STUDIES IN CREATINE AND CREATININE METABOLISM.

V. THE METABOLISM OF CREATINE.

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In 1906 Folin (1) published a memorable contribution to the subject of creatine metabolism in which, as a result of experiments upon human subjects, he concluded that the animal organism cannot convert creatine into creatinine. The conclusion was also reached that while creatinine is a waste product, creatine is a food and that there is no biological relationship between these compounds. Klercker (2) reached quite similar conclusions independently of Folin's observations.

In view of the very recent admirable summary of the literature on creatine and creatinine given by Hunter (3), it is quite unnecessary for us to present an account of the mass of data which has accumulated since Folin's first contribution. A number of investigators have reported that traces of extra creatinine may appear in the urine following creatine administration and that with very large doses of creatine (Rose and Dimmitt, 4) appreciable quantities of extra creatinine may be eliminated. Such findings, together with the very excellent and suggestive work of Myers and Fine upon the relationship between muscle creatine and urinary creatinine elimination, have led many to question seriously Folin's original conclusions. Yet all the evidence presented on the origin of urinary creatinine from creatine during the 16 years which have elapsed since Folin's paper, has not been very convincing. Folin himself has never abandoned his original position, and there are many who would agree with him that practically no positive evidence has been offered that creatine is the normal biological source of creatinine. Even those most strongly favoring the view

that creatinine arises from creatine would have to admit that the evidence for this view must be selected piecemeal here and there, wherever it can be found. Direct, clear-cut evidence has been wholly lacking. The failure to find conversion of appreciable amounts of creatine into creatinine, together with the fact that "creatinuria" is a definite entity which is usually unrelated to changes in the creatinine elimination, are facts which have made Folin's position almost unassailable for those who forget the chemical relationship of the compounds involved.

The work reported in the present paper was a result of the conclusions reached by Behre and Benedict (5) that creatinine does not exist in appreciable amounts, if at all, in blood, while creatine (or some combination of it) can be demonstrated in this fluid, and accumulates in large amounts after ablation of the kidney function. Prior to these findings we had held the position taken by Folin, but the work on creatine and creatinine in blood forced us to a different view-point. We concluded that creatine, or some simple derivative of it, must be the precursor of creatinine, and therefore sought for loopholes in previous investigations which might open a way to attack the problem, so that some direct answer might be obtained to the fundamental questions involved. A careful consideration of all the facts available led us to conclude that there was one basic defect in the strongest argument advanced by Folin and his followers against the origin of urinary creatinine from creatine. Creatine was administered and did not increase the urinary creatinine. *But the creatine administered was never accounted for in any way whatever.* Here lay the weakness in Folin's argument. It is true that, reasoning from the known facts of metabolism, it seemed safe to assume that a nitrogenous compound would surely give rise to its end-products in the body during the period covered by Folin's experiments. Yet Folin himself demonstrated that creatine was practically or wholly unique among nitrogen compounds in that it failed to reappear in any form—even undetermined nitrogen—during the period of his observation. *Obviously, if metabolized at all, the creatine must ultimately give rise to something. Then so long as it remains wholly unaccounted for, there is no justification for the conclusion that it may not give rise to creatinine.*

As a result of these considerations we were led to undertake the experiments reported in the present paper.

The general plan of the work was to bring dogs into approximate nitrogenous equilibrium upon a constant diet, and then to administer a small amount of creatine daily until an appreciable quantity of the administered creatine was accounted for in some form in the urine. The results obtained are, we believe, of definite interest in connection with the basic questions of creatine and creatinine metabolism.

The work was carried out during the spring and summer of 1922. The experimental animals were healthy female dogs. Each was kept in a metabolism cage and fed upon a constant diet throughout the experiment. The animals were catheterized and weighed once daily before feeding. Periods were marked for the feces by administration of bone-black. Total nitrogen was determined by the macro Kjeldahl method, using potassium and copper sulfates as catalyzers. Creatinine was determined by the original Folin method, using bichromate as standard. With very few exceptions the urines were diluted to the same volume each day and the reaction and colorimetric reading were thus carried out under rigidly fixed conditions. Creatine in the urine was converted to creatinine and determined as such by the procedure commonly employed in this laboratory (6). The creatine employed was prepared in the laboratory from creatinine obtained from urine (7) and was recrystallized until free from any detectable trace of creatinine.

