Since a urine-to-plasma ratio of less than 1.0 for an uncharged molecule indicates tubular reabsorption against a concentration gradient, these results constitute evidence for the active reabsorption of methylurea and acetamide. The data for urea are similar to those reported by others (*1, 3*) and also show active reabsorption.

The ratio for thiourea, U_t/P_t, measured in two fish was greater than 1.0 (average, 2.74). These results for thiourea confirm those of Clarke and Smith (*5*) and provide no evidence for active reabsorption of thiourea. The ratio for inulin, U_i/P_i, as measured in four fish was always greater than 1.0 (average, 6.6; range, 2.12 to 15.0), while urine flow varied from 0.03 to 1.69 ml/hr.

If the results obtained for the shark are compared with those for the frog (*6*) it is apparent that the substance thiourea, which is actively secreted in frog tubules (from blood to tubular lumen), is not actively reabsorbed (from tubular lumen to blood) in the shark tubules; while the two substances acetamide and methylurea, which are not secreted by the frog tubules, are actively reabsorbed by the shark tubules.

In the mammalian kidney, Rabinowitz and Kellogg (*7*) found that acetamide and methylurea can enhance the ability to produce a concentrated urine. The effect of these compounds were similar to the well-recognized effect of urea, whereas no effect was found with thiourea. Truniger and Schmidt-Nielsen (*8*) found that methylurea, acetamide, and urea accumulate in the renal medulla in concentrations higher than that in the urine in rats on a diet low in protein and high in salt, while thiourea does not accumulate in the medulla.

The various results suggest that the two mechanisms for transporting urea across the renal epithelium, namely, the secretory mechanism in the frog tubules and the reabsorptive mechanism in the shark tubules, do not share a common carrier since they handle urea-related compounds differently. The similarity between the results in mammals and shark are in agreement with the concept of an active reabsorption in the collecting ducts as suggested by previous results (*8, 9*).

A facilitated diffusion system for urea in elasmobranch erythrocytes was described recently by Murdaugh *et al.* (*10*), but the results of those authors cannot readily be interpreted on the same basis as our results.

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Inulin and Albumin Absorption from the Proximal Tubule in Necturus Kidney

Abstract. *In the kidney of the amphibian,* Necturus, *appreciable quantities of inulin and human serum albumin are transported from the tubular lumen of the proximal tubules into the blood. These findings suggest that inulin may not be a satisfactory indicator for measuring net water movement in the kidney of this species.*

The time course of fluid absorption from the proximal tubules of the *Necturus* kidney was measured by sequential photography of isolated columns of fluid in the tubule. Water absorption in these experiments, which were all performed on adult "summer" necturi obtained in April, May, and June, 1964, was found to be considerably in excess of that previously determined in the same species by the stopped flow microperfusion technique (*1*) in which C^{14}-labeled inulin is used to measure net water movement. To investigate this discrepancy, we examined the possibility that inulin may be absorbed from the tubular lumen. Although it has been widely accepted by renal physiologists that inulin is not reabsorbed by the renal tubule, evidence in support of this viewpoint is entirely indirect (*2*). Our results show that appreciable quantities of this molecule are indeed absorbed in the *Necturus* proximal tubule, but, conversely, inulin initially introduced into the blood does not cross into the tubule. These findings indicate that inulin may not be a satisfactory indicator for measuring net water movement in the proximal tubule of *Necturus*.

Segments of the proximal and the distal portions of the proximal tubule are usually visible on the ventral surface of the *Necturus* kidney. These can be filled with oil, and the column then can be split by injection of isosmotic perfusion fluid containing 100 m*M* NaCl. This procedure allows the isolation of columns of perfusion fluid 0.5 to 0.6 mm long in either of these segments of the proximal tubule. To record the changes in volume of the perfusate, we took photographs throughout a 20-minute period at 2-minute intervals, using a condenser-actuated flash discharge, according to the procedure described by Gertz (*3*). The length and the diameter of the column of perfusion fluid were measured on projections of the photographic negative, so that the perfusate volume could be calculated. After applying a suitable meniscus correction, the logarithm of the volume of perfusate relative to its initial volume [ln (v/v_0)] was found to be a linear function of time over the course of the experiments. The relative volumes at 20 minutes, expressed as percentage water absorption [$(1-v_{20}/v_0)$ 100], were, for the proximal segment, 73, 70, 37, 48, 35; and for the distal segment, 44, 51, 43. These data show that there was no significant difference in water absorption between the proxi-

mal and distal segments of the proximal tubule. The mean net water movement, 50 ± 5 percent, was almost twice the value of 27 percent previously obtained by the stopped flow microperfusion technique in which inulin-carboxyl-C^{14} is used as a reference substance.

We also measured water movement in the present group of animals, using C^{14}-labeled inulin in conventional stopped flow microperfusion experiments. The results, as presented in the first two columns of Table 1, show that the mean water movement of 21 ± 4 percent was somewhat less than the 27 percent usually found. In these experiments we injected a known initial volume of fluid into the tubule, collected all the perfusion fluid remaining in the tubule after 20 minutes and measured its volume in a calibrated constant-bore capillary tube. The mean value of the water absorbed, 67 ± 3 percent, was somewhat greater than the value of 50 ± 5 percent determined by the photographic method, though this difference is not significant ($p = 0.1$).

