THE HYDROLYSIS OF PHOSPHOCREATINE AND THE ORIGIN OF URINARY CREATININE

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Shaffer (1), Myers and Fine (2), and Hahn and Meyer (3) adduced evidence that muscle creatine is the precursor of urinary creatinine. This was questioned by Chanutin and Kinard (4) but was conclusively proved by Bloch, Schoenheimer, and Rittenberg (5, 6) by isotope tracer evidence, which also showed that creatinine was the only normal urinary constituent containing any significant amount of body creatine nitrogen.[1]

Although Bloch, Schoenheimer, and Rittenberg had settled the issue of the relation between creatine and creatinine, the site and mechanism of creatinine formation and the place of phosphocreatine in the process were still open questions. The crux of all three questions is the fact that the amount of creatinine normally excreted is greater than can be accounted for by the spontaneous (*i.e.*, non-enzymatic) rate of dehydration of creatine at the pH and temperature of the body. Thus Myers and Fine (7) found that the creatine in finely ground muscle was transformed to creatinine more than 3 times as fast as in pure solutions of creatine. Hammett (8) measured the velocity constants of the transformation at 38° and pH 6.9 of the creatine in buffered extracts of rat brain and muscle and in similarly buffered solutions of pure creatine. The velocity constants in the brain suspensions ranged from 0.00083 to 0.00148, average 0.00117; in muscle 0.00091 to 0.00119, average 0.00104; and in creatine solutions 0.00058. (The time unit of these constants is 1 hour.) The percentage of creatine converted in 24 hours calculated from these constants was, in the brain and muscle extracts, 2.8, and in the creatine solution 1.4.

The rate of conversion of creatine to creatinine *in vivo* is of the same order of magnitude as observed by Hammett in brain and in muscle extracts. Table I is an assembly of the data obtained by direct observation on this point.[2]

[1] Excretion of creatine and creatinine into the intestine and their degradation there were not examined.

[2] Included in Table I are data obtained by Chanutin and Kinard (4), who concluded that, "A statistical analysis of the data obtained in the dog, rat, rabbit, and guinea pig indicates an absence of any relationship between creatinine elimination and the percentage concentration of muscle creatine." We have calculated the correlation coefficient, r, and the value of t (the probability coefficient of r), grouping

TABLE I

Rate of Conversion of Creatine to Creatinine in Vivo

Animal	Average total creatine in body	Average urinary creatinine in 24 hrs.	Creatine converted to creatinine in 24 hrs.	No. of animals	Correlation coefficient r*	t†	P‡	Bibliographic reference No.
	mg.	*mg.*	*per cent*					
Dog	23,020§	477.5	2.41	7	0.951	6.86	<0.01	(4)
Guinea pig	1,197§	27.5	2.66	21	0.880	8.07	<0.01	(4)
Rabbit	3,188	67.7	2.46	10	0.967	10.72	<0.01	(2)
"	6,070§	150.4	2.88	11	0.349	1.12	0.3	(4)
Rat	568§	11.4	2.33	17	0.800	5.6	<0.01	(4)
"	1,026	13.8	1.56	14	0.897	7.04	<0.01	(9)
"			2.03	3				(6)
"			2.70‖					(6)
In vitro from creatine			1.06-1.33					

* Correlation coefficient between the average total body creatine and the average 24 hour urinary creatinine; $r = \frac{\Sigma xy}{\sqrt{\Sigma x^2 \cdot \Sigma y^2}}$, where x and y are deviations of individual values of body creatine and 24 hour urinary creatinine, respectively, from their average values for the series.

† $r\sqrt{\frac{n-2}{1-r^2}}$.

‡ Probability of obtaining the observed value of r or higher, on the assumption that body creatine and urinary creatinine are unrelated.

§ Estimated as follows: average concentration of muscle creatine × body weight × 0.40.

‖ Calculated from the rate of decrease of isotopic nitrogen in urinary creatinine and in terminal muscle creatine.

in one series all their data on the four animals. The value of r is 0.787 and of t 9.37, which indicates a very high correlation. The statistical treatment of Chanutin and Kinard was inadequate in two respects; (1) they compared the concentration of muscle creatine, which varies little in healthy adult animals with any one species, with the total creatinine elimination, which is affected greatly by the body weight which varies considerably, and (2) the variations within each group of animals of the same species were over too small a range for the number of animals within the group. Table I shows that, when the total body creatine and the urinary creatinine are compared, their data yield high correlation coefficients, except in the case of the rabbit in which again the number of animals was too low for the small range of variation within the group. In the group of rabbits used by Myers and Fine (2) the range of variation was much larger and a high correlation coefficient is obtained. Kinard *et al.* (9) found later in an independent study on the rat a high correlation between total body creatine and creatinine elimination. Any inference in the conclusion of Chanutin and Kinard, quoted above, that body creatine is not related to urinary creatinine was disproved conclusively by Bloch and Schoenheimer (5, 6) by means of creatine labeled with isotopic nitrogen.

