XIX. CREATINE EXCRETION IN RUMINANTS.

BY JOHN BOYD ORR (BARBOUR SCHOLAR).

From the Physiology Department, Glasgow University.

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PRELIMINARY.

A CONSIDERABLE amount of evidence has accumulated to show that privation of carbohydrate or interference with its metabolism leads to an excretion of creatine in the urine. Thus Cathcart [1907] observed creatine in the urine of a starving subject, a like observation being made about the same time by Benedict [1907]. Cathcart [1909] later, found that in inanition the output of creatine is unaffected by feeding proteins or fat, but ceases on feeding carbohydrate. Mendel and Rose [1911, 1] caused the creatinuria of starvation in the rabbit to cease by giving dextrose or starch; protein or fat, singly or combined having no effect.

Excretion of creatine has also been shown in pathological conditions, natural or induced, that interfere with the metabolism of carbohydrate, e.g. diabetes mellitus [Krause, 1910], phloridzin poisoning [Taylor and Cathcart, 1910], destruction of carbohydrate in the tissues by injection of sodium selenite [Cathcart and Orr, 1914, 1] and in various conditions affecting the glycogenic function of the liver, e.g. hydrazine poisoning [Macadam, 1913].

Under the condition of privation of carbohydrate or its faulty metabolism, the acetone bodies that appear in the urine give rise to an error in the Folin method of quantitative analysis of creatinine and creatine, the former appearing less and the latter correspondingly greater than the true amounts [Greenwald, 1913; Graham and Poulton, 1914]. In most of the work referred to above this error was not guarded against and the amounts of creatine shown are doubtless exaggerated.

The creatinuria of starvation and of phloridzin poisoning have been re-investigated, the error of analysis due to acetone bodies being eliminated, and the original finding confirmed, though the creatine is less in amount and later

in appearance than was formerly supposed [Cathcart and Orr, 1914, 2]. Further, the results obtained on rabbits by Mendel and Rose [1911, 1] are not affected by acetone bodies, which do not readily occur in rabbit's urine.

In all these researches the question turned upon the increased excretion of creatine in privation of carbohydrates in subjects that normally excreted no creatine or only small quantities. It was thought that further information might be obtained by experimenting in the opposite direction, i.e. giving a high carbohydrate diet to an animal that normally excretes creatine in large quantities and observing whether any quantitative relationship existed between the metabolism of carbohydrate and the creatine excretion. This method has the distinct advantage of getting rid of complications arising from the acetonuria of carbohydrate privation. To this end the following experiments were undertaken on ruminants (goats and sheep).

METHOD.

During each experiment the animal was put in a metabolic cage of such construction that the urine could be collected separately from the faeces. At the close of each experiment it was turned out to graze so that it might be in a normal condition at the commencement of the next period. Urine was collected in twenty-four hour periods and examined immediately at the close of the twenty-four hour period of collection. As no catheterisation was practised there was marked variation in the twenty-four hourly excretions. An approximation to the daily secretion is obtained by giving the average daily excretion of each separate period during which the conditions of experiment were constant. As the urine was examined on 142 days this method of presenting the results tends also to brevity and lucidity. The methods of analysis were: total nitrogen—Kjeldahl; creatine and creatinine—Folin.

I. PRELIMINARY EXPERIMENTS.

Creatine has been found in the urine of cattle [Lindsay, 1911]. The figures however are rather scanty and variable. Through the kindness of Dr Leonard Findlay several ruminants were rendered available and some preliminary work was done to ascertain to what extent creatine is normally excreted in ruminants. Table I gives the results obtained: the figures are the average daily amounts excreted for the periods stated.

TABLE I.

Creatine excretion in ruminants.

(Daily averages.)

Animal	Length of exper.	Tot. N g.	Creatinine g.	Creatine g.	Creatinine Creatine ratio	Diet
He-goat, age 6 mon.	19 days	2.04	0.220	0.198	1.1	Bran, hay, turnips
Sheep, ewe, non-	4 „	2.34	0.424	0.196	2.1	Hay and turnips
lactating {	4 „	?	0.446	0.397	1.1	Hay
Goat A, lactating, {	7 „	7.61	0.620	0.457	1.4	Hay, oats, bran
non-pregnant {	8 „	10.30	0.660	0.336	2.0	Hay, turnips, bran

In goat A, the total nitrogen figures include both milk and urine.

In the creatinine excretion of each animal there appeared a certain degree of uniformity not shown in the case of creatine which varied between 40 and 90 % of the amount of creatinine.

The urine was usually alkaline. To ensure that alkalinity of the urine had no influence in altering the proportion of creatinine and creatine several urines—dogs' and human—were treated with inorganic alkalis. No change was detected in the creatine or creatinine content. On nine days in the course of these experiments the urine was acid to litmus paper without any change in the proportion of creatinine or creatine being observed. The loss of creatinine which occurs in alkaline urines appears to be due not to the alkalinity *per se* but to organisms that flourish in an alkaline medium.