There are three possible explanations for the discrepancy between the two determinations of water movement, that made by inulin and that made by the collected volume: (i) incomplete collection of the perfusate, an appreciable fraction remaining behind in the kidney; (ii) contamination of the inulin by polymers of low molecular weight which could readily diffuse out of the tubule; or (iii) absorption of inulin by the tubular epithelium. The first possibility could be excluded by measuring the amount of inulin that remained in the kidney. The portion of the kidney which contained the perfused tubule was excised and macerated in 1 ml of 0.1*N* HNO_3. The suspension was first shaken for 24 hours at room temperature; and after centrifugation, 0.5 ml of the supernatant was counted in a liquid scintillation counter. By a similar procedure Page and Solomon (*4*) recovered 98.6 percent of the C^{14}-labeled inulin in papillary muscle. As shown in Table 1, an average of only 13 percent of the C^{14} remained in the kidney, too small a fraction to account for the discrepancy between the two methods.

Three experiments were carried out in doubly perfused kidneys, prepared as described by Cullis (*5*), in order to assess the possibility that our inulin contained small fragments of C^{14}-labeled material. In this preparation inulin perfusing the kidney via the portal system does not pass through the glomerulus and appear in the urine, as has been shown by Giebisch in *Necturus* (*6*) and Richards, Bott, and Westfall in the frog (*7*). The experimental procedure was divided into two parts. First the portal system was perfused for 1 hour with a solution containing a high concentration of C^{14}-labeled inulin; the C^{14} which passed across the tubular epithelium and appeared in the urine was counted. In these experiments the concentration of C^{14} in the collected urine averaged only 0.3 percent (range, 0.2 to 0.5 percent) of that in the perfusate, indicating that a negligible fraction of the perfusing inulin molecules were able to cross the tubular wall. This demonstrates that our C^{14}-labeled inulin did not contain polymers small enough to cross the tubular epithelium by simple passive diffusion.

The preparation was then perfused with frog Ringer solution for three 20-minute periods in order to wash out the C^{14}-labeled inulin. No radioactivity could be detected in the wash solution collected in the last period. Several proximal tubules, usually four, were then blocked with oil and filled with a 100 m*M* NaCl solution containing C^{14}-labeled inulin, while perfusion of the vascular system was continued. At the end of 40 minutes all the fluid that had perfused the vascular system was collected. This fluid contained 48 ± 6 percent of the radioactivity initially injected into the tubules. This portion of the experiment indicates that a mechanism exists which is able to transport inulin from the tubule into the blood stream.

This conclusion is also supported by the results of two stopped flow microperfusion experiments in intact animals in which large amounts of C^{14}-labeled inulin were injected into single tubules. In these two experiments (animals 1 and 5, Table 1), the concentration of C^{14} in each milliliter of the plasma collected at the end of the experiment represented 5 percent and 15 percent, respectively, of the total C^{14}-labeled inulin injected into the tubule. These figures provide qualitative evidence that in the intact animal as in the perfused kidney significant quantities of inulin are transported from the tubular lumen to the bloodstream.

It seemed unlikely that there would be in the *Necturus* proximal tubule a mechanism uniquely responsible for the observed absorption of inulin. To investigate the possibility that other large molecules might also be absorbed in similar fashion we performed stopped flow microperfusion experiments, similar to those described above, in which the C^{14}-labeled inulin was replaced by I^{131}-labeled human serum albumin (20 g/liter). The results in Table 2 show that the mean net water movement determined by the change in luminal albumin concentration was 21 ± 5 percent at the end of a 20-minute period, in excellent agreement with the water movement determined with inulin (21 ± 4 percent). In these experiments, also, the net water movement determined volumetrically was 54 ± 5 percent, far in excess of that measured by the change in I^{131} concentration. This figure is comparable with the value of 67 ± 3 percent in the inulin experiments and the net movement of 50 ± 5 percent determined photographically.

Table 1. Water absorption in the proximal tubule measured by stopped flow microperfusion. Results are expressed as percentages.

Animal No.	Water absorbed		Inulin	
	By inulin	By volume	Recovered from tubule	In kidney tissue
1	28	58	25	17
2	22	63	37	21
3	10	61	31	10
4	13	76	24	9
5	33	76	36	6
		Mean		
	21	67	31	13
		Standard error		
	4	3	3	3

The amount of I^{131} remaining within the kidney tissue, plus that recovered from the tubular lumen, accounted for only 71 ± 3 percent of the injected I^{131}. The average content of I^{131} in each milliliter of the plasma at the end of the 20-minute perfusion period was equivalent to 4.9 ± 0.8 percent of the total radioactivity injected into the tubule, indicating that significant amounts of the I^{131} lost from the tubule had been transported to the blood.