The faster rate of conversion of creatine to creatinine in the tissues than in pure solutions of creatine suggested that an enzyme might be responsible. No evidence of such an enzyme has been found in repeated searches (3, 7, 8, 10). Hahn and Meyer estimated that creatinine elimination in man could be accounted for satisfactorily simply by the spontaneous (*i.e.* non-enzymatic) dehydration of creatine in pure solution, but they used too high a figure for the creatine concentration of human muscle in their estimate, and they took no account of the fact that most of the creatine in the body is not free, but is "bound" as phosphocreatine. Hahn and Meyer's own figure of the rate constant for the spontaneous conversion of creatine to creatinine at pH 7 and 38°, $K = 0.000538$, corresponds to 1.3 per cent conversion in 24 hours, which is lower than the rates observed *in vivo* (Table I), in tissue extracts (7), and in tissue autolysates (8).

In view of the conclusive evidence that the rate of creatinine elimination is related to the amount of body creatine, nearly all of which is in the muscles and in the form of phosphocreatine, we investigated the rate of creatinine formation in buffered aqueous solutions of phosphocreatine and compared it with the rate in similar solutions of creatine. It was found that creatinine is formed spontaneously much faster from phosphocreatine than from creatine. The rate from phosphocreatine at pH 7 and 38° is, in buffered solutions without an enzyme, within the range of rates of transformation of creatine to creatinine observed *in vivo*. It is, therefore, unnecessary to postulate the intervention of an enzyme in this process. That possibility is not excluded, but if there is an enzyme, its activity *in vivo* is low.

EXPERIMENTAL

Phosphocreatine was isolated from rabbit muscle by the method of Fiske and Subbarow (11), and synthesized by the method of Zeile and Fawaz (12). Both preparations were purified in the form of the calcium salt. The same results were obtained with both the natural and synthetic preparations.

Creatine was purified by the method described by Hunter (13).

Creatinine was determined by the alkaline picrate method. We found in solutions containing a large excess of creatine over creatinine that a slow intensification of the color occurs after the initial rapid development. The slow increase in color is, presumably, a consequence of a slow conversion of creatine to creatinine in the alkaline picrate. Accordingly, readings were taken at intervals and the extrapolated zero time value gave the creatinine present before the addition of the alkaline picrate. As an example of the order of magnitude of the extrapolation, the color at the end of an hour

corresponded to 11.5 per cent more creatinine than the extrapolated zero time value.

Inorganic phosphate in the presence of phosphocreatine was determined both by the indirect and by the direct precipitation methods of Fiske and Subbarow. The two methods gave essentially the same hydrolysis constants of phosphocreatine when the room temperature at which the determination was carried out was not too high. We came to prefer the direct method, as the laboratory temperature was often above 25°, and the solutions were warmed somewhat during the colorimetry. Under these conditions the indirect method, as Fiske and Subbarow pointed out, gives values of inorganic phosphate (in the presence of phosphocreatine) which are too high and inconsistent.

The colorimetry of both the creatinine and of the inorganic phosphate was carried out either in a König-Martens (visual) or a Beckman (electrical) spectrophotometer.

0.1 M maleic acid-sodium hydroxide buffers were used.

The preservative employed, after a number were tried, was 0.1 per cent phenol; it did not interfere with either the creatinine or phosphate determinations.

Mathematical Analysis

Three reactions ensue when phosphocreatine is dissolved in approximately neutral solution and at temperatures near to that of the body.

(A) Phosphocreatine → inorganic phosphate + creatinine

(B) Phosphocreatine → inorganic phosphate + creatine

(C) Creatine → creatinine

On the assumption that the three reactions are of the first order, and with the concentration of phosphocreatine designated as [PC], of inorganic phosphate as [P], of creatinine as [Cn], and of creatine as [Cr], the rate of creatinine formation in reaction (A) is

$$\left(\frac{d\mathrm{Cn}}{dt}\right)_1 = k_1[\mathrm{PC}] \tag{1}$$

and in reaction (C)

$$\left(\frac{d\mathrm{Cn}}{dt}\right)_2 = k_2[\mathrm{Cr}] \tag{2}$$

If the initial concentration of phosphocreatine is designated as a, at any time during the reaction

