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ON THE RENAL TUBULAR EXCRETION OF CREATININE[1] IN NORMAL MAN

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The demonstration that in man the renal excretion of exogenous creatinine[1] takes place in part by an active tubular process depends upon two experimental findings (1). The plasma clearance of creatinine in the normal individual is invariably in excess of the simultaneously determined inulin clearance, which is accepted as a valid measure of glomerular filtration rate (2). Furthermore, raising the plasma creatinine from low to high concentrations depresses its clearance toward that of inulin, which appears to be its limiting value. However, contrary to similar findings in the dogfish (3) and chicken (4), this depression is not completely reversible.

More recently, both the presence and the significance of the curvilinear relationship between plasma concentration and renal excretion of creatinine have been questioned. Winkler and Parra (5) observed that, following the ingestion of a single dose of creatinine, its clearance and the creatinine/sucrose clearance ratio behave rather erratically, but are generally depressed as the experiment progresses. These authors believe that the curvilinear relationship, as previously described, is not a dependent one. They suggest that its presence in the original experiments was incidental to an experimental routine which usually resulted in the high plasma concentrations being observed later than the low ones. Findley (6) later re-examined the relationship between plasma concentration and renal excretion over a range of plasma values of 1.0 to 14.0 mgm. per cent. His findings are precisely the same as those previously reported by Cope (7). That is, a linear relationship between these two variables obtains if one arbitrarily corrects the observed plasma concentration by — 0.5 mgm. per cent of assumed non-creatinine chromogenic material. He did not consider the depression of the creatinine

clearance at higher plasma levels valid evidence of tubular excretion, since the concentrations necessary to demonstrate the phenomenon were " far beyond the physiological range." The linear relationship limited to the lower plasma values was then advanced as evidence opposing the renal tubular excretion of creatinine. This conclusion would favor Rehberg's (8) original contention that creatinine is excreted solely by glomerular filtration.

More recently, Rehberg (9) has called attention to the demonstration by Abdon (10) that the administration of creatinine leads to the appearance in the plasma of a substance resembling creatinphosphoric acid. It is Rehberg's belief that the tubules do not participate in the excretion of preformed creatinine but rather that they may transfer creatinine which has its origin in the plasma in some more complex compound, such as that described by Abdon. This is an important consideration, since this type of compound would yield true creatinine in ordinary plasma filtrates and behave as such toward both specific and non-specific analytical methods.

EXPERIMENTAL METHODS

The subjects were healthy males selected from the wards of the Third (New York University) Medical Division of Bellevue Hospital. They had been admitted for minor illnesses from which they had completely recovered at the time these observations were made. In general, conditions were quasi-basal. The patients were at complete bed rest the morning of the experiment and had not received food for 16 hours. They were hydrated by the administration of 1000 ml. of water, 120 and/or 60 minutes prior to the first period. In long experiments a high urine flow was sustained by the administration of 250 to 500 ml. of water between groups of observations.

All urine collections were by catheter with completeness of collection insured by bladder washouts. The blood samples were obtained by puncture of the antecubital vein at the middle of each experimental period. The samples were centrifuged at once and the plasma precipitated as soon as separation was effected. Heparin was used as the anticoagulant. The urine samples were

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[1] In this report, unless specifically stated to the contrary, the term creatinine may be taken to mean exogenous creatinine.

diluted to the expected U/P ratio of inulin and this diluted urine subjected to the same precipitation reagents as the plasma. The routine of analyses was much the same as in our previous work (1, 2), except that for inulin determinations the Folin (11) sugar method was used. All analyses were in duplicate and the mean of these was used in the calculation of the clearances.

Three general types of experiments were performed. In all, the plasma concentration of inulin was elevated to and maintained at approximately 100 mgm. per cent by appropriate priming and sustaining infusions. When creatinine was given intravenously, it was included in the inulin infusion, or it was injected into the lumen of the infusion tubing when a single intravenous injection was given. When given by mouth, the creatinine was dissolved in water, chilled and made palatable by the juice of a lemon. The details of experimental procedure and typical plasma concentrations can be obtained by reference to Tables I, II, and III.

