XXXIX. THE INFLUENCE OF FLESH-FEEDING ON URINARY CREATININE.

BY DAVID BURNS AND JOHN BOYD ORR (Barbour Scholar).

From the Department of Physiology, Glasgow University.

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PREVIOUS WORK ON THE CONVERSION OF CREATINE TO CREATININE.

In recent years a considerable amount of work has been done to ascertain whether either a conversion of exogenous creatine to creatinine or the converse takes place within the body.

It is generally agreed that creatinine given by the mouth or injected hypodermically is excreted as such to, at least, 80 %, and that there are no indications of its conversion to creatine [Myers and Fine, 1913, 1915; van Hoogenhuyze and Verploegh, 1905].

The question of the conversion of creatine to creatinine however, must still be considered unsettled. Czernecki [1905], Folin [1906], Klercker [cited by Folin, 1906], Lefmann [1908], and Folin and Denis [1914] found no evidence of a conversion. On the other hand, Pekelharing and van Hoogenhuyze [1910], Myers and Fine [1913, 1915], Towles and Voegtlin [1912], and van Hoogenhuyze and Verploegh [1905] found evidence of a conversion, which, however, is small in amount, being in most cases under 5 %, the rest of the creatine being either excreted unchanged or remaining unaccounted for.

Some workers (*e.g.* Levene and Kristeller [1909], McCrudden [1912] and Taylor [1915]) have published results of experiments in which importance is attached to variations in the excretion of creatinine while the subjects of the experiments were upon a flesh-containing diet.

These have either ignored the possible influence of meat-eating on the creatinine excretion, or have assumed, from the work of Folin [1905], that creatinine is of purely endogenous origin, and that its regular excretion is quite unaffected by the ingestion of creatine in any form.

Work done on rabbits is of doubtful value and need not be considered. The daily fluctuations of the excreted creatinine due to the impossibility of obtaining an exact twenty-four hour's secretion of urine, are too great in view of the small amount of conversion which is claimed to be obtained.

Only experiments, in which creatine was given to a subject on a creatine-free diet are worthy of attention.

The most extensive research on this subject is probably that of van Hoogenhuyze and Verploegh [1905], who, in a series of twelve experiments, took each 2 g. of creatine by the mouth, and concluded that creatinine appeared in the urine in small quantity.

An analysis of these results does not seem to bear out this conclusion. One of the six experiments on Verploegh is omitted as it is admittedly in error; and one on van Hoogenhuyze is omitted because a subsequent rise in the creatine excretion, not connected with the experiment, makes comparison impossible.

From 14th September to 3rd October the average creatinine excretion of Verploegh, excluding the days on which the creatine was taken, was 1.917 g. The average of the three creatine days and the following days was 1.905, a decrease of 0.012 g. For van Hoogenhuyze, the figures for the same period were—non-creatine days 1.886 g.; creatine and subsequent days 1.881 g., a decrease of 0.005 g. From the 25th July to 15th February Verploegh's average excretion, excluding creatine days, was 1.996 g. On the two creatine and following days the average was 1.988 g., a decrease of 0.008 g. From 25th January to 9th February van Hoogenhuyze's figures were—non-creatine days 1.886 g.; creatine and subsequent days 1.881 g., a decrease of 0.005 g.

From 25th January to 9th February van Hoogenhuyze's figures were—non-creatine days an average of 1.842 g.; creatine and following days 1.871 g., an increase of 0.029 g.

In view of the amount of creatine taken—2 g.—and of the daily fluctuation in the excretion, the results are not such as would lead to the conclusion that the conversion of creatine to creatinine occurs in the body.

These authors, and the others who conclude that creatinine is a derivative of creatine appear to have been unduly influenced by the work of Gottlieb and Stangassinger [1907, 1908], who believed that they had found in the liver, and to a lesser degree in other organs, an enzyme which converted creatine to creatinine. Valid objections to the acceptance of their results were raised by Mellanby [1908] and by Twort and Mellanby [1913], who showed

that the chemical conditions and presence of bacteria were sufficient to account for the changes observed, and who, under strictly aseptic conditions, were unable to confirm their results. Dakin [1907] could find in the extract of liver, kidney, or duodenal mucous membrane no ferment acting upon creatine. It is interesting that Rothmann [1908] who repeated the work of Gottlieb and Stangassinger, found on one occasion almost twice as much creatinine as could be accounted for by the creatine added.