$$[\mathrm{PC}] = a - [\mathrm{P}] \tag{3}$$

and

$$[\mathrm{Cr}] = [\mathrm{P}] - [\mathrm{Cn}] \tag{4}$$

The total creatinine formed at any time during the reaction is, from equations (1) and (2),

$$\int (d\mathrm{Cn})_1 + \int (d\mathrm{Cn})_2 = [\mathrm{Cn}] = k_1 \int [\mathrm{PC}]\, dt + k_2 \int [\mathrm{Cr}]\, dt \tag{5}$$

which may be rewritten according to equations (3) and (4)

$$[\mathrm{Cn}] = k_1 \int (a - [\mathrm{P}])\, dt + k_2 \int ([\mathrm{P}] - [\mathrm{Cn}])\, dt \tag{6}$$

If equation (6) is rearranged,

$$k_1 = \frac{[\mathrm{Cn}] + k_2 \int [\mathrm{Cn}]\, dt - k_2 \int [\mathrm{P}]\, dt}{at - \int [\mathrm{P}]\, dt} \tag{7}$$

[Cn] is the concentration of creatinine (total) at time t. k_2 was obtained independently from buffered solutions of creatine. $\int [\mathrm{Cn}]\, dt$ was computed by graphical integration of the plot of [Cn] against time. To obtain $\int [\mathrm{P}]\, dt$ the rate constant of total inorganic phosphate formation from phosphocreatine was determined first; $\int [\mathrm{P}]\, dt$ was then computed by graphical integration of the plot against time of inorganic phosphate liberated.

For convenience of computation, the initial concentration of phosphocreatine was set at 100; [Cn] then became the per cent of phosphocreatine converted to creatinine and [P] the per cent of phosphocreatine hydrolyzed in the sum of reactions (1) and (2).

Results

A test of the validity of equation (7) is the constancy of k_1 at different times during the hydrolysis of phosphocreatine at any given pH and temperature. The same test in another form is the agreement between observed and calculated values of [Cn].

The rate constants of the spontaneous conversion of creatine to creatinine in aqueous solution, k_2 in equation (7), obtained by different authors and ourselves, are collected in Table II. The values we obtained lie between those calculated from the data of Myers and Fine (2) and those reported by Hammett (8) and by Hahn and Meyer (3). The values at 38° at or near the same pH are in fairly good agreement; this is appreciated better when the rate constants are expressed as pK values. Thus Hahn and

TABLE II

Rate Constants of Spontaneous Conversion of Creatine to Creatinine

pH	3.96	4.90	5.86	6.00	6.50	6.90	7.00	7.01	7.00	7.50
Temperature, °C.	38	38	38	38	38	38	36	38	38	38
Rate constant	0.00711	0.00207	0.000907	0.000606	0.000507	0.000525*	0.00021†–0.00030	0.000538	0.000442	0.000412
Bibliographic reference No.	(3)	(3)	(3)	Authors	Authors	(8)	(2)	(3)	Authors	Authors

* Hammett (8) gave k from his data as 0.00058; but the small amount of creatinine, which his figures show was present initially in the creatine used, was not taken into account. The value of k above is obtained when this is done.

† The values of k ascribed to Myers and Fine (2) were calculated from their data.

Meyer give, at pH 7.01, pK_2 3.27; we found, at pH 7.00, pK_2 3.35. Neither Hammett nor Hahn and Meyer mention the necessity of extrapolating to zero time when determining creatinine in the presence of a great excess, 30 to 50 times, of creatine. Our having done so might account for half the difference between their values and ours. Probably the main reason for the difference lies in the fact that the precision of the alkaline picrate method for determining creatinine in low concentrations (0.2 to 1.0 mg. per cent) is not better than ±5 per cent in the lower portion of this range and ±2.5 per cent in the higher portion. The precision was lowered further in all the determinations of k_2 by the great excess of creatine present. The above differences in the values of k_2 at any one pH have a negligible influence on the value of k_1 in equation (7) and may, therefore, be taken as sufficiently concordant for this purpose.