In three experiments we examined the plasma for the presence of a labile combination of creatinine or derivative with phosphate following the administration of creatinine. After a control sample of blood, 10 grams of creatinine were administered by mouth and further blood samples taken at 1, 2, and 3 hours. The samples were handled in a cold room, $+1.0 - +2.0°$ C., with all apparatus and reagents cooled to this temperature by a stay overnight at the low temperature. The bloods were centrifuged at high speed for 2 minutes and the plasma separated and precipitated immediately with trichloracetic acid. The filtrates, obtained with the aid of a vacuum pump, were then neutralized to pH 7.5 to 8.0 with NaOH. The entire operation, i.e., vein puncture to the neutralization of the filtrate, required less than 5 minutes. The low temperature obviated the necessity of an anticoagulant. At the end of the experiment the neutralized filtrates were brought to room temperature and the method of Fiske and Subbarow (12) was used to determine the presence of a labile phosphoric acid compound. The rate of color development in this method was determined by readings each minute on an Evelyn photoelectric colorimeter. This rate was precisely the same in the test samples as in the control, and not significantly different from standard phosphate solutions which had their salt content enriched by dilution with trichloracetic acid, neutralization with NaOH and subsequent acidification.

EXPERIMENTAL RESULTS

On the depression of the creatinine clearance by simple elevation of the plasma creatinine concentration. Figure 1 presents the relationship between the plasma creatinine concentration and the creatinine/inulin clearance ratio in a series of seven experiments derived from our previous study (1). Each dot represents the mean ratio observed in three consecutive 10 to 20-minute
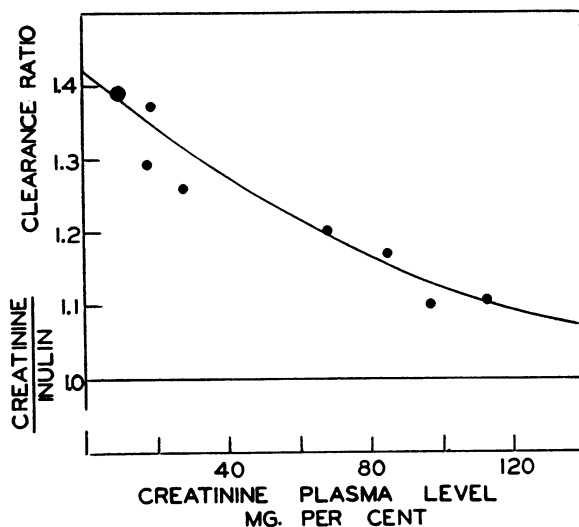

FIG. 1. THE RELATIONSHIP BETWEEN THE PLASMA CONCENTRATION OF CREATININE AND THE CREATININE/ INULIN CLEARANCE RATIO

For full explanation, see text, p. 170.

periods started within 20 minutes after a single injection of creatinine; the circled dot is the mean of a large series of experiments at plasma concentrations varying from 5 to 15 mgm. per cent. The curve is constructed from the eighty observations in our original series of data and represents the mean of this relationship. These observations, for the most part, were from experiments which proceeded from low to high plasma concentrations by a series of two or three doses of creatinine. This comparison indicates that the creatinine clearance, and the creatinine/inulin clearance ratio, are dependently related to the plasma creatinine concentration as described by this curve. That is, the depression at the high plasma concentrations is a true expression of the relationship of the two variables.

On the depression of the creatinine clearance related to the duration of observations at low plasma concentrations of creatinine. Our data indicate that, after the oral administration of a single dose of creatinine, the initially elevated creatinine clearance may progressively fall. It should be stressed, however, that this phenomenon is wholly separable from that described in the previous section. We have attempted to define the circumstances of the fall experimentally and thereby gain some insight into the mechanism responsible for it. The experiments shown in

Tables I, II, and III are typical of the data as a whole. The lack of any systematic changes in the inulin clearances permits the presentation of our results in the form of creatinine/inulin clearance ratios or calculations derived from this term.

TABLE I

An experiment wherein a constant intravenous infusion of creatinine was administered throughout

Subject: P. F. Experiment number 8C.
0–10 minutes. Priming infusion 100 ml. of 12.0 per cent inulin, 3.0 per cent creatinine in 0.85 per cent saline.
10 minutes-end. Sustaining infusion, 4.5 per cent inulin, 0.6 per cent creatinine in 0.85 per cent saline at 4.0 to 4.5 ml. per minute.

