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Renal Excretion of 3-O-Methyl-D-glucose* (33017)

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Recently the literature has revealed that many substances move bidirectionally across the renal tubules of mammalian kidney. These include most weak organic acids (1, 2), ions such as Na^+, K^+, Ca^{2+}, and PO_4^{3-} (3–6) and even D-glucose (7,8). Evidence has also been presented in our previous papers for the existence of a common renal carrier system for sugars which reabsorbs D-glucose and also has the capacity of secreting L-hexoses and 3-*O*-methyl-D-glucose (3MG). The movement of sugars in either direction was shown to be sensitive to the inhibitory action of phlorizin (9,10). The 3MG, the methyl analog of D-glucose, has been a very useful tool for studying sugar transport in several biological systems. This is principally due to the fact that mammalian organisms are incapable of metabolizing this sugar analog as they would D-glucose (11–13). The nature and direction of transport of 3MG across the intestinal mucosa (14,15), erythrocyte membranes (16) and Ehrlich ascites tumor cells (17) has been found to be very similar to that of D-glucose transport in each of these systems. However, in the sugar transport system of the renal tubules, 3MG behaves more similarly to L-glucose than D-glucose. This was interpreted by us as being due to similarities in the structural configurations of 3MG and L-glucose (9). Dissimilarity of 3MG transport and D-glucose transport has also been reported by Kleinzeller and his co-workers (18). They found that rabbit kidney slices did not accumulate 3MG, but did accumulate several other sugars including D-glucose.

Recently Stolte *et al.*, using the microperfusion technique of perfusing single renal tubules, found that 3MG was absorbed from the perfusate at approximately 50% of the rate determined for D-glucose absorption in rats (19). This suggests that species difference may also dictate the characteristics of 3MG transport in the kidney. It was the purpose of this investigation to reexamine the tubular transport of 3MG in both dogs and rats and to demonstrate correlations which might exist between the transport of D-glucose and 3MG, in order to add further evidence for our proposal of a bidirectional sugar carrier in the renal tubular epithelium.

Experimental Method. Material. Nonlabeled 3MG was purchased from Calbiochem (Calif.) and ^{14}C labeled 3MG from New England Nuclear Corp. (Boston). Thin-layer chromatography indicated that both substances used in these experiments were probably homogenous compounds since each gave single spots with identical R_f values. The recovery of radioactivity from 3MG-^{14}C spots was always in the range of 93–97%. Inulin was obtained from Nutritional Biochemicals Corp. (Cleveland) and was subjected to chromatography by the procedure of Merck (20). Single spots were obtained from inulin samples indicating the probable absence of other carbohydrates as contaminants.

Methods. Dogs anesthetized with pentobarbital and rats with "Inactin" (ethyl-1-methyl-propyl-malonyl-thiourea) were used in T value determination of 3MG. In all

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experiments 3MG, with ^{14}C-labeled 3MG added as tracer, was given to the animal intravenously in a prime dose and sustaining infusion. The rate of infusion varied with the prime dose and ranged from 1.0 to 1.5% of the prime dose per minute. The stop flow technique of Malvin *et al.* was used to study the localization of tubular transport of 3MG. The procedures for all experiments performed here were exactly the same as have been used in the studies of L-glucose secretion (10).

Analysis. The concentration of inulin in diluted urine samples and protein free filtrates of plasma was determined by the method of Roe *et al.* (21). The concentration of 3MG in urine and plasma samples was determined by their radioactivity based upon the specific activity of the injected 3MG solution. The radioactivity was analyzed by the method of Bray (22) with a Nuclear-Chicago liquid scintillation counter.

Urine samples were chromatographed (23) and the spots were scraped from the plates and counted in liquid scintillation vials. For each sample a single spot was obtained which had an R_f value identical to that of the 3MG standard solution. The recovery of radioactivity was in the range from 90 to 98% for each sample. Therefore, it is reasonable to assume that the radioactivity which was counted actually represented the 3MG which had been infused into the animals. Determination of protein binding with the ultrafiltration technique of Toribara *et al.* (24) showed that 3MG was not protein bound at plasma concentrations in the range from 0.12 to 1.51 m*M*.