The method for administration of the creatine was carefully considered. We finally adopted oral administration by mixing the creatine with the food as the method to be tried first. It seemed probable that the experimental period would be long, and the oral administration was far preferable from the practical standpoint to any injection procedure. It is time-saving, and avoids infection and chance of conversion of creatine to creatinine during sterilization of the solutions. We soon found that we could check up the question of conversion of creatine to creatinine in the gastro-intestinal tract, as well as that of absorption of the creatine as such. As we shall show later in this paper, the oral method of administration is ideal from every standpoint for the experiments which we carried out upon dogs.

In each experiment the animal was placed upon the weighed diet, and became accustomed to the cage and routine of the experiments many days before the first periods which we have reported in the present paper.

The results of the experiments are recorded in Tables I to III. Table I presents a detailed report of our first experiment. Including the preliminary period of 9 days, this experiment lasted 128 days. There were ten periods of 7 days each, during which the dog received 0.620 gm. of creatine daily (containing 1 molecule of water of crystallization). This was equivalent to 0.470 gm. expressed as creatinine. The ten periods of creatine administration were followed by seven after periods of 7 days each.

We believe that a detailed study of Table I will be found of interest. We may summarize the main facts brought out as follows:

The dog had been on the diet for 24 days before the preliminary period reported. The nitrogen balance was + 1.3 gm. for a period of 7 days preceding the first period recorded in the table. The preliminary period reported shows a plus nitrogen balance of 0.7 gm. for 7 days. The creatinine output is quite constant for this (and the preceding unreported periods) and averages 405 mg. per day. During the 1st week of the creatine administration there is no apparent effect on the creatinine elimination, except that on the last day of the period an increase of about 20 mg. above the average level is to be noted. Such a slight increase is typical of results previously reported after creatine administration. The nitrogen balance of the first creatine period is +2.7 gm. This represents a retention of nitrogen equal to more than twice that contained in the creatine. None of the creatine given appears as such in the urine. Since the urinary nitrogen is definitely below that of the preliminary period we may infer that the creatine is practically wholly retained in some form during this period. The second period of creatine administration develops several points of real interest. The creatinine in the urine is definitely above that of the preceding period, but shows its maximal height during the 1st day. The creatinine for this second period averages about 40 mg. higher per day than is found during the control periods. This increase appears to us as still too small to warrant concluding that there is conversion of creatine into creatinine, but it begins to be very suggestive.

TABLE I.

Nitrogen, Creatinine, and Creatine Elimination after Creatine Administration.

Dog 1. Female.

The dog received daily the following diet:

	gm.
Cracker meal	150
Evaporated milk	100
Casein	24

(6.65 gm. nitrogen)

Weight of dog.	Daily output in the urine.				Remarks.
	Total nitrogen.	Creatinine.	Creatine (expressed as creatinine).	Total nitrogen for period.	
kg.	*gm.*	*mg.*	*mg.*	*gm.*	
14.4	5.711	418			Preliminary period of 9 days duration. No creatine given.
	5.571	405			
	6.000	413	0	Urine = 50.686	
	5.600	405		Feces = 8.282	
14.4	5.934	405		Hair = 0.115	
	5.700	397		Total = 59.083	
14.3	5.460	405			
	5.350	397		Food N = 59.850	
	5.360	401	0	Balance = +0.767	
	5.631	405	0		Average daily output.
14.4	5.740	405	0	Urine = 38.626	First period of creatine administration. 0.620 gm. of creatine ($C_4H_9N_3O_2 \cdot H_2O$) was mixed with the food each day. This quantity of creatine would represent 0.470 gm. of creatinine.
	5.124	393	0	Feces = 6.316	
	5.418	397	0	Hair = 0.992	
	5.600	405	0	Total = 45.034	
	5.500	381		Food N = 46.55	
	5.644	405			
14.3	5.600	426	0	Creatine N = 1.215	
				Total = 47.765	
				Balance = +2.731	
	38.626	2,812	0		Total for 7 days.
14.4	5.518	401	0		Average for 1 day.

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