipette so as to perfuse constantly. Furthermore, the use of relatively large volumes of coloured perfusate and the constant observation by 2 persons enabled leakage at the puncture site to be identified. For normal kidneys, where proximal or distal recovery of ferrocyanide or inulin approaches 100%, barely perceptible leaks of the perfusion solution at

the puncture site resulted in a reduction of the average recovery in 10 instances to 89.4 ± 2.8. Quite obvious leaks, on the other hand, lowered proximal ferrocyanide recovery to 1.4% in 1 instance and urine recovery to 6.5% and 15% in 2 cases in the 1 h following injection. It is debatable whether in two of the experiments reporting diminished ipsilateral recovery, in which the injected volumes were only 2 nl (20) and 4 nl (4), any such leakage would have been observable.

Some information is available which indirectly relates to the leakiness of the proximal tubule in acute renal failure kidneys. Measurements of tubular reabsorption obtained in the proximal convolutions using the split-oil drop method [9] have shown that fluid reabsorption is extensively decreased after 60 min ischaemia [20], 24 h and 48 h following 2–4 mg/kg and 4 mg/kg mercuric chloride [19,21] and 72 h after 0.7–1.5 mg/kg potassium dichromate [3]. In this experimental situation, the reabsorption of a saline droplet introduced into a column of black stained oil, is visualised by the rate of approach of the two half columns of oil. In the acute renal failure tubules, the rate of approach was slower than normal, indicating depressed reabsorption. However, were tubular permeability increased more than active transport depressed, this would be manifest as an increase in the rate of approach of the oil columns. This rapid disappearance of a saline droplet, albeit infrequent, has been observed in the necrotic tubules 48 h after 4.0 mg/kg mercuric chloride injection [21] and 2 days following potassium dichromate administration (Gertz, unpublished observations).

It may therefore be concluded that tubular leakage can exist, for the rapid disappearance of a saline drop in the split-droplet situation, where leakage at the puncture site cannot occur because of the interposition of the oil blocks, provides proof of it. Nevertheless, the pertinent question is whether this situation is prevalent in experimental acute renal failure or is a seldom occurence. The 2 reports of accelerated saline reabsorption describe the function of tubules, observed in vivo to be severely necrotic, the neighbouring, non-necrotic tubules showed diminished, not enhanced reabsorptive properties [21]. Furthermore, the necrotic tubules had nearly no tubular flow and autoradiographic examination of them following intra-arterial injection of ^{3}H inulin showed that no filtered fluid reached them [21]. Thus, tubular friability may only be associated with the severest forms of nephron disruption in which glomerular filtration no longer occurs. In two of the investigations claiming extensive tubular leakage of microinejcted inulin [20,19], an enhanced proximal tubular reabsorption with the split-drop method was not in evidence, and neither of these models, at the time of studied, is characterised by extensive necrosis. In the present investigation, in models also not displaying any degree of tubular necrosis, serious inulin losses did not occur. As such, it would seem that early in the course of less severe renal damage, tubular leakage is not a feature of pathological significance.

In conclusion, although the difficulty in reobtaining intra-tubularly injected inulin from acute renal failure kidneys is well documented, the cause is not clear. The role of tubular leakage has not been unequivocally demonstrated in most cases, and no data is available which permits an assessment of the degree to which artifacts, resulting from the application of micropuncture techniques to kidneys displaying a multitude of features other than epithelial changes, may influence the results. The radiochemical determination of marker substances, such as ferrocyanide and inulin, when confined to the early stage of milder forms of acute renal failure, would seem to give data which is quantitatively reliable, thus permitting accurate determination of single nephron filtration rate, tubular fluid reabsorption and whole kidney filtration rate. In such models, the restricted inulin clearance measured must be taken as reflecting a decrease in filtration rate at the glomerulus.