Period	Concurrent time	Urine flow	Plasma concentration		Plasma clearance		Creatinine/Inulin clearance ratio	C/I ratio−1.0	Tubular activity Fraction of control
			Inulin	Creatinine	Inulin	Creatinine			
	minutes	*ml. per minute*	*mgm. per cent*	*mgm. per cent*	*ml. per minute*	*ml. per minute*			*mean*
1	26.5–34	15.2	75.0	11.2	126	186	1.48	0.48	1.00
2	–50.5	16.2	75.4	10.6	135	199	1.47	0.47	
3	–62	15.5	75.0	10.3	131	193	1.47	0.47	
4	117.5–128	15.2	83.9	11.4	133	187	1.41	0.41	0.83
5	–139.5	14.9	86.8	11.7	124	176	1.42	0.42	
6	–152	15.7	90.9	12.4	136	182	1.34	0.34	
7	205.5–215.5	14.4	98.1	13.85	132	176	1.33	0.33	0.77
8	–228.5	13.2	98.1	13.5	123	173	1.41	0.41	
9	–240	7.92	97.4	13.5	129	171	1.33	0.33	

TABLE II

An experiment showing the fall in the creatinine clearance after a single oral dose of creatinine

Subject: P. F. Experiment number 5C.
0 minutes. 12 grams of creatinine by mouth.
30–40 minutes. Priming infusion: 100 ml. of 12.0 per cent inulin in 0.85 per cent saline.
40 minutes-end. Sustaining infusion: 4.5 per cent inulin infusion in 0.85 per cent saline at 4.0–4.5 ml. per minute.

Period	Concurrent time	Urine flow	Plasma concentration		Plasma clearance		Creatinine/Inulin clearance ratio	C/I ratio−1.0	Tubular activity Fraction of control
			Inulin	Creatinine	Inulin	Creatinine			
	minutes	*ml. per minute*	*mgm. per cent*	*mgm. per cent*	*ml. per minute*	*ml. per minute*			*mean*
1	51–66	13.7	90.9	9.4	137.0	195	1.42	0.42	1.00
2	–83	13.1	112.5	10.7	130.5	189	1.45	0.45	
3	–98.5	13.3	121.6	11.6	129.0	188	1.46	0.46	
4	148–162	15.8	125.0	10.5	141	179	1.27	0.27	0.60
5	–179	13.2	125.6	10.0	129.3	163	1.26	0.26	
6	–193	9.09	125.6	9.5	129.0	161	1.25	0.25	
7	239–253	8.56	122.3	7.3	128.0	153	1.20	0.20	0.37
8	–266.5	3.41	121.5	7.0	128.8	150	1.16	0.16	
9	–280	2.22	121.0	6.7	131.7	149	1.12	0.12	

TABLE III

An experiment showing the effect of a second small dose of creatinine on depressed creatinine clearances

Subject: P. F. Experiment number 7C.
0 minutes. 13.0 grams creatinine by mouth.
290–300 minutes. Priming infusion: 100 ml. 12.0 per cent inulin in 0.85 per cent saline.
300–371 minutes. Sustaining infusion: 4.5 per cent inulin in 0.85 per cent saline at 4.0–4.5 ml. per minute.
371–381 minutes. Priming infusion: 100 ml. 4.5 per cent inulin, 3.0 per cent creatinine in 0.85 per cent saline.
381 minutes-end. Sustaining infusion 4.5 per cent inulin, 0.8 per cent creatinine in 0.85 per cent saline at 4.0–4.5 ml. per minute.

Period	Con-current time	Urine flow	Plasma concentration		Plasma clearance		Creatinine/Inulin Clearance ratio
			Inulin	Cre-ati-nine	Inulin	Cre-ati-nine	
	minutes	*ml. per minute*	*mgm. per cent*	*mgm. per cent*	*ml. per minute*	*ml. per minute*	
1	320–337.5	12.2	82.0	7.98	126	143	1.13
2	–342.5	12.2	82.1	7.31	129	151	1.17
3	–368.5	12.4	77.4	6.93	126	144	1.14
4	393–404	12.7	101.2	17.85	117	160	1.37
5	–417	13.6	95.6	16.80	125	167	1.34
6	–429	14.5	95.6	16.85	128	168	1.31

In Figures 2 and 3 we have plotted the individual clearance ratios of the initial periods which serve as controls in the experiments presented in Figures 4 and 5. These show the variations to be expected in the creatinine/inulin clearance ratio in three short consecutive periods. This is usually less than ± 5.0 per cent of the mean. The absolute values of the ratios have much the same distribution as those we have previously reported (1, 2). The data contained in these figures indicate that the mode of administration, *i.e.*, intravenously or per os, is not a determining factor in the absolute magnitude of the initial ratios. The lack of systematic variation permits the use of the mean of the initial ratios as a standard of reference for the study of subsequent change in the system.