In any case, the utmost caution should be exercised in drawing far-reaching conclusions from such autolytic experiments, as we do not know that the chemical changes, which occur in an isolated organ in the process of dissolution, are strictly analogous to those that take place in the living body, where the chemical action may be influenced by the activities of other organs of the body.

The influence which an alleged creatinase exerts in converting creatine to creatinine within the body may be disregarded until more certain proofs of its existence are forthcoming.

Though the evidence that creatinine is derived from creatine is wanting, it appears likely that an intimate connection exists between these bodies. This view is indicated by their close resemblance in chemical composition; their mutual connection with the metabolism of muscle [Spriggs, 1907; Shaffer, 1908; Brown and Cathcart, 1909; Pekelharing, 1911]; and by the fact that in the urine of the bird creatine replaces the creatinine found in the urine of mammals [Paton, 1910, 1, 2; Paton and Mackie, 1912].

EXPERIMENTAL.

At the suggestion of Professor Noël Paton, this research was undertaken to ascertain to what extent, if any, the excretion of creatinine is affected by the eating of flesh, and to obtain further evidence of the conversion or non-conversion of ingested creatine to creatinine.

Some of the previous workers used in their experiments flesh, which contains from 3 to 4 % of creatine, while others used pure creatine. In the present series of experiments the influence of flesh and of creatine were investigated separately.

The subjects of the experiment, normal healthy males in the third or fourth decade of life, were put upon a creatine-free diet for several days. Then, on a given day, a weighed amount of flesh or of creatine was added to the diet, after which the creatine-free diet was continued.

Creatine and creatinine were estimated by Folin's colorimetric method, and the total nitrogen by Kjeldahl's method.

The results are recorded below:

A. The influence of Flesh Feeding.

TABLE I.

	Amount of urine cc.	Total nitrogen g.	Creatinine g.	Creatine g.	Remarks
Exp. I.	1840	13.22	1.41	—	Creatine-free diet
D. B.	1690	10.53	1.40	—	Creatine-free diet
	1897	15.13	1.50	0.06	227 g. uncooked meat in diet (i.e. 0.68 g. creatine)
	1300	11.88	1.43	0.01	Creatine-free diet
	1500	11.55	1.41	—	Creatine-free diet
Exp. II.	1510	11.74	1.63	—	
D. B.	1545	12.32	1.61	—	
	1150	13.17	1.65	0.1	114 g. uncooked meat in diet (0.34 g. creatine)
	1495	13.19	1.64	0.018	
	2280	11.91	1.61	0.02	
Exp. III	770	13.1	1.45	0.04	Creatine-free diet
M. C.	645	12.4	1.48	0.167	227 g. meat cooked
	850	12.2	1.40	0.200	Creatine-free diet
	700	11.5	1.35	—	Creatine-free diet
Exp. IV	1150	12.6	1.6	—	Creatine-free diet
N. M.	900	11.7	1.62	—	Creatine-free diet
	1100	12.9	1.86	0.001	227 g. meat (cooked)
	1100	12.9	1.64	0.002	Creatine-free diet
	900	11.7	1.46	—	Creatine-free diet

In the foregoing experiments, while the diet was creatine-free, except on the days when meat was given, it was only approximately fixed in amount and in composition.

In the two following experiments a fixed diet of known composition was maintained. In Exp. V, it consisted of oatmeal, pease-meal, white bread, dried skimmed milk, sugar, butter, and cocoa, and contained, approximately, protein 63 g., fat 80 g., and carbohydrate 320 g.