Results. T value determination in dogs and rats. Table I summarizes the results obtained from 3 experiments with dogs to determine T values for 3MG as a function of plasma 3MG concentration. Plasma samples were taken at the beginning and at the end of each urinary collection. The concentration of 3MG in the two samples varied less than 10% of their mean and it was therefore assumed that a steady state condition had been approximately reached at that time. The plasma concentration was determined by interpolation to the midpoint of the urine collection time and used in all calculations. A positive T value, indicating tubular secretion, was obtained in all determinations from each dog tested. A tubular transport maximum (T) was not reached over a plasma concentration range from 0.08 to 1.51 m*M*.

Table II summarizes the results from four experiments to determine the T value of 3MG in rats as a function of plasma 3MG concentration. With only one exception, negative T values, indicating tubular reabsorption, were found. The magnitude of the 3MG reabsorption in rats was about 10% of the 3MG filtration rate.

Effects of drugs upon the tubular secretion of 3MG in dogs: Five experiments were performed to study the effect of various intravenously infused agents upon the secretion of 3MG in dogs. The results are summarized in Table III. With only one exception (Expt. 4), positive T values were obtained during the control periods of each experiment. In the four experiments in which D-glucose was infused intravenously, the T values increased to levels greater than those observed during the respective control periods. In the one experiment in which positive T values were not found during the control periods, the infusion of D-glucose caused the appearance of relatively large positive T values. After the infusion of D-glucose and concomitant increase in 3MG secretion, phlorizin was added to the infusion solution producing, in every case, either a reduction or an abolition of the 3MG secretion. A typical experiment (Expt. 3) is shown in Fig. 1 to illustrate the effect of infusing D-glucose or phlorizin upon the T value of 3MG at approximately constant plasma 3MG concentration and glomerular filtration rate (GFR). In two experiments (Expts. 5 and 6) 2,4-dinitrophenol (DNP) was infused intravenously, after which the T values of 3MG remained constant or fell only slightly. In Expt. 5 in which the T value fell slightly the plasma concentration of 3MG also fell and could possibly account for the decrease in secretory rate observed.

Site of tubular secretion of 3MG. Three

TABLE I. *T* Value Determination of 3MG in Three Female Dogs.[a]

Dog wt. (kg)	Dose given: Prime (μmole/kg)	Dose given: Infusion (μmole/min)	Urine flow (ml/min)	C_{In} (ml/min)	3MG: *UV* (μmole/min)	3MG: *P* (m*M*)	3MG: *T* (μmole/min)
10.9	50	10.9	3.35	48.3	9.07	.16	+ .89
	50	21.5	3.5	53.1	20.7	.35	+1.89
	100	32.6	3.6	48.8	38.2	.65	+6.20
	100	40.3	3.1	48.7	56.0	1.00	+6.97
	125	46.1	3.15	47.0	71.2	1.34	+8.22
9.55	25	4.5	3.37	32.1	3.41	.08	+ .84
	25	9.5	3.8	32.9	6.73	.17	+1.01
	50	14.3	3.2	27.9	12.2	.32	+3.08
	100	19.0	2.8	28.9	21.2	.58	+4.69
	100	28.4	2.67	26.2	29.2	.79	+8.22
11.4	25	5.7	5.0	37.6	4.88	.08	+1.53
	25	11.4	5.3	35.0	9.16	.18	+2.64
	50	17.1	4.5	31.6	15.8	.33	+5.18
	100	29.0	3.7	31.9	27.8	.65	+6.77
	125	37.0	3.4	29.4	39.0	1.04	+8.41
	125	51.2	3.3	31.9	49.0	1.51	+ .43

[a] C_{In} = inulin clearance; UV = excretory rate or (urine concentration × urine flow rate); P = plasma concentration; $T = U \cdot V - P \cdot C_{In}$ = transport rate.

experiments were performed with the stop flow technique. In each experiment the excretory pattern for 3MG was observed before and after infusion of phlorizin. In all experiments the U/P ratios for 3MG were larger than the U/P ratios of inulin and were reduced after infusion of phlorizin. One of these experiments is shown in Fig. 2. There were two peaks in the values for $(U/P_{3MG})/(U/P_{In})$ seen during the control experiments; one corresponding to urine from the distal tubules and another corresponding to urine from the proximal tubules. The peak in the values for U/P_{In} denotes the region of the tubule in which water was removed, i.e., the distal tubules. The appearance of the creatinine, which was injected after ureteral clamping, denotes urine from the early portion of the proximal tubules or urine which was filtered after the clamp was opened. Phlorizin produced a flattening of the peaks by reducing the