Typical experiments which examine the change in the tubular excretion of creatinine with time are given in Tables I and II; a graphical summary of all experiments in Figures 4 and 5. We have taken the creatinine/inulin clearance ratio — 1.0 as the measure of tubular activity in these figures. Each point is the mean of three experimental periods and is plotted as the fraction of the initial or control level of activity. Figure 4 shows that,
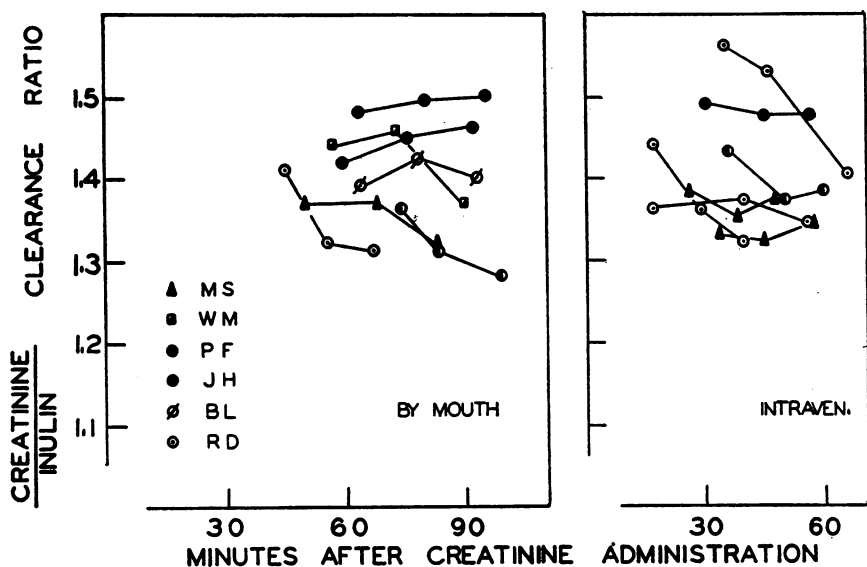
FIGS. 2 AND 3. CREATININE/INULIN CLEARANCE RATIOS OBSERVED SHORTLY AFTER
THE ADMINISTRATION OF CREATININE

Each point is an experimental observation; the three periods in each experiment are
connected by lines. In the experiments illustrated by Figure 2, a single dose of
creatinine (10 to 13 grams) was administered by mouth; in those of Figure 3, a
smaller amount (3.0 grams) was given intravenously and the plasma concentration
maintained by a constant intravenous infusion. The plasma concentrations in both
types of experiments were essentially the same.

when the plasma creatinine concentration is maintained by a constant intravenous infusion, there may be no marked depression of tubular activity as the experiment progresses.[2] However, in those experiments where the creatinine was administered in a single oral dose, the depression, though variable, is quite definite. The detailed data indicate that the extent of this fall is not correlated with the absolute value of the initial ratio.

Our experiments do not reveal the maximal depression of tubular activity that may be expected, since practical considerations limit the duration of observation. Five hours after the oral administration of 13 grams of creatinine the plasma concentration in a normal man of average size is usually 5.0 to 7.0 mgm. per cent. At this time

we have observed ratios varying from 1.11 to 1.24 (see control ratios, Figure 6). Eight hours after a large single intravenous injection the ratio is in the same range (cf. 1, Figures 1 and 2). From this we are inclined to believe that, when there is a moderate concentration of creatinine in the blood, it is unlikely that the ratio will fall to 1.0; i.e., that all tubular excretion of creatinine will cease.