The possibility existed that the technique of stopped flow microperfusion might injure the tubule either by pressure or in some undefined way. If this were the case, we would expect I^{131}-labeled albumin introduced into the blood stream to find its way into tubules blocked with oil according to the usual technique. Tubules in four animals were filled with 100 m*M*

Table 2. Water absorption measured by albumin in the proximal tubule. Each experiment was conducted in a separate animal, animals 6 through 11. Results are expressed as percentages.

Water absorbed		Albumin		Injected albumin per ml plasma
By albumin	By volume	Recovered from tubule	In kidney tissue	
28	66	49		4.6
20	44	69	7	5.0
1	50	50	12	4.0
33	62	37	33	
21	34	67	15	3.8
20	65	44	21	7.1
		Mean		
21	54	53	18	4.9
		Standard error		
5	5	5	5	0.8

NaCl, and a large amount of I^{131}-labeled albumin was injected into the animal intravenously. At the end of the usual 20-minute perfusion period the concentration of the radioactivity in the collected perfusate averaged 1.0 ± 0.6 percent of that in the plasma at the same time. This suggests that the absorption of I^{131}-labeled albumin could not be ascribed to damaged tubules. However, distention of the tubule might produce channels sufficiently large for the passage of albumin. Fluid is reabsorbed from the tubule in these experiments. If the flow went through such channels, entrance of albumin might be prevented. Such a mechanism for the observed rectification of albumin transport seems unlikely, but it is not excluded by these studies.

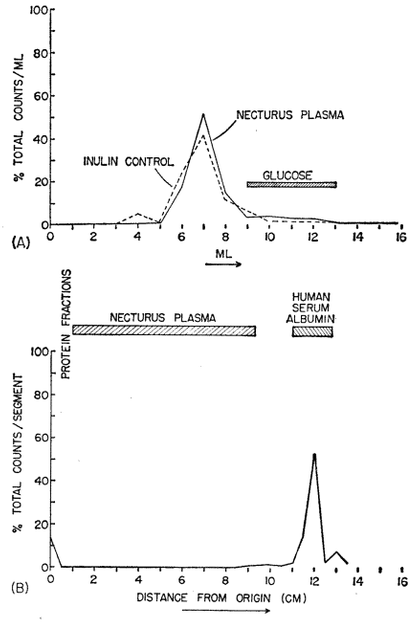

Fig. 1. *A*, Gel filtration of C^{14}-labeled inulin in plasma collected from perfused *Necturus*. The bar indicates the fractions in which added glucose molecules were found. *B*, Electrophoretic fractionation of I^{131}-labeled albumin in plasma collected from perfused *Necturus*. The top bars indicate the positions in which human serum albumin and *Necturus* plasma proteins were found.

Further experiments were conducted to determine whether inulin and albumin were metabolized in their passage from the tubule into the blood. Single tubules were perfused under free flow conditions for a period of 60 minutes in order to accumulate enough radioactivity to permit analysis, and the animal was killed. In the four experiments with inulin, 0.5 ml of the plasma was filtered through a Sephadex G-25 (medium) column, which "excludes" those molecules whose molecular weight is approximately 5000 or greater (*8*). Figure 1*A* shows the results of a typical experiment. Essentially all the radioactivity is in a peak whose position coincides with the peak obtained when a solution of labeled inulin is fractionated on the same column. These results demonstrated that the C^{14} in the blood represents inulin with essentially the same molecular weight distribution as that added to the tubule.

In the case of I^{131}-labeled human serum albumin, 0.25 ml of plasma was analyzed according to a modification of the disc electrophoresis method described by Raymond (*9*). The results in Fig. 1*B* show that the peak of the radioactivity coincides with the position of the peak obtained when human albumin was added directly to the plasma. These fractionation techniques suggest that both molecules have been transported from the lumen of the tubule into the blood with no essential alteration in physical characteristics.

Since the finding that inulin is absorbed by the kidney tubule was unexpected, we examined the considerable body of evidence summarized by Smith (*2*) which suggested that inulin was not reabsorbed. As he was careful to point out, this evidence is indirect; our studies represent the first direct examination of the behavior of inulin in single tubules by experiments *in situ*. Our evidence indicates that substantial amounts of two chemically dissimilar large molecules are transported out of the lumen of the *Necturus* proximal tubule by a mechanism which does not involve any apparent alteration in their physical properties. Since the fractional rate of absorption is qualitatively the same for these two molecules it would seem likely that a single process is responsible. One such process, for which Maunsbach (*10*) has presented evidence in electron microscopic studies, is pinocytosis. Such a process would have escaped detection by the experiments cited by Smith and would not have been excluded by the rigorous criteria used to establish inulin as a measure of glomerular filtration rate.

Note added in proof: We have recently completed a further series of experiments using I^{131}-labeled human serum albumin on "winter" necturi obtained in November 1964. In a series of seven animals, only 0.6 ± 0.4 percent of the albumin was found in each milliliter of plasma, far less than the 4.9 ± 0.8 percent in our earlier experiments (Table 2) on summer necturi. Two summer necturi remained and gave results of 2.6 and 5.7 percent, comparable to those obtained previously on the same series of animals. This suggests that the quantitative importance of this process is subject to biological variation such as the seasonal differences observed by Kinter (*11*) in the transport of para-amino-hippurate by renal *Necturus* tubules.

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