URINARY EXCRETION OF CREATINE IN THE SHEEP

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Summary

Creatinuria was found in normal rams, wethers, and ewes on ordinary diets. This finding emphasizes the need for caution in interpreting urine creatine concentration or creatine to creatinine ratios used as diagnostic aids in muscular dystrophy in sheep. No change in creatine output per 24 hr was observed when the ewes were fasted for 6 days. Values are given for the ratio creatine clearance to creatinine clearance in rams, wethers, and ewes and for creatine clearance in ewes.

I. Introduction

Two early reports of Lindsay (1912) and Orr (1918) described the presence of creatine in the urine of fed sheep, but Palladin (1924) found it only in the urine of fasting animals. Hunter (1928) explained these results and his own (Hunter 1914) by suggesting that the fed sheep were actually losing condition. Green (1918) also found creatine in the urine of fed sheep.

Creatinuria is found in cases of human muscular dystrophy and Whiting, Willman, and Loosli (1949), Bacigalupo *et al.* (1952), Draper, James, and Johnson (1952), and Hartley (1953) found creatine in the urine of lambs with muscular dystrophy—both naturally occurring cases and cases induced by artificial diets, and it seemed that the presence of creatine in urine may have been of diagnostic value in this condition in lambs. Although some of these authors quoted urine creatine concentrations in lambs which were fed the artificial diets with added *dl*-α-tocopherol and which did not develop muscular dystrophy, none of them quoted values from normal lambs on ordinary diets. In preliminary investigations, using the same method as the earlier workers, the present authors found that the urine of lambs from a few weeks of age onwards and of adult sheep contained a substance reacting like creatine. This method is notoriously subject to error, and the present work, using a method more specific and reliable than those available at the time of these early reports, was undertaken to clarify these differences.

II. Materials and Methods

Aged Corriedale ewes and wethers were fed oaten chaff (diet B, 4 per cent. protein), and two-tooth Corriedale × Merino rams and one aged Dorset ram were given mixed feed consisting of (by weight) 40 per cent. lucerne chaff, 40 per cent. oaten chaff, 10 per cent. bran, and 10 per cent. crushed oats with fine salt added to 0·1 per cent. (diet A, 8 per cent. protein). Water was allowed to all groups freely throughout.

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Ewes were catheterized and the urine collected directly into chilled (crushed ice) receivers, 5 minutes' collection usually being sufficient. Rams and wethers, however, cannot be catheterized directly, and urine from these animals was collected by suffocating them until urine was passed; a portion was then transferred to the ice-bath. The samples were maintained at 0°C until applied to chromatography paper (no longer than 1–2 hr). Blood samples were collected from the jugular vein into heparin.

Urinary creatine was estimated quantitatively by the method of Eden, Harrison, and Linnane (1954), which involves paper chromatographic separation of creatine from interfering related compounds. The optical densities of the final solutions were read on a Beckman model DU spectrophotometer. 10–20 μl (multiples of 5 μl with drying between applications) was a convenient urine volume to use. The solvent (75 per cent. aqueous ethanol) was allowed to run 7–9 hr at room temperature. Each urine sample was checked for the presence of α-naphthol–diacetyl-reacting substances other than creatine by cutting the dried papers into 2-cm strips and dipping these into the reagent as described by Eden, Harrison, and Linnane. Similar R_F values for creatine were obtained. In no case was there any spot other than that corresponding to known creatine. The method was also tested with normal male adult human urine, which is generally considered creatine-free: no creatine was present (five samples). Creatinine gave a slow positive reaction at R_F 0·66 by virtue of its conversion to creatine (verified by alkaline picrate).

Plasma creatine was determined as by Eden, Harrison, and Linnane but with corrections for mono- and unsubstituted guanidines (Setchell, unpublished data 1960).

Plasma and urinary creatinine concentrations were obtained by the alkaline picrate method as modified by Hare (1950) with the use of Lloyd's reagent.

III. Results

Creatine was present in considerable amounts in the urine of all the groups (Table 1), and in the wethers only was the output of creatine less than that of creatinine. The higher concentration of creatine in the urine of the ewes after 6 days of fasting is due to diminished urine volume: the creatine excretion per day in this group was similar before and after fasting. The creatine to creatinine renal clearance ratios indicate that about 70 per cent. of creatine passing the glomerulus was resorbed by the tubules, assuming creatinine clearance is a measure of glomerular filtration rate in the sheep (Shannon 1937; Schmidt-Nielsen *et al.* 1958).