After the creatinine/inulin clearance ratio has fallen, a second dose of creatinine usually elevates it to or towards the level characteristic of initial observations. Table III gives the details of one such experiment, while six are summarized graphically in Figure 6. It may be important that in two of the experiments the first period after creatinine seems significantly higher than subsequent ones; in these two it is actually higher than that observed initially in other experiments (see J. H., Figures 2, 3, 6). When there has been no depression in the ratio, a second dose of creatinine does not disturb the system. These negative experiments need no special presentation. The plasma concentrations in this group of experi-

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[2] The errors inherent in the evaluation of this type of renal tubular activity are too great to permit a more precise description of the changes in activity we have observed. These figures (i.e., 4 and 5) indicate first, a definite change in activity in the experiments where creatinine was given in a single oral dose, and secondly, that this change was greater than when creatinine was given by constant intravenous infusion. They do not permit the conclusion that no change takes place in the latter case.
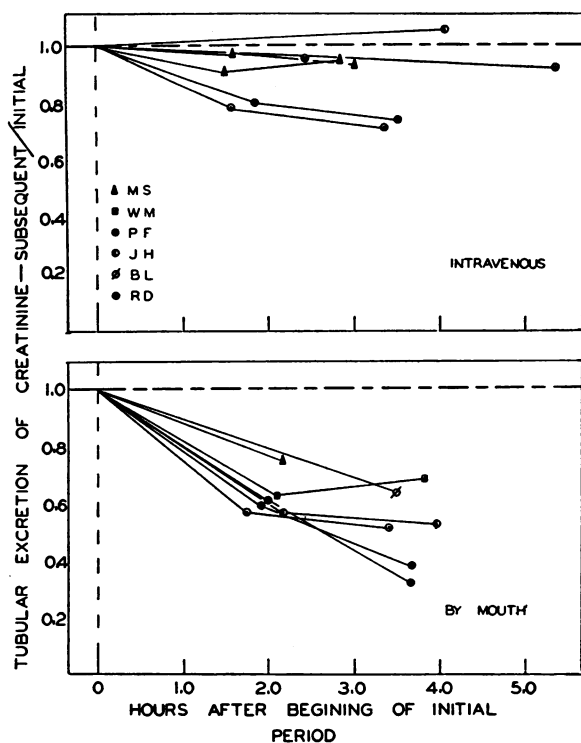
FIGS. 4 AND 5. THE CHANGE IN TUBULAR EXCRETION OF CREATININE RELATED TO THE DURATION OF THE OBSERVATIONS

Figure 4 illustrates those experiments where the plasma concentration was maintained by a constant intravenous infusion of creatinine; Figure 5, those where a single dose of creatinine was administered by mouth.

Each point is the mean of a group of three short experimental periods which expresses the state of tubular activity at that time as the fraction of that observed initially. To better indicate the duration of the experiments, zero in the time scale is taken as the mid-point of the first period; the mean of the second group is plotted as the mid-point of the second group of periods, while the mean of the third group is plotted at the mid-point of the last period. The reader is referred to the text (p. 171) and to Tables I and II for further explanation.

ments are given in the legend of Figure 6. It will be noted that the plasma concentrations are sufficiently high to obviate any serious error from the endogenous chromogenic material.

On the presence of a phosphocreatine-like substance in the plasma after the administration of creatinine in man. In three experiments we have examined the plasma for the presence or absence of labile organic phosphate, 1, 2, and 3 hours after the oral administration of 10 grams of creatinine. In no sample was there evidence of

such a substance. Nor was there any systematic change in the concentration of plasma inorganic phosphate.