It was continued for six days. On the seventh day 125 g. of meat were added to the diet. An analysis of 125 g. of the same flesh was made by the method described by Brown and Cathcart [1909]. There was found 0.38 g. of creatine and no creatinine. The meat was eaten raw to avoid the possibility of a conversion to creatinine taking place in the cooking. Observations

made by Noël Paton show this to occur to a small and varying extent¹. In Exp. VI the pease-meal of the diet was replaced by bread. Table II gives results of the urinary analysis.

TABLE II.

	Amount of urine cc.	Total nitrogen g.	Creatinine g.	Creatine g.	Remarks
Exp. V	1017	10.35	1.46	—	Fixed creatine-free diet
J. B. O.	1360	10.46	1.52	—	Fixed creatine-free diet
	1400	10.08	1.44	—	Fixed creatine-free diet
	960	12.52	1.62	0.05	125 g. uncooked meat extra (0.38 g. creatine)
	1184	13.60	1.47	—	Fixed creatine-free diet
	757	10.96	1.32	—	Fixed creatine-free diet
Exp. VI	1000	8.90	1.70	—	Fixed creatine-free diet
J. B. O.	710	9.72	1.77	—	Fixed creatine-free diet
	680	9.99	1.85	—	170 g. uncooked meat added to diet (0.5 g. creatine)
	685	9.61	1.60	—	Creatine-free diet

B. *The ingestion of creatine.*

One experiment on the ingestion of pure creatine was made.

The diet consisted of the same ingredients as in Exp. V, and contained approximately of protein 60 g., fat 79 g. and carbohydrate 315 g. It was continued for ten days. On the third, sixth and ninth days 0.5 g. of creatine was taken dissolved in water. The creatine used was a preparation of Merck's.

(It was analysed and found to contain a trace of creatinine.)

Table III gives the record of the urinary analysis.

¹ We are indebted to Prof. Noël Paton for the following figures which indicate the varying extent to which cooking causes the conversion of creatine to creatinine in flesh.

Material	Total creatinine %	Creatinine %	Creatine %
Salt beef cooked	0.234	0.109	0.125
Horse-flesh cooked at knacker's	0.244	0.047	0.197
Mince cooked at home	0.260	0.096	0.164
Steak cooked in laboratory			
[Raw]	0.346	0.0194	0.3266
Grilled	—	0.0262	0.3198
Stewed	—	0.0210	0.3250
Boiled	—	0.0217	0.3243
Steak	0.315	0.0214	0.2936
Valentine's meat juice	0.696	0.338	0.358

TABLE III.

	Amount of urine cc.	Total nitrogen g.	Creatinine g.	Creatine g.	Remarks
Exp. VII	980	8.74	1.78	—	
J. B. O.	670	8.71	1.8	—	
	640	7.96	1.8	—	
	890	8.68	1.77	0.03	0.5 g. creatine
	760	8.71	1.8	—	
	730	8.71	1.808	—	0.5 g. creatine
	660	8.46	1.8	—	
	790	10.46	1.8	0.2	0.5 g. creatine

DISCUSSION OF RESULTS.

A. *Flesh*. In every instance, there follows on the ingestion of the meat an increased excretion of creatinine, which is confined chiefly to the day on which the meat is eaten. The following table summarises the series, showing the average in the preliminary days of creatine-free diet and the amount excreted on the day on which the meat was taken.

TABLE IV.

Creatinine in grams.

Exp.	Subject	Preliminary period average	Day of meat ingestion	Increase	Amount of meat ingested
I	D. B.	1.405	1.5	0.095	227 g. raw
II	D. B.	1.62	1.65	0.03	114 g. raw
III	M. C.	1.43	1.48	0.05	227 g. cooked
IV	N. M.	1.61	1.86	0.25	227 g. cooked
V	J. B. O.	1.47	1.62	0.15	125 g. raw
VI	J. B. O.	1.735	1.85	0.115	170 g. raw

It is seen that there is no parallelism between the amount of flesh eaten and the increase in the excretion of creatinine. The results show that estimations of the excretion of creatinine, while a subject is upon a flesh-containing diet, are quite devoid of value, even though the amount of flesh in the diet be kept constant.