TABLE II. *T* Value Determination of 3MG in Male Rats.[a]

Exp. no.	Rat wt. (kg)	Dose given: Prime (μmole/kg)	Dose given: Infusion (μmole/min)	Urine flow (ml/min)	C_{In} (ml/min)	3MG: *UV* (μmole/min)	3MG: *P* (m*M*)	3MG: *T* (μmole/min)
1	.400	100	.6	.25	2.74	.55	.197	+ .01
		200	.8	.20	1.67	.70	.531	— .18
2	.350	200	1.05	.14	.94	.33	.495	— .13
		200	1.3	.13	.89	.62	.942	— .22
3	.370	600	2.2	.22	2.25	2.41	1.12	— .11
		300	3.3	.20	1.73	2.52	1.57	— .20
4	.410	400	1.6	.27	2.25	5.63	2.59	— .22
		200	2.8	.23	10.02	1.93	5.89	—1.36

[a] See Table I for explanation of symbols.

TABLE III. 3MG Steady-State Experiments in Dogs.

Exp. no.	Dog wt. and sex (kg)	Dose given		Urine flow (ml/min)	C_{In} (ml/min)	3MG		
		Prime (μmole/kg)	Infusion (μmole/min)			UV (μmole/min)	P (mM)	T (μmole/min)
1	7.7 f	50	5.8	7.3	31.9	4.94	.154	+ .03
			5.8	8.4	31.6	5.24	.149	+ .54
			D-glucose infusion (1.1 mmole/min)					
			5.8	9.1	35.8	5.64	.129	+1.02
			5.8	8.7	35.7	5.36	.132	+ .67
			D-glucose infusion + phlorizin (1 μmole/min)					
			5.8	8.6	35.7	4.95	.135	+ .14
			5.8	8.2	35.7	5.01	.137	+ .13
2	11.8 m	75	13.3	3.3	41.7	10.8	.250	+ .40
			13.3	3.5	39.7	9.65	.232	+ .44
			D-glucose infusion (1.1 mmole/min)					
			13.3	3.4	42.2	9.71	.219	+ .46
			13.3	3.1	35.3	9.02	.205	+1.8
			D-glucose infusion + phlorizin (1 μmole/min)					
			13.3	3.2	31.9	7.23	.215	+ .38
			13.3	2.8	30.1	6.93	.227	+ .09
3	10.9 f	103	16.8	3.2	30.6	14.7	.401	+2.45
			16.8	3.0	25.3	14.4	.381	+3.60
			D-glucose (1.1 mmole/kg and 0.72 mmole/min infusion)					
			16.8	3.65	25.3	15.5	.348	+6.68
			16.8	3.95	26.6	15.1	.331	+6.31
			D-glucose infusion + phlorizin (2 μmole/kg and 0.35 μmole/min infusion)					
			16.8	4.7	35.4	12.1	.366	— .85
4	8.3 f	206	25.7	2.8	22.8	16.3	.72	— .11
			25.7	3.0	24.9	17.2	.69	.0
			D-glucose (1.1 mmole/kg and 0.46 mmole/min infusion)					
			25.7	3.65	23.5	22.7	.71	+5.99
			25.7	4.1	24.9	23.5	.69	+6.31
			D-glucose infusion + phlorizin (2 μmole/kg and 0.5 μmole/min infusion)					
			25.7	4.2	27.8	19.8	.76	—1.43
5	9.1 f	100	13.65	2.1	8.72	6.99	.41	+3.42
			13.65	1.8	6.58	5.70	.40	+3.07
			2,4-DNP (1 μmole/kg and 0.99 μmole/min infusion)					
			13.65	1.1	4.04	4.20	.38	+2.63
			13.65	.9	3.32	3.89	.38	+2.63
6	10.0 f	100	15	3.1	15.9	8.81	.39	+2.46
			15	2.8	14.5	6.29	.37	+2.93
			2,4-DNP (5.4 μmole/min infusion)					
			15	1.4	8.6	5.86	.36	+2.60
			15	1.1	6.9	5.07	.35	+2.55