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REFERENCES

1. Bank, N., Mutz, B. F., Aynedjian, H. S.: The role of "leakage" of tubular fluid in anuria due to mercury poisoning. J. Clin. Invest. **46**, 695–704 (1967)
2. Barenberg, R. L., Solomon, S., Papper, S., Anderson, R.: Clearance and micropuncture study of renal function in mercuric chloride treated rats. J. Lab. Clin. Med. **72**, 473–484 (1968)
3. Biber, T. U. L., Mylle, M. M. A., Baines, A. D., Gottschalk, C. W., Oliver, J. R., MacDowell, M. C.: A study by micropuncture and microdissection of acute renal damage in rats. Am. J. Med. **44**, 664–705 (1968)
4. Blantz, R. C.: The mechanism of acute renal failure after uranyl nitrate. J. Clin. Invest. **55**, 621–635 (1975)
5. Daugharty, T. M., Ueki, I. F., Mercer, P. M., Brenner, B. M.: Dynamics of glomerular ultrafiltration in the rat. V. Response to ischemic injury. J. Clin. Invest. **53**, 105–116 (1974)
6. Eisenbach, G. M., Steinhausen, M.: Micropuncture studies after temporary ischemia of rat kidneys. Pflügers Arch. **343**, 11–25 (1973)

7. Flamenbaum, W., McDonald, F. D., DiBona, G. F., Oken, D. E.: Micropuncture study of renal tubular factors in low dose mercury poisoning. Nephron **8**, 221–234 (1971)
8. Flamenbaum, W., Hamburger, R. J., Huddleston, M. L., Kaufman, J., McNeil, J. S., Schwartz, J. H., Nagle, R.: The initiation phase of experimental acute renal failure: An evaluation of uranyl mitrate-induced acute renal failure in the rat. Kidney Int. **10**, Suppl. 6, 115–122 (1976)
9. Gertz, K. H.: Transtubuläre Natriumchloridflüsse und Permeabilität für Nichtelectrolyte im proximalen und distalen Konvolute der Rattenniere. Pflügers Arch. **276**, 336–356 (1963)
10. Gottschalk, C. W., Morel, F., Mylle, M.: Tracer microinjection studies of renal tubular permeability. Am. J. Physiol. **209**, 173–178 (1965)
11. Hanssen, O. E.: The relationship between glomerular filtration and length of the proximal convoluted tubules in mice. Acta Path. Microbiol. Scand. **53**, 265 (1961)
12. Hayman, J. M., Shumway, N. P., Dumke, P., P., Miller, M.: Experimental hyposthenuria. Clin. Invest. **18**, 195–212 (1939)
13. Hilger, H. H., Klümper, D., Ulrich, K. J.: Wasserrückresorption und Ionentransport durch die Sammelrohrzelle der Säugetierniere. Pflügers Arch. **267**, 218 (1958)
14. Lorentz, W. B., Lassiter, W. E., Gottschalk, C. W.: Renal tubular permeability during increased intrarenal pressure. J. Clin. Invest. **51**, 484–492 (1972)
15. Mason, J., Olbricht, C., Takabatake, T., Thurau, K.: The early phase of experimental acute renal failure. I. Intratubular pressure and obstruction. Pflügers Arch. **370**, 155–163 (1977)
16. Nomiyama, K., Foulkes, E. C.: Some effects of uranyl acetate on proximal tubular function in rabbit kidney. Toxicol. Appl. Pharmacol. **13**, 89–98 (1968)
17. Oken, D. E.: On the passive back flow theory of acute renal failure. Am. J. Med. **58**, 77–82 (1975)
18. Stein, J. H., Gottschall, J., Osgood, R. W., Ferris, T. F.: Pathophysiology of a nephrotoxic model of acute renal failure. Kidney Int. **8**, 27–41 (1975)
19. Steinhausen, M., Eisenbach, G. M., Helmstädter, V.: Concentration of lissamine green in proximal tubules of antidiuretic and mercury poisoned rats and the permeability of these tubules. Pflügers Arch. **311**, 1–15 (1969)
20. Tanner, G. A., Sloan, K. L., Sophasan, S.: Effects of renal artery occlusion on kidney function in the rat. Kidney Int. **4**, 377–389 (1973)
21. Thiel, G., Hugenin, M., Brunner, F., Peters, L., Peters, G., Eckert, H., Torhorst, J., Rohr, H. P.: Etude du mecanisme de l'insuffisance renale aigue a $HgCl_2$ chez le rat. J. Urol. Nephrol. **79**, 967–977 (1973)

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