B. *Creatine*. Of the occasions on which pure creatine was given, the first shows a decrease in the output of creatinine of 0.03 g.; the second a slight increase of 0.008 g., and the third, no change. These small variations are within the limits of the normal fluctuation. This experiment appears to afford no evidence that ingested creatine is converted within the body to creatinine.

Why, then, is there an increased creatinine output after a flesh diet, and

why does not a similar increase take place after the ingestion of pure creatine? There are at least, three possible explanations:

(a) Creatine (or creatinine) may be "protected" when administered in flesh from destruction in the alimentary canal, and so a greater absorption of these bases may take place. Twort and Mellanby [1913, p. 45] have shown that flesh may "protect" added creatine from destruction (*in vitro*) by certain creatine-destroying bacteria of the intestinal canal. They suggest that this bacterial action is the cause of the loss of creatine and the retention of creatine nitrogen when pure creatine is given by the mouth to a subject on a creatine-creatinine-free diet with a moderate or low nitrogen intake. Against this must be put the finding of MacNeal and Chace [1913], viz. that, under normal conditions, the upper portion of the gut is comparatively sterile. Myers and Fine [1915] urge that, in any case, the loss of creatine by bacterial action, would be inappreciable, because of the rapid rate of absorption.

(b) In addition to the preformed extractable creatine, another precursor of creatinine may exist in flesh which after absorption, at a later stage of metabolism, may yield creatinine. One of us [Burns, 1916, 1] has shown that in the developing chick guanidine is formed in muscle, in point of time, before creatine and reaches its highest concentration at the point when creatine appears in detectable amounts. Further, the administration of the salts of guanidine [Burns, 1916, 2] may produce a small increased daily output of creatinine per kilo. body weight. Flesh contains not only creatine (or some bodies which post-mortem give rise immediately to creatine and more slowly to creatinine) but free guanidine, methyl-guanidines and complexes which are readily oxidised to guanidine. It might well be that under different conditions of oxidation, *e.g.* at different stages of metabolism these various catabolites might be produced [Ditman and Welker, 1909].

(c) The increased creatinine excretion after the ingestion of flesh may be due to the specific dynamic action of the various products of flesh catabolism. It is well known that any factor which increases the heat production of an animal increases the creatinine output, *e.g.* fevers [Leathes, 1907] and artificial hyperthermia [Myers and Volovic, 1913].

The increased creatinine output got by Myers and Fine [1915] after the ingestion of pure creatine may possibly be explained as the result of the diuresis. Burns and Orr [1914] pointed out that the production of a diuresis by the ingestion of water markedly and immediately raised the creatinine coefficient. The administration of 1 g. of creatine (in 250 cc. of warm water) to Myers increased the volume of urine from 570 to 910 cc. and produced no increase

in the creatinine or creatine of the urine. The addition of 2 g. of creatine raised the urine volume to 1330 cc. and its creatinine content by 0.03 g. The same amounts of creatine taken by Fine resulted in urinary alterations of water 645 cc. to 1100 and 1205 cc.; of creatinine 1.29 to 1.26 and 1.32 g. (20 and 29 % of the ingested creatine was eliminated as such in this case.)

Burns and Orr [1914] obtained similar increases in the creatinine output on the ingestion of water.

Exp.	Amount of water	Creatinine g.		Creatine g.	
		Before	After	Before	After
III.	3 litres	1.31	1.33	—	—
VII.	„	1.33	1.36	0.01	0.01

SUMMARY.

1. The ingestion of flesh causes an increased excretion of creatinine and generally the appearance of creatine in the urine.
2. The amount of the increase is not proportional to the amount of flesh taken.
3. Although cooking increases, to a variable extent, the amount of creatinine present free in flesh, cooked meat does not necessarily cause a greater increase in urinary creatinine than uncooked flesh.
4. Pure creatine administered per os is only to a slight extent eliminated as such and does not markedly alter the creatinine excretion.

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