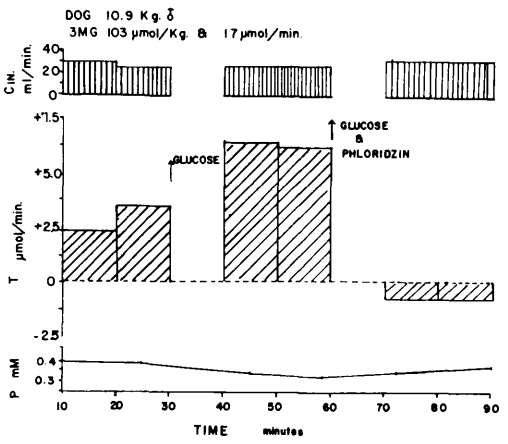

FIG. 1. Effect of intravenously infused D-glucose and phlorizin upon the rate of renal secretion of 3MG at approximately constant plasma concentration and glomerular filtration rate. The 4-glucose dose: 1.1 mmole/kg prime followed by an infusion of 5% of the prime dose per minute. Phlorizin dose: 2 μmole/kg prime followed by an infusion of 1% of the prime dose per minute. P = plasma concentration of 3MG. T = rate of transport of 3MG. C_{In} = inulin clearance or glomerular filtration rate.

ratio, $(U/P_{3MG})/(U/P_{In})$ to unity or smaller.

Discussion. Data obtained from these experiments confirm our previous finding that 3MG is secreted by the renal tubules of dog kidney (9). Although a maximum transport capacity (T_m) could not be demonstrated in these studies, the existence of a T_m for the secretory process cannot be precluded. The range of plasma concentrations of 3MG used in these experiments was evidently too narrow and a T_m could possibly be observed if the plasma level were sufficiently increased. It should be pointed out that the parameter for transport which was observed in these experiments was the T value of the compound. The T value is actually a *net* rate of transport and is therefore the sum of all of the processes which affect the movement of 3MG at all levels of the nephron. The net rate is probably dependent upon such diverse factors as glomerular filtration rate, tubular permeability, passive diffusion in either direction and possibly active transport in either direction. Therefore it is possible that a T_m could not be demonstrated for 3MG, as has been the case for sugars which are reabsorbed, presumably by active processes (25, 26).

Previously we have proposed that the transport of hexoses in the dog kidney is by means of a bidirectional carrier system (9, 10). If so, there should be certain correlations between the movement of one sugar in one direction and the movement of another sugar in the opposite direction. This hypothesis has been substantiated by our work with L-glucose (10) and by the present studies with 3MG. As seen in Fig. 1 and Table III, an intravenous infusion of D-glucose caused a very significant increase in the secretory rate of 3MG in dogs and the infusion of phlorizin inhibited both the original level of the 3MG secretion and that which appeared after D-glucose infusion. There are two possible interpretations of the data from these experiments. The first is, that an increased rate of D-glucose trans-

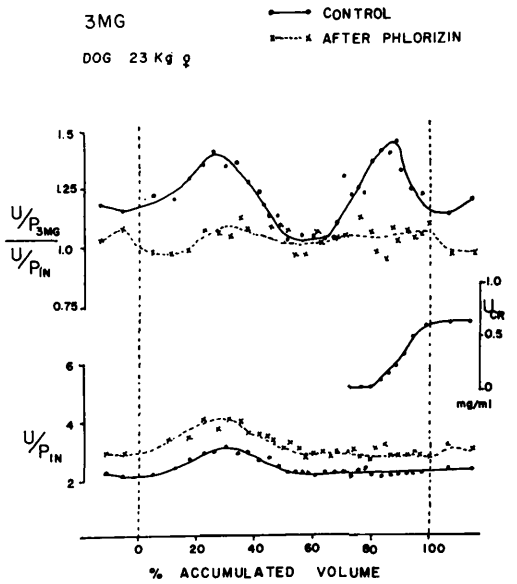

FIG. 2. Patterns of 3MG excretion obtained by stop flow analysis before and after an intravenous infusion of phlorizin (0.5 μmole/min). $(U/P_{3MG})/(U/P_{In})$ = ratio of the urine-to-plasma ratios of 3MG and inulin. U/P_{In} = urine-to-plasma ratio of inulin. U_{Cr} = concentration of creatinine in the urine samples. Solid lines denote the control patterns whereas the broken lines denote the patterns obtained after infusion of phlorizin. All curves were drawn by inspection.