To ascertain the average creatine excretion under conditions as nearly normal as possible of goat B, in milk, that chosen for the following experiments, the animal was taken from grazing, put up in the metabolic cage and fed on turnips, oats and hay for five days. It was then let loose to graze for seven days and put up for a further period of nine days on the diet as before. The averages for fourteen days appear in Table II.

TABLE II.

Normal creatine excretion in goat B (lactating).

(Daily averages.)

Length of exper.	Tot. N urine + milk g.	Creatinine g.	Creatine g.	Creatinine Creatine ratio	Milk cc.	Diet
14 days	5.04	0.85	0.31	2.7	79	Hay, turnips, oats

II. INFLUENCE OF CARBOHYDRATE ON CREATINE EXCRETION.

Experiment I. This experiment was divided into four periods of five days each during which the diet was changed from a high protein and low carbohydrate to a low protein and high carbohydrate diet. The composition of the diets was as detailed below:

Period	Diet	Protein g.	N approx. (prot./6.25) g.	Fat g.	Carbo- hydrate g.
1	"Plasmon," 250 g. Bean meal 75 g. Turnip 200 g.	200	32	1.6	66
2	Potatoes, 1400 g.	14.98	2.4	1.4	224
3	Potatoes, aver. 1800 g.	19.26	3.1	1.8	288
4	Potatoes, 2200 g.	23.54	3.8	2.2	352

The composition of the potato diets is calculated from the average percentage of digestible nutrients given by Hendry [1913], viz. protein 1.07 %, carbohydrate 16.02 %. The figures given by Kellner [1909] vary as follows: protein 0.9 to 1.3 %; carbohydrate 12.5 to 24.6 %.

The animal was milked daily and the total nitrogen of the milk estimated, so that the combined nitrogen excretion of urine and milk might give some indication of the protein utilised in the periods of low protein diet. Table III gives the averages of the five day periods of the twenty consecutive days.

TABLE III.

Influence of carbohydrate on creatine excretion. Goat B (lactating).

(Daily averages.)

Period	Intake		Output					Change in wt. of goat kilos
	N approx. g.	Carbo- hydrate g.	Tot. N urine + milk g.	Creatinine g.	Creatine g.	Creatinine ratio	Milk cc.	
1	32	66	28.11	0.91	1.13	0.8	219	loss 0.05
2	2.4	224	5.04	0.80	0.38	2.1	192	„ 2.2
3	3.1	288	4.08	0.98	0.23	4.2	176	„ 0.3
4	3.8	352	2.90	0.93	none	—	81	gain 0.3

Experiment II. In the foregoing experiment the flow of milk decreased from 219 to 81 cc. per day, so that an amount of material formerly drained off from the economy in the milk remained for consumption in the tissues. To obtain results uncomplicated by variations in milk flow this experiment (II) was delayed until lactation had ceased. The course of events during cessation of lactation is recorded in Experiment III. To eliminate any of the effects of the high protein diet being carried over to the subsequent period the animal

was put upon a natural diet of turnips, hay and oats for an initial period of six days. It was then put upon a high carbohydrate diet of potatoes, which for the first period of three days was less than the animal's requirements. The diet was increased as follows:

Period	Length of Period	Diet	Protein g.	N approx. prot./6·25 g.	Fat g.	Carbo-hydrate g.
1	6 days	Turnips, hay, oats		<i>ad lib.</i>		
2	5 "	Potatoes, 960 g.	10·3	1·6	1	154
3	3 "	" 2000 g.	21·4	3·4	2	320
4	3 "	" <i>ad lib.</i>	?	?	?	?

The results are given in Table IV.

TABLE IV.

Influence of carbohydrate on creatine excretion. Goat B (non-lactating).

(Daily averages.)

Period	Length of period	Intake		Output				Change in wt. of goat kilos
		N approx. g.	Carbo-hydrate g.	Tot. N g.	Creatinine g.	Creatine g.	Creatinine ratio	
1	6 days	?	?	2·30	0·85	0·14	6	loss ·1
2	3 "	1·6	154	3·39	0·90	0·20	4·5	" 1·6
3	3 "	3·4	320	3·14	0·91	0·18	5	" ·1
4	3 "	3·4 +	320 +	2·35	0·92	none	—	" ·3

The low creatine excretion in the initial period of six days is believed to be due to the fact that the animal had just ceased lactation, the last milk having been drawn off three days before the beginning of the experiment.

III. INFLUENCE OF CESSATION OF LACTATION ON CREATINE EXCRETION.

The following experiment intervenes in time between those recorded in Tables III and IV. The diet consisted of hay, oats and turnips. During the period, the animal was twice put to graze in the open, as a result of which the milk flow tended to return. While in the metabolic cage the flow steadily decreased. During the period the goat increased in weight from 32·7 to 35·4 kilos. The results are recorded in Table V.