IV. Discussion

The present findings of creatinuria in sheep on a normal diet confirms those of Lindsay (1912) and Orr (1918) and contradict those of Palladin (1924). In any case, the agreement in Palladin's paper of the creatinine values before and after autoclaving (the difference assumed to be due to creatine) is so good as to be almost incredible. The values for the ratio of urinary creatine to creatinine are even higher with the present method than older values, calculated from the literature and summarized in Table 2, for sheep and some other ruminants.

TABLE 1

URINE AND PLASMA CREATINE AND CREATININE CONCENTRATIONS AND CLEARANCES IN THE SHEEP

Values given are means and standard errors of the means

No. of Sheep and Diet	(C) Creatine Concn. in Urine (μg/ml)	Amount of Creatine Excreted in 24 Hr (g)	Creatine Concn. in Plasma (μg/ml)	(D) Creatine Clearance (ml/min)	(E) Creatinine Concn. in Urine (μg/ml)	Amount of Creatinine Excreted in 24 Hr (g)	Creatinine Concn. in Plasma (μg/ml)	(F) Creatinine Clearance (ml/min)	Ratio D/F	Ratio C/E
5 rams, diet A	1060±330	—	26·0±2·1	—	678±200	—	6·4±0·7	—	0·39±0·12	1·56±0·15
5 wethers, diet B	660±340	—	24·2±0·9	—	1220±340	—	7·6±0·2	—	0·22±0·03	0·72±0·12
4 ewes, diet B	620±140	1·10±0·21	26·2±1·1	32·5±8·9	560±60	0·99±0·08	6·8±0·3	109·9±9·7	0·29±0·06	1·2 ±0·3
4 ewes, fasted 6 days	2450±290	1·16±0·14	30·1±0·2	29·3±1·2	1775±260	0·84±0·13	7·3±0·4	82·9±11·0	0·36±0·03	1·5 ±0·1

The occurrence of creatine in normal sheep urine may be simply a reflection of the higher plasma creatine concentration in sheep than in man: 24–30 μg/ml compared with 6·6 and 8·1 μg/ml for two samples of pooled human plasma by the present method and less than 6 μg/ml for human males by autoclaving and alkaline picrate (Tierney and Peters 1943).

That creatinuria is a normal occurrence for sheep emphasizes the need for caution in interpreting urine creatine concentrations or creatine to creatinine ratios used as diagnostic aids in muscular dystrophy.

TABLE 2

URINARY CREATINE TO CREATININE RATIOS FOR SOME RUMINANTS

Ratios quoted are means and standard errors of the means

Animal	Sex	Fed or Fasted	Creatine/ Creatinine Ratio	No. of Obser- vations	Reference
Sheep	?	Fed	0·88±0·16	4	Lindsay (1912)
	Female	Fed	0·70±0·22	2	Orr (1918)
	Female	Fasted	0·80±0·13	13	Hunter (1914)
	Wether	Fed	0·58±0·08	15	Green (1918)
	Wether	Fed	0·0 ±0·0	11	Palladin (1924)
	Wether	Fasted up to 6 days	Up to 1·87	25	Palladin (1924)
Goat	Male	Fed	0·91	1	Orr (1918)
	Female	Fed	0·61±0·11	2	Orr (1918)
Cattle	Male	Fed	0·55±0·29	4	Lindsay (1912)
	Female	Fed	0·90±0·32	4	Lindsay (1912)
	?	Fed	0·69±0·04	30	Nagy (1935)
(Calves)	?	Fed	0·72±0·12	12	Hart *et al.* (1911)
Camel	Female	Fed	0·45±0·14	5	Smith and Silvette (1928)
Dromedary	Male	Fed	0·32±0·06	2	Smith and Silvette (1928)
Llama	Male	Fed	0·37±0·20	2	Smith and Silvette (1928)
Alpaca	Male	Fed	0·68	1	Smith and Silvette (1928)

Our results also differ from those of Palladin (1924) in the effect of fasting on the creatine excretion, and this may possibly be due to body condition, animals in good condition probably drawing on fat reserves before protein.

The urinary creatine to creatinine ratio in the wethers was significantly less than that for the ewe and ram groups ($P<0·01$). This may be related to the observations of Wilkins and Fleischmann (1945), who found that methyltestosterone enhances creatine synthesis in the human in some way unrelated to its role as a methyl donor, even though these workers were unable to demonstrate methyltestosterone-induced creatinuria in laboratory animals and in pigs.

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VI. References

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