To compute $\int [P]\,dt$ in equation (7), it is necessary to obtain the rate constant of the liberation of inorganic phosphate from phosphocreatine in the sum of reactions (A) and (B).[3] Table III contains the value of k_P obtained at 20.5° and 38° over a pH range from 5.4 to 7.5. Natural and synthetic phosphocreatine gave the same values (within the experimental error) of k_P at the same pH and temperature. The plot of pk_P against pH, including in one array the values of both natural and synthetic phos-

[3] This constant, k_P, is equal to the sum of the constants of the liberation of inorganic phosphate in reactions (1) and (2), where k_{P_A} and k_{P_B} respectively are the rate constants for reactions (1) and (2). The proof is as follows: The rate of liberation of phosphate in reaction (1) is

$$\left(\frac{dP}{dt}\right)_A = k_{P_A}(a - [P]) \tag{8}$$

and in reaction (2)

$$\left(\frac{dP}{dt}\right)_B = k_{P_B}(a - [P]) \tag{9}$$

The rate of liberation of phosphate in the sum of reactions (1) and (2) is

$$\left(\frac{dP}{dt}\right)_A + \left(\frac{dP}{dt}\right)_B = (k_{P_A} + k_{P_B})(a - [P]) \tag{10}$$

Equation (10) may be written

$$\frac{dP}{dt} = k_P(a - [P]) \tag{11}$$

and since

$$\left(\frac{dP}{dt}\right)_A + \left(\frac{dP}{dt}\right)_B = \frac{dP}{dt} \tag{12}$$

$$\therefore k_P = k_{P_A} + k_{P_B} \tag{13}$$

phocreatine, gives at 20.5° a straight line from pH 5.4 to 7.0, and at 38° a straight line from pH 5.8 to 6.5 and a shallow curve from pH 6.5 to 7.5. In Table IV the values of pk_P interpolated from the curves are compared with the observed values at the same pH. There is no consistent trend of disagreement between the values obtained from natural and synthetic phosphocreatine and the corresponding interpolated values. The two preparations may be considered as identical by this criterion.

TABLE III

Hydrolysis Constants of Liberation of Inorganic Phosphate from Phosphocreatine in Buffered Aqueous Solutions

Temperature, 20.5°			Temperature, 38°		
Source of phosphocreatine	pH	Hydrolysis constant	Source of phosphocreatine	pH	Hydrolysis constant
Natural	5.4	0.0298*	Natural	5.80	0.103
"	5.8	0.0109*	Synthetic	6.00	0.0634
Synthetic	6.0	0.00711	Natural	6.43	0.0272
"	6.21	0.00439	Synthetic	6.50	0.0233
Natural	6.4	0.00283*	"	6.70	0.0149
Synthetic	6.5	0.00290	Natural	6.96	0.0101
"	6.55	0.00252	Synthetic	6.96	0.0103
"	6.7	0.00139	"	7.00	0.0101
"	7.02	0.00057	"	7.20	0.00686
			Natural	7.50	0.00553

* Calculated from Fiske and Subbarow's value at 22° (11), multiplied by 2.303 (since their values are for the expression, $k = (1/t) \log (a/(a - x))$ instead of $k = (1/t)\ln(a/(a - x))$ and modified for the difference in temperature between their and our determinations. The equation used to obtain the temperature coefficient is

$$\log \frac{k_2}{k_1} = \frac{E}{2.303R}\left(\frac{T_2 - T_1}{T_1 T_2}\right)$$

where k_1 and k_2 are the velocity constants at temperatures T_1 and T_2 (on the absolute scale) respectively, R is the gas constant (1.989), and E is the energy of activation. E, calculated from interpolated points on smooth curves, of the rate constants obtained at 20.5° and 38°, is here 23,170 calories. When T_1 and T_2 are 20.5° and 22° respectively, $\log k_1 = \log k_2 - 0.087$.

Table V summarizes the data on creatinine formation in solutions of phosphocreatine at different hydrogen ion concentrations. The initial concentration of phosphocreatine was, in every case, 1.43×10^{-3} molal. Creatinine formed, expressed as equivalent per cent of initial phosphocreatine, was plotted against time, and $\int [\mathrm{Cn}]\,dt$ was computed graphically from a smooth plot through the experimental points. In determining the plot, greater weight was given the points at the end of the run than at the beginning. $\int [\mathrm{P}]\,dt$ was computed by graphical integration of

the curve of the liberation of inorganic phosphate given by the values of k_P at each pH. The values of k_P and of k_2 used were obtained by interpolation of smooth plots of pk_P and of pk_2 against pH.

The calculated values of creatinine formed were interpolated from curves given by the calculated values of k_1, which, in every case, were near to the mean of the observed values of k_1. The differences between the observed and calculated values of creatinine formed are within the experimental error of the creatinine determination. There was no consistent difference in this respect between the natural and synthetic phosphocreatine. By this criterion, therefore, as well as by the identity of their k_P values, the two preparations behaved the same.