RESULTS.

I. CREATINE. (1) *Normal Excretion.* In all the animals put under observation there occurs, except on the high carbohydrate diet, an excretion of relatively large quantities of creatine. In the various animals and on the

various diets the amount fluctuates between rather wide limits. No constancy appears in the creatinine/creatinine ratio, which in the preliminary experiments recorded in Table I varies between 2.1 and 1.1.

TABLE V.

Cessation of lactation, goat B.

(Daily averages.)

Length of period	Milk cc.	Tot. N urine + milk g.	Creatinine g.	Creatine g.	Creatinine Creatine ratio	Remarks
5 days	120	6.12	1.01	0.51	2	Milk flow constant
7 "	154		goat at grass			" increasing
9 "	55	3.62	0.77	0.28	2.8	" decreasing
10 "	11		goat at grass			" increasing
7 "	6	5.33	1.02	0.26	3.9	" decreasing
7 "	1	2.82	0.88	0.23	3.8	" "
6 "	nil	2.30	0.85	0.14	6	" ceased

(2) *Influence of Carbohydrates.* The highest creatine excretion—1.13 g. per day—occurs on the diet with the abnormally low carbohydrate content—66 g. per day. In both Tables III and IV as the carbohydrate content of the diet is increased, the creatine excretion decreases. The close relationship that exists is shown in Table VI.

TABLE VI

	Period	1	2	3	4
Table III	{ Carbohydrate in diet, g.	66	224	288	352
	{ Creatine excret., g.	1.13	0.38	0.32	nil
Table IV	{ Carbohydrate in diet, g.	?	154	320	<i>ad lib.</i>
	{ Creatine excret., g.	0.14	0.20	0.18	nil

The decrease is more rapid in Table III. This may be because the effects of the increased carbohydrate ingestion are augmented by the increased retention of material in the blood due to the reduction in the milk flow, which occurred during the course of the experiment.

(3) *Influence of Cessation of Lactation.* Table V shows a decreased creatine output accompanying the decreased milk flow. The two declines do not run parallel and the greatest decrease in the creatine occurs on the six days after the flow had completely ceased. During the period covered by Table V the animal increased in weight by 2.7 kilos.

(4) *Influence of Proteins.* In periods 2, 3 and 4 an increased nitrogen intake of—in g. per day—2.4, 3.1 and 3.8 coincides with a decreasing creatine output of 0.38, 0.23 and nil respectively: a like decreasing creatine output

occurs simultaneously with an increasing nitrogen intake in Table IV. On the other hand, the highest creatine excretion occurs on the highest protein intake, and in general in both Tables III and IV the decreased creatine excretion is accompanied by a decreased total nitrogen excretion.

In Table III on the fifteen days of periods 2, 3 and 4 the total nitrogen excreted amounts to 60.1 g. while the total nitrogen intake is 46.5 g.—a loss of 13.6 g., indicating a catabolism of about 85 g. of tissue protein. There is no indication of this endogenous protein catabolism producing any addition to the excretion of creatine, which during the period decreases to zero.

II. CREATININE. The excretion of creatinine shows remarkable uniformity, compared with the wide fluctuations shown by the excretion of total nitrogen and of creatine. Thus, in Table I, in the two periods of the ewe the amount, in g. per day, is 0.424 and 0.446 and in goat A 0.62 and 0.66. In goat B the highest is 1.02 and the lowest 0.77.

Taking the preliminary period of goat B, Table II, as the normal, we have 0.85 g. per day in an animal of 35 kilos, i.e. 24 mg. per kilo, a ratio somewhat similar to that found in dogs, and in man, suggesting a similar metabolism.

DISCUSSION OF RESULTS.

1. *Production of Creatine.* The fact that in a period of progressively increasing intake of protein of constant composition there occurs a decreasing creatine excretion (Tables III and IV) seems to indicate that the creatine, of that period at least, is not derived from the catabolism of exogenous protein. On the other hand the continuous excretion, in ruminants, of large quantities of creatine on a normal diet, as well as on a creatine-free high protein diet, points to a production of creatine from a source other than the catabolism of endogenous protein.

These results suggest that while quantities of creatine may appear as a consequence of the rapid disintegration of muscle tissue, or of the catabolism of certain exogenous proteins, these are merely irregular and adventitious increments to a constant creatine production in the tissues.

The results provide no means of estimating the amount produced. If, as is suggested below, creatine is utilised, the amount excreted would merely be the surplus of production over utilisation. In the fourteen days' period of goat B, Table II, the daily average on a diet of turnips, oats and hay is 0.31 g. On the lowest carbohydrate diet it is 1.13 g. while on the highest carbohydrate diet the excretion reaches zero. Provided there is no creatine of exogenous

origin, there appears to be the possibility of the production and utilisation of over a gram per day.