DISCUSSION

We reaffirm the curvilinear relationship between plasma creatinine concentration and its renal excretion and the independent nature of such evidence in demonstrating the active participation of the renal tubules in the excretion of this substance. Although such a relationship is not essential in theory, it has been demonstrated in every system of renal tubular excretion where adequate examination has been made. Furthermore, where
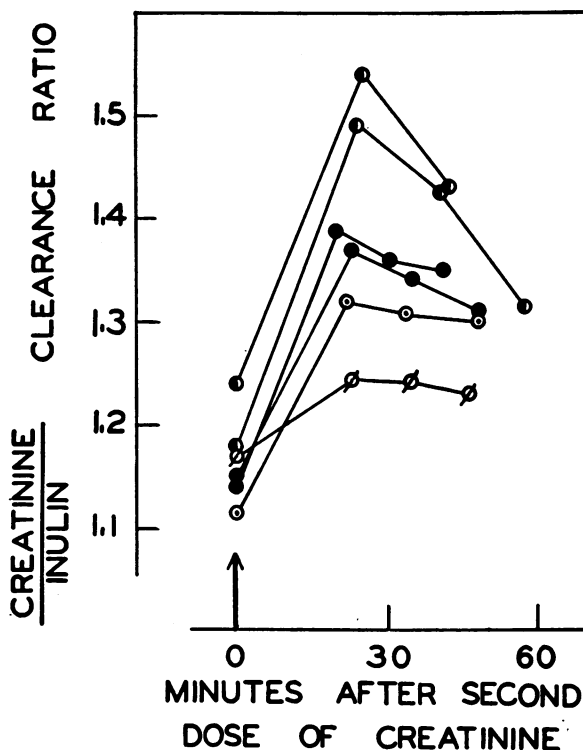

FIG. 6. THE EFFECT OF A SECOND DOSE OF CREATININE ON THE DEPRESSED CREATININE/INULIN CLEARANCE RATIO

The control values (zero time) are the means of three experimental periods obtained approximately 5 hours after the oral administration of 13 grams of creatinine. Subsequent points in each experiment are individual clearance ratios observed (at times indicated by the abscissa) after a small intravenous injection of creatinine. The change in plasma concentration (mgm. per cent) from the first group of periods to the second is given by the following mean values: P.F., 4.0 → 17.20; 8.60 → 20.6; B.L., 7.60 → 21.3; J.H., 4.9 → 16.0; 5.2 → 14.3; R.D., 6.9 → 15.3.

such a relationship has been described there is no evidence to controvert the presence of a process of tubular excretion (13). It is true that to demonstrate this relationship in the case of creatinine, plasma concentrations in excess of the physiological range are essential. However, the quantitative relationships which produce such a situation in no way diminish the forcefulness of the argument.

The origin of this curvilinear relationship is probably similar to that observed in other systems of renal tubular transfer; i.e., it is due to an internal cellular limitation which manifests itself in a maximal rate of transfer (13). This possibility is somewhat strengthened by the observation that a maximal rate characterizes the tubular excretion of creatinine in the dogfish (14) and the chicken (4), and seems likely to be present in the aglomerular toadfish (15). In man, although the data are consonant with such a concept (cf. 1, Figure 5), the complications which serve as a basis for this report make its establishment and accurate evaluation difficult.

It is the process of tubular excretion itself, or the circumstances which may affect it, that must be examined for an explanation of the changes in the creatinine clearance not related to plasma concentration. Among the more obvious possibilities which may be responsible for this phenomenon are the following: (1) The mechanism of transfer might become " fatigued " incident to the continued presence of a high plasma creatinine. This impairment would express itself in an inability of the tubular mechanism to continue the transfer of creatinine with the same facility as was observed initially. (2) A process of adaptation or accommodation to the high plasma concentration might also produce the same result. In this view, essentially that of Miller and Winkler (18), little or no activity of the mechanism at endogenous plasma concentrations would be replaced for a time by a relatively high degree of activity on the presentation of a sudden increment in plasma concentration; this activity subsequently diminishing with simple maintenance of the elevated level. (3) The nature of the circulating creatinine might differ at endogenous levels, immediately after administration and sometime later. In this view, the renal mechanism itself would not be responsible for the apparent change in its activity.

The expression " fatigue " is used in the chemico-physiological sense. That is, accompanying activity of a system there occurs an expenditure or dissipation of some prerequisite for normal activity, or the accumulation of some substance as the result of activity which in itself impairs the subsequent working of the system. Physiological properties such as accommodation or adaptation are less easy to define in terms that would have any meaning in relation to a mechanism responsible for the tubular excretion of a substance. However, in the systems examined up to the present time, including those responsible for the transfer of creatinine in the aglomerular toadfish, dogfish and chicken, there is no evidence to indicate the presence of any of these properties. Nor do our present results indicate that they are contained in the system under examination. The restoration of the depressed tubular excretion toward the initial level by a second dose of creatinine, and the maintenance of essentially full activity [2] in the experiments where the plasma concentration was maintained by constant intravenous infusion seem to dismiss these possibilities.