TABLE IV

Comparison of Hydrolysis Constants of Liberation of Inorganic Phosphate of Natural and Synthetic Phosphocreatine

Temperature, 20.5°				Temperature, 38°			
Source of phosphocreatine	pH	pK observed	Interpolated value on smooth curve, pK	Source of phosphocreatine	pH	pK observed	Interpolated value on smooth curve, pK
Natural	5.4	1.53*	1.51	Natural	5.80	0.99	0.95
"	5.8	1.96*	1.93	Synthetic	6.00	1.20	1.18
Synthetic	6.0	2.15	2.14	Natural	6.43	1.57	1.59
"	6.21	2.36	2.36	Synthetic	6.50	1.63	1.66
Natural	6.4	2.55*	2.56	"	6.70	1.83	1.82
Synthetic	6.5	2.54	2.67	Natural	6.96	2.00	2.00
"	6.55	2.60	2.72	Synthetic	6.96	1.99	2.00
"	6.7	2.86	2.87	"	7.00	2.00	2.03
"	7.02	3.24	3.21	"	7.20	2.16	2.17
				Natural	7.50	2.26	2.33

* From Fiske and Subbarow's data; see the footnote to Table III.

Equation (7) and the numerical value of the rate constant k_1 are based on the hypothesis that phosphocreatine is hydrolyzed in part to creatinine and inorganic phosphate and in part to creatine and inorganic phosphate, and the creatine thus formed is independently dehydrated to creatinine. This process is formulated in reactions (A), (B), and (C) above. It is implied that the prevailing concentrations of creatine and creatinine are so far from their equilibrium ratio that reactions (A), (B), and (C) may be considered as irreversible and of the first order.

In this hypothesis the rate of formation of creatinine directly from phosphocreatine is

$$\int d[\mathrm{Cn_I}] = k_1 \int (a - [\mathrm{P}])\, dt \qquad (14)$$

where $[Cn_1]$ is the creatinine coming directly from phosphocreatine, k_1 is the rate constant of that process, a is the initial concentration of phosphocreatine, and [P] the concentration of total inorganic phosphate liberated.

TABLE V

Creatinine from Phosphocreatine at 38°; Creatinine Expressed As Equivalent Per Cent of Initial Phosphocreatine

Initial concentration of phosphocreatine in every case was 1.43×10^{-3} molal, corresponding to 18.7 mg. per cent of creatine or 16.2 mg. per cent of creatinine. Equation (7) and the values of k_P and k_2 at each pH were used for the calculation of k_1. (N) designates that natural and (S) that synthetic phosphocreatine was used.

Time	Creatinine		k_1	
	Observed per cent	Calculated per cent	Observed	Calculated
	pH 6.0; k_P = 0.0634; k_2 = 0.000606			
hrs.				
4.2	1.1 (N)	1.0	0.0029	0.0026
19.4	3.4 (S)	3.4	0.0026	0.0026
24.1	3.85 (N)	3.95	0.0025	0.0025
46.9	6.1 (S)	5.8	0.0029	0.0027
67.6	7.0 "	7.1	0.0026	0.0027
71.5	6.8 (N)	7.3	0.0023	0.0026
Mean			0.0026	0.0026
	pH 6.44; k_P = 0.027; k_2 = 0.00051			
19.3	2.6 (S)	2.3	0.0016	0.0014
24.2	3.0 (N)	2.8	0.0015	0.0014
48.1	4.7 "	4.8	0.0014	0.0014
67.5	6.7 (S)	6.1	0.0016	0.0014
71.6	6.4 (N)	6.4	0.0014	0.0014
92.7	7.6 (S)	7.5	0.0014	0.0014
95.8	7.3 (N)	7.7	0.0013	0.0014
Mean			0.0015	0.0014
	pH 6.91; k_P = 0.00983; k_2 = 0.00045			
20.0	2.3 (S)	2.0	0.00123	0.00106
23.1	1.9 (N)	2.3	0.00087	0.00106
44.4	4.3 (S)	4.2	0.00110	0.00107
47.0	3.8 (N)	4.4	0.00091	0.00107
69.0	5.4 (S)	6.1	0.00093	0.00107
70.8	5.6 (N)	6.2	0.00094	0.00106
91.3	7.1 (S)	7.6	0.00097	0.00106
94.9	7.7 (N)	7.8	0.00103	0.00105
119.0	9.2 (S)	9.3	0.00104	0.00105
Mean			0.00100	0.00106