On the whole the results would seem to lend support to the view that creatine is produced within the tissues in relatively large amounts, a conclusion arrived at by Benedict and Osterberg [1914], from results obtained on a dog under the influence of phloridzin.

2. *Fate of Creatine.* In so far as the creatine under discussion is being produced within the metabolic field, it is probably in a more favourable position for conversion to creatinine if such takes place, than is creatine given by the mouth or injected subcutaneously. The relatively uniform excretion of creatinine gives no indication of any conversion. If creatinine is derived from creatine the amount converted depends upon some constant metabolic process which is not affected by the amount offered for conversion.

Van Hoogenhuyze and Verploegh [1908] concluded that creatine is usually broken down in the liver. Noël Paton and Mackie [1912] ligatured the portal vein and its radicles in a duck and found no change in the creatine excretion. Towles and Voegtlin [1911] in a dog with an Eck's fistula found no change in the creatine or creatinine excretion. Dakin [1907] and Twort and Mellanby [1913] failed to discover any ferment in the liver capable of acting upon creatine. The influence of the liver in the metabolism of creatine is evidently indirect and possibly solely by virtue of its glycogenic function.

The question as to the fate of creatine cannot be regarded as settled by assuming either its destruction in the liver or its conversion to creatinine.

In the present experiments it has been shown that in an animal normally excreting creatine, the excretion decreases *pari passu* with the addition of carbohydrate to the diet, a result which is the complement of that arrived at by the workers referred to above, viz. the excretion of creatine in carbohydrate privation in an animal normally excreting no creatine. A close relationship therefore appears between the metabolism of carbohydrate and that of creatine. It can be stated at least, that increased carbohydrate ingestion and decreased creatine excretion are in some way causally connected.

Two possible views are considered. (1) Creatine is only produced in the absence of carbohydrate. (2) Creatine or its precursor is constantly being produced but is utilised in the metabolism of carbohydrates.

According to the first view, the excretion of creatine is an indication of the break-down of muscle tissue, and carbohydrate exerts its influence on the excretion by virtue of its presence preventing the necessity for this break-down. In all probability this source supplies at least a portion of the creatine

excreted in starvation, in which the excretion of creatine is in some degree proportional to the break-down of tissue protein [Noël Paton, 1910]. As has been suggested above, however, the quantities of creatine excreted in ruminants cannot wholly be accounted for by the catabolism of endogenous protein, so that this view seemingly fails to cover all the facts as now presented.

The second view—the utilisation of creatine, a conclusion arrived at by Cathcart [1912], from the results of work done in this laboratory, and later suggested by Benedict and Osterberg [1914]—finds support in the fact that on the high carbohydrate and low protein diet, where there is evidence of a break-down of tissue protein and, presumably, the liberation of creatine, there appears in the urine no additional creatine, the excretion of which during the period decreases to zero.

If, as has been suggested, there occurs a constant production of creatine, the decrease in the excretion concomitant with the increased ingestion of carbohydrates points to a utilisation of creatine in the metabolism of carbohydrate.

If this view were correct one would expect to find, that, in cases of excessive consumption of carbohydrate, the creatine in the tissues would be utilised in excess of the production and the stock diminished. Myers and Fine [1913] record a decrease in the percentage of creatine in muscle from 0.520 to 0.482 on feeding on a pure carbohydrate diet. On the other hand, when carbohydrate is not presented for consumption there would be an accumulation and excretion of creatine. Demant [1879] observed an increase in the creatine content of muscle during starvation, an observation confirmed by modern methods by Mendel and Rose [1911, 2], while the excretion of creatine in inanition has been repeatedly observed, *vide supra*.

It may be, that creatine or whatever body represents it in the tissues serves as a connecting link securing the incorporation of the carbohydrate molecule within the protoplasm complex in some glucoside-like combination, in which the catabolism of the carbohydrate moiety occurs.

CONCLUSIONS.

1. In ruminants creatine is normally excreted in relatively large quantities.
2. The amount of creatine excreted is in inverse proportion to the amount of carbohydrate in the diet.
3. Cessation of lactation causes a decrease in the amount of creatine excreted.

4. There is a source of creatine other than the catabolism of protein either exogenous or endogenous.

5. Arguments based on the results are advanced in support of the view, that creatine is formed in relatively large quantities, that it is utilised in the tissues and that its utilisation is intimately connected with the metabolism of carbohydrate.

This paper is an extract from a section of a thesis submitted for the M.D. degree of Glasgow University in May 1914.

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Further research on the excretion of creatine on ruminants was being carried out in the summer of 1914. It is hoped to resume the work as soon as an opportunity is afforded.

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