Our results would be consonant with the viewpoint that a derivative of creatinine is formed subsequent to its administration to man. It is presumed that such a substance would react in plasma filtrates to ordinary chemical methods as does creatinine itself, but would be handled less efficiently by the mechanism of tubular transfer than the parent substance.[3] This type of change would be quantitatively more important in those experiments where a single oral dose is given than in those where the plasma concentration is sus-

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[3] It is impossible to state how large a portion of the creatinine would have to undergo the change we suggest. If the material is simply handled less efficiently by the tubules, or not at all, it would be expected in the plasma in proportion to the lowering of tubular excretion; i.e., 3 hours after creatinine by mouth it would make up a minimum of 50 per cent of the total analyzed concentration. On the other hand, it might act in a wholly different fashion. In terms of our conception of the cellular limitations in such systems of transfer (cf., 12, p. 81), it could have a high affinity for the cellular element of the system and a slow rate of dissociation in the second reaction. In this case, minimal plasma concentrations would have a marked depressing effect upon tubular excretion. If the latter is the case, it poses an almost unsolvable problem, since material producing this effect need not have the chemical properties of creatinine.

tained by a constant intravenous infusion. In the former the creatinine would be exposed to this change for a longer duration of time.

The indirect nature of our evidence permits no more than a tentative suggestion that this may be the case. It is true that the body is capable of performing chemical operations on creatinine as evidenced by its incomplete recovery after administration (16). This experimental fact acquires added significance from the demonstration that this loss is not due to simple hydration to creatine (17). But there is no evidence that this loss is connected with the formation of a substance which conditions the tubular excretion of creatinine. The type of compound described by Abdon (10), *i.e.,* a labile compound containing phosphoric acid appearing in the plasma subsequent to creatinine administration, would satisfy the requirements of our experiments. We have, however, been unable to confirm Abdon's findings

There are two lines of evidence—one experimental, the other chemical—which are in conflict with our interpretation. These would place the site responsible for the changing tubular excretion locally in the tubular mechanism itself. Individuals with renal insufficiency severe enough to cause creatinine retention are stated to have, contrary to the normal, a high creatinine/inulin clearance ratio at endogenous plasma concentrations (18) and, subsequent to creatinine administration, to show no depression in creatinine clearance related to the duration of observation (19). The high ratios observed in this type of patient are not comparable to the normal, since the absolute rate of glomerular filtration has suffered a marked reduction. The lack of elevation in the ratio on the administration of creatinine seems the exception rather than the rule. The experimental data available do not justify the conclusion that, contrary to the normal, after the administration of creatinine no change in tubular excretion takes place which may be related to the duration of the experiment. In eight of the twelve experiments which are purported to demonstrate this (19), there was a fall in the absolute value of the creatinine clearance. Although creatinine/sucrose clearance ratios were more or less constant in those where it was observed, the sucrose clearances were far too erratic to permit their use as an adequate standard of reference.

The second point of conflict stems from a careful study of the chemical nature of plasma creatinine based upon what seems to be a specific enzymatic method of analysis (20). This method does not disclose a significant amount of non-creatinine Jaffé-reacting material in plasma filtrates derived from normal man either before or after the administration of creatinine (18, 20). It may be that the study of rates of reaction with this method rather than simple destruction or non-destruction of chromogenic material would aid in the solution of this conflict.[3]

SUMMARY

The process responsible for the renal tubular excretion of creatinine in normal man has been re-examined. In order to isolate the tubular process for examination, glomerular filtration rate has been measured simultaneously with the observations on creatinine excretion.

1. We reaffirm the dependent curvilinear relationship between plasma concentration and the tubular excretion of this substance.

2. We confirm the fact that after the oral administration of a dose of creatinine (13 grams) the initially high clearance falls progressively with time.

3. If, after this fall, a second dose of creatinine is administered, tubular excretion is raised to or toward the initial level of activity.

4. When the plasma concentration is maintained by the constant intravenous infusion of creatinine, tubular excretion is usually maintained at a level close to that observed initially.

5. It is tentatively suggested that the phenomena described under 2 and 3 are not due to a change in the renal tubular mechanism itself, but rather that, subsequent to its entry into the body, some of the creatinine undergoes a change which makes it less readily transferable by the tubular mechanism.

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