TABLE V—*Concluded*

Time	Creatinine		k_1	
	Observed per cent	Calculated per cent	Observed	Calculated
pH 7.21; k_P = 0.00686; k_2 = 0.00043				
hrs.				
20.1	2.65 (S)	2.3	0.0014	0.0012
44.4	5.1 "	4.9	0.0013	0.0012
68.9	6.6 "	7.1	0.0011	0.0012
91.3	8.8 "	8.9	0.0012	0.0012
119.1	11.0 "	11.1	0.0012	0.0012
Mean			0.0012	0.0012
pH 7.50; k_P = 0.00550; k_2 = 0.00041				
23.2	2.35 (N)	2.45	0.0011	0.0011
47.0	4.8 "	4.8	0.0011	0.0011
71.5	6.95 "	7.0	0.0011	0.0011
95.0	9.0 "	9.0	0.0011	0.0011
Mean			0.0011	0.0011

The same numerical result as from equations (7) and (14) is obtained on the hypothesis that phosphocreatine in solution consists of two forms which are always in equilibrium with each other. One form, a_1, gives rise on hydrolysis to creatinine and inorganic phosphate, the other, a_2, to creatine and inorganic phosphate. Equation (14) is modified to

$$\int d[Cn_1] = k'_1 \int ([a_1] - [P_1])\, dt \tag{15}$$

which the experimental data showed to be equal to

$$\int d[Cn_1] = k'_1 \int \alpha(a - [P])\, dt \tag{16}$$

where α is the fraction of the total phosphocreatine in the form of a_1.

From equations (14) and (16)

$$k_1 = k'_1\alpha \tag{17}$$

α can be evaluated from the values of k_1 and of k'_1 at any one time in the course of the reaction, or by solving for a_1 in equation (15) at two different times during the reaction. Both methods gave the same value of α, which was constant, at any one pH, during the whole time (up to 120 hours) that the reaction was followed. α varied with pH; it was 0.042, 0.051, 0.104, 0.173, and 0.200 at pH values of 6.0, 6.44, 6.91, 7.21, and 7.50 respec-

tively. On the hypothesis as defined above, of two forms of phosphocreatine, a_1 and a_2, in equilibrium the variation of α with pH indicates that hydrogen ion enters into the equilibrium.

The fact that, at any given pH, α is constant throughout the course of the reaction excludes the possibility that the phosphocreatine we used was a mixture, *i.e.* that a_1 and a_2 were not in equilibrium. If that were the case, since k'_2 (the rate constant for the hydrolysis of phosphocreatine to creatine and inorganic phosphate) is larger than k'_1, α would have varied throughout the reaction. The experimental data showed that this was not the case.

The possibility that the phosphocreatine we used was a mixture is excluded also by the following additional evidence. If it were a mixture,

TABLE VI

Rate Constants (Interpolated) at 38° of Liberation of Inorganic Phosphate from Phosphocreatine, of Creatinine Directly from Phosphocreatine, and of Creatinine from Creatine over pH Range 6.0 to 7.5

pH	k_P	k_{PA}	k_{PB}	k_1	k_2
6.0	0.063	0.0026	0.060	0.0026	0.00061
6.5	0.022	0.0014	0.021	0.0014	0.00050
7.0	0.0092	0.0011	0.0081	0.0011	0.00044
7.5	0.0055	0.0011	0.0044	0.0011	0.00041

k_P = the rate constant of liberation of total inorganic phosphate.

$k_{P_A} = k_1$ = the rate constants of liberation of creatinine directly from phosphocreatine and of the accompanying inorganic phosphate in that reaction alone.

$k_{P_B} = k_P - k_{P_A}$ = the rate constant of liberation of inorganic phosphate accompanying the liberation of creatine from phosphocreatine.

k_2 = the rate constant of conversion of creatine to creatinine.

we might expect the proportions of a_1 and a_2 to be different in different preparations of natural and synthetic phosphocreatine, and accordingly their values of k_P and of k_1 to be different. We used three different preparations of synthetic phosphocreatine and one from rabbit muscle. Fiske and Subbarow obtained theirs from cat muscle. All preparations gave the same values of k_P over the whole experimental range of pH, and all of our preparations gave the same values of k_1. In fact, as shown below, even the *in vivo* rates of creatinine formation in a number of different animals are in accord with that of phosphocreatine *in vitro*.

In the present communication we are concerned primarily with the relation between the rate of spontaneous, *i.e.* non-enzymatic, formation of creatinine from phosphocreatine and the rate of creatinine excretion in the urine. For this purpose it is convenient to use equation (7), as all the terms on the right-hand side are obtained without hypothesis;

the constancy of k_1 at any one pH for all values of t sanctions the use of the equation.

In the literature there are no values of the hydrolysis constants of phosphocreatine over the physiological range of hydrogen ion concentration. These are given in Table VI and also those we obtained of the conversion of creatine to creatinine.