port, which results from the increased plasma concentration, could cause the countertransport of 3MG and therefore an increase would be seen in the 3MG secretory rate. The other possibility is that the increased level of D-glucose in the tubular lumen could competitively inhibit a reabsorptive component of 3MG transport and in that way make the net *T* value become a larger positive number. However, if the second possibility is valid, the infusion of phlorizin should also cause an increase, rather than a decrease, in the rate of renal excretion of 3MG in the dog. Yet it is possible that the transport of 3MG involves both active secretory and active reabsorptive processes with both of these systems being phlorizin sensitive. In such a case, the effect of phlorizin upon the net urinary excretion of this compound would be unpredictable and dependent upon which process is predominant. Perhaps in the dog kidney, the secretory rate is much greater than the reabsorptive rate (if a reabsorptive system actually exists) accounting for the normally observed positive *T* values. It should be noted here that in two of the four experiments with dogs, negative *T* values were observed for 3MG after the infusion of phlorizin. This indicates that when the secretion of 3MG is totally inhibited by phlorizin, the 3MG in the tubular lumen can move into the tubular cells or blood, possibly by diffusion or an active process. No matter how interpreted, the data seem to clearly indicate the existence of a bidirectional sugar transport system in the renal tubules of dogs.

Using the microperfusion technique, Stolte *et al.* (19) have found that the proximal tubules of rat kidney are capable of reabsorbing 3MG and that the rate of reabsorption is approximately 50% of that determined for D-glucose. Our results with the steady state experiments in rats agree qualitatively with theirs, namely, that 3MG is reabsorbed in the rat kidney. However, as we have stated above, the *T* value as determined in our experiments if actually a net value calculated from tubular secretion minus tubular reabsorption. A negative *T* value could also mean that in the rat the tubular reabsorption is greater than the tubular secretion, as has been the case in experiments to study the transport of organic acids (1,2).

Another correlation between the reabsorption of D-glucose and the secretion of 3MG is seen in the data of Table III. In two dogs an intravenous infusion of 2,4-DNP did not inhibit the secretion of 3MG, whereas Mudge and Taggart (27) have shown that similar doses of 2,4-DNP can totally inhibit the organic acid secretory mechanism. This indicates that the secretion of 3MG is not related to the organic acid secretory process and that it is possible that it might be related to the D-glucose reabsorptive system which is also unaffected by 2,4-DNP (27).

Moreover, the stop flow experiments in dogs have shown that 3MG is secreted and that the site of secretion is localized in the proximal tubules, where D-glucose is known to be reabsorbed (28,29). The inhibitory action of phlorizin upon 3MG secretion was again demonstrated with this technique. It can be seen in Fig. 2 that there is also a secretory peak which corresponds to the distal portion of the nephron. This is possibly a phenomenon which is due to "reabsorption replacement" as mentioned by Malvin (30).

Data from all experiments presented here point out the possibility of a common carrier system in mammalian kidney for 3MG, D-glucose and probably several other sugars. This carrier system operates bidirectionally and the direction and magnitude of transport for a given sugar will depend upon the structural conformation of the sugar and the animal species. For example, D-glucose exhibits the greatest affinity for the influx system, i.e., the reabsorptive process. Using the microperfusion technique, Ullrich and his co-workers have found that the net movement of D-glucose in rat kidney is due to an active reabsorptive process and also a component of passive efflux (7). On the contrary, the net movement of L-glucose is due to an active secretory component and a passive influx component (influx meaning movement out of the tubular lumen) (10,31). The transport of 3MG is not as clear-cut as that of D-glucose of L-glucose since,

secretion is the predominant process in the dog and reabsorption is the predominant process in the rat with movement in the opposite direction probably occurring in each case.

Summary. The renal tubular transport of 3-*O*-methyl-D-glucose (3MG) was studied in dogs and rats. The 3MG was found to be actively secreted by a phlorizin sensitive mechanism in dogs. The nature of the renal handling of 3MG in rats was found to be different from that found in dogs, since, 3MG was reabsorbed by the renal tubules of rats at a rate of about 10% of the 3MG filtration rate. This was interpreted as a result of a reabsorptive process having a larger magnitude than a simultaneously operating secretory process for the same compound. The secretion of 3MG in dogs was found to occur in the proximal tubules and was not inhibited by 2,4-DNP. It was significantly augmented by an intravenous infusion of D-glucose. The results of these experiments were taken as evidence for the existence of a bidirectional sugar transport system in the renal tubules of dogs. This system would be capable of reabsorbing D-glucose and also capable of secreting 3MG and L-glucose.

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