DISCUSSION

In the body most of the creatine (98 per cent) is in the skeletal muscles. Its concentration is kept constant within a range of 400 to 600 mg. per cent in different species. Since the concentration of creatine is kept constant, the per cent converted in 24 hours is $kat = k \times 100 \times 24$. If it were all free creatine and no enzymatic action postulated, the amount converted to creatinine in 24 hours at pH 7.0 would be, with the value of k_2 in Table VI, $24 \times 0.00044 \times 100 = 1.06$ per cent. If it were all phosphocreatine, the value would be $24 \times 0.0011 \times 100 = 2.64$ per cent. The latter is within the range of rates of conversion of creatine to creatinine *in vivo* (Table I), whereas the rate of conversion of creatine *in vitro* is definitely below, approximately one-half, that *in vivo*.

Rosengart (14) found that the formation of creatinine was greatly accelerated in minced muscle at 38° in the presence of fluoride, iodoacetate, or HCN, as did Myers and Fine and Hammett in unpoisoned autolyzing muscle extracts. Rosengart tried to explain this fact by a non-hydrolytic dephosphorylation of phosphocreatine; he later accepted the explanation proposed here, *i.e.* the cleavage of phosphate from phosphocreatine in reaction (1) above.

Some of the data presented by Hammett (8) in 1924 on the rate of appearance of creatinine in autolyzing rat muscle extracts afford a direct comparison with the rate we observed in phosphocreatine solutions at the same pH and temperature. At pH 6.9 and 38° Hammett found at 10, 20, and 30 hours incubation the following percentages of the total creatine converted to creatinine: 1.03, 2.02, and 3.06, respectively. A smooth plot through our experimental data at the same pH and temperature gave for the same time intervals 1.04, 2.03, and 2.98 per cent. After 30 hours incubation Hammett's values are higher than we observed; the explanation is very probably an increase in acidity in Hammett's autolysates, as they were not strongly buffered. Hydrolysis of phosphocreatine itself increases the acidity, and a strong buffer is needed to minimize the latter change. Glycolysis and other autolytic processes also contribute to the increasing acidity. Even after 100 hours incubation Hammett's figure is 9.88 per cent of the original total creatine converted to creatinine; ours (without change in pH) is 8.13 per cent. The agreement is remarkable.

Probably most, but not all, of the creatine in muscle exists as phosphocreatine. In Table VII are collected data in the literature on total creatine and phosphocreatine in the resting muscles of a number of vertebrate species. The ratio of creatine as phosphocreatine to total creatine appears to vary from 43 to 80 per cent. The true values are probably in the upper part of the range; some hydrolysis of phosphocreatine is unavoidable during removal and analysis of muscle; Riesser and Hansen (24) claim that values of phosphocreatine in muscle obtained by Fiske and Subbarow's method, as most of those in Table VII were, are too low; their method gave values 180 per cent of that of Fiske and Subbarow.

TABLE VII
Creatine and Phosphocreatine in Muscle

Animal	Creatine	Phosphocreatine; as creatine	Ratio of phosphocreatine creatine to total creatine
	mg. per cent	*mg. per cent*	*per cent*
Cat	500 (13)	215–363 (11)	43–73
Dog	380 (15)	170 (15)	45
Frog			70–74 (16)
"			80 (17)
Human	309–485 (18)		
"	400 (19)	211–239 (20)	44–68
Rabbit	419–510 (21)	193–291 (21)	
"		301 (20)	59–72
Rat	287 (9)		
"	450 (10)		
"	460 (22)	211 (22)	44–74
"	480 (4)		
"		164–240 (23)	

The figures in parentheses are bibliographic references.

If we assume, as an average figure, that 60 per cent of the creatine in muscle normally is bound as phosphocreatine, then the creatinine excretion in 24 hours, assuming no enzymatic conversion of creatine or of phosphocreatine to creatinine, is $24 \times 100\ (0.0011 \times 0.6 + 0.00044 \times 0.4) = 2.02$ per cent of the total creatine in the body. This figure is toward the lower end, but within the range of estimates in Table I.

The hydrolysis rate constants used in the foregoing computations were those at pH 7.0. It may be that the normal resting pH of muscle is lower. Dubuisson (25) gives a range of 6.19 to 7.18 in frog muscle. It seems unlikely that the pH is below 6.0, as Rous (26) reported from his observations with indicators. At pH 6.0, assuming 60 per cent of the total creatine as phosphocreatine, 4.3 per cent of the creatine in both forms would be converted spontaneously to creatinine. As this figure is far above any

observed in animals, the normal resting pH of muscle on the average is probably distinctly above pH 6.0. In any event it is clear that the observed rates of creatinine formation from phosphocreatine *in vitro* are sufficient to account for the amounts of creatinine in the urine of normal animals.

The following facts argue against the participation of an enzyme in the immediate process of creatinine formation *in vivo*. The ratio of daily urinary creatinine to total body creatine is nearly the same in different species (Table I). The ratio is close to that observed in autolyzing muscle extracts and to that observed and calculated by means of equation (7) in solutions of phosphocreatine not containing any tissue extract.

The main lines of the formation of creatine in the animal body may be considered as established (27–31). Normally the creatine formed is retained temporarily in the body as phosphocreatine; the end-product of its metabolism is urinary creatinine. The evidence presented above indicates that creatinine is formed directly by a non-enzymatic cleavage of phosphocreatine, presumably wherever the latter substance exists in the tissues, and hence mainly in the skeletal muscle.

This conception of creatine-creatinine metabolism is in accord with nearly all the data in the literature on normal and abnormal creatinine excretion and creatinuria and on the effects of feeding creatine or increased amounts of its physiological precursors in normal and in pathological conditions; it is in accord with the urinary and muscle findings in the different myopathies. Space does not permit presentation of the data and discussion of them here.

A few findings reported in the literature are not in accord. Outstanding among these is the condition in birds. The major fraction of the creatine plus creatinine they excrete is creatine (7, 32). There has been little study of this aspect of the metabolism of birds, and further investigation is needed. There are some features of creatine formation in birds which differ from those in other animals (27).

In some experiments on man after feeding creatine the data reported give more extra creatinine in the urine than could have arisen spontaneously in the time interval if all the creatine had been converted to phosphocreatine, and, of course, much more than could have arisen directly from creatine (33–35). In most short period experiments little or no extra creatinine was excreted after feeding moderate amounts of creatine. An explanation may be found in the experiments recently reported in a note by Fisher (36). When rabbit kidney was perfused with creatine (10 to 30 mg. per cent) and α-phosphoglycerol, large amounts of creatinine appeared in the perfusate. The requirement of α-phosphoglycerol suggests that the creatine was first converted to phosphocreatine. There may be

an enzyme in kidney which accelerates the decomposition of phosphocreatine to creatinine and inorganic phosphate. Lohmann (10) found no evidence of a phosphatase for phosphocreatine in heart or skeletal muscle. It is unlikely that the kidney is the sole or main site of creatinine formation *in vivo*. If it were, extra creatine production or the feeding of creatine would be followed by increased excretion of creatinine, which is rarely the case. Against the hypothesis of kidney as the main site of formation of urinary creatinine also are the observations of Chanutin and Silvette (37) on the rapid accumulation of creatinine in the blood of nephrectomized rats, the rise in blood creatinine in clinical and experimental kidney disease, and the concordance of the observed ratio of urinary creatinine to total body creatine with that calculated by means of equation (7) and the total creatine in the muscles.

Assigning to phosphocreatine the rôle of immediate precursor of urinary creatinine is, of course, only a modification of the view propounded more than 30 years ago by Shaffer (1), Myers (2, 38), and Spriggs (39) that body creatine is the precursor. Our findings are in accord also with the mechanism of the reaction suggested by Lipmann (40).[4]

SUMMARY

1. The following reactions ensue in aqueous solutions of phosphocreatine at 38°; (A) phosphocreatine → creatinine + inorganic phosphate; (B) phosphocreatine → creatine + inorganic phosphate; (C) creatine → creatinine.

2. They are first order reactions and proceed independently of each other.

3. An equation is developed by means of which the rate constants of reactions (A) and (B) can be determined. The rate constant of reaction (C) was determined independently. The values of the rate constants at 38° over the pH range 6.0 to 7.5 are given. Reaction (A) is 3 to 4 times faster than reaction (C).

4. The combined rate constants of reactions (A) and (C) are sufficient, without postulating the intervention of an enzyme, to correlate quantitatively the reported concentrations of phosphocreatine in muscle and the creatinine excretion in all animal species on which data are available, except birds, and the rate of formation of creatinine in autolyzing muscle.

5. The concept of phosphocreatine as the main immediate precursor of

[4] Our work with phosphocreatine was nearly completed and had been put aside at the time the above suggestion was published. We were unaware of it when we referred to the present work in a brief reference (41). We now wish to thank Dr. Lipmann for drawing our attention to his illuminating suggestion.

urinary creatinine does not, as it stands, account for the excretion in birds of much more creatine than creatinine, nor for some few instances in man, in which more creatinine has been reported excreted than can be acccunted for by postulating its prior transformation to phosphocreatine and subsequent non-enzymatic hydrolysis of the latter.

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