XCVI. STUDIES IN CREATININE AND URIC ACID METABOLISM.

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INTRODUCTION.

IN regard to the origin of urinary creatinine, the theory most generally accepted at present is that creatinine arises from creatine, but it is only the recent work of Benedict and Osterberg [1923] which has provided definite evidence in favour of this view and their work still requires confirmation.

Folin still maintains the essential independence of the two and Mellanby holds the view that creatinine is formed in the liver from substances brought to it by the blood stream. On the whole, the evidence does not enable one to come to any definite and final conclusion in regard to the origin of urinary creatinine.

The idea underlying the following work was that creatinine might arise from some analogous ring compound. Mellanby [1908] writes: "From a chemical consideration it is more probable that the ring formation of creatinine comes direct from a tissue breakdown and that this ring is then hydrated to a creatine chain by muscle rather than that the creatine chain is dehydrated to form the creatinine ring." Creatinine may be regarded as a derivative of glyoxaline or iminazole.

The iminazole ring points to relations with other iminazole derivatives such as histidine and the purines. The results below show the effects on urinary creatinine of ingestion of uric acid in man and in the dog.

EXPERIMENTAL METHODS.

(a) The subjects of the experiments were young men, students and laboratory workers. During the period of experiment the diet of each individual was as free from purine and creatine as possible. No meat was eaten and on the whole no animal food except eggs, milk and occasionally fish. Besides this the diet consisted of potatoes, cabbage, peas, etc. and bread and butter. There was no restriction in regard to drinking tea or coffee as these beverages contain

too small an amount of purine (caffeine) to affect the excretion of uric acid to any appreciable extent.

The urine was collected for exact 24-hour periods. A few drops of chloroform in each collecting bottle acted as a preservative. The periods ended at 9 a.m. and the creatinine and uric acid were estimated as soon as possible afterwards.

Creatinine was determined by means of Folin's colorimetric method. The picric acid used was purified by crystallisation from benzene as described by Benedict [1922]. The colorimeter was placed in a specially constructed dark box and the source of light, a daylight lamp, ensured a constant illumination.

Uric acid was estimated by Cole's modification of the Hopkins method [1926].

(b) In the experiments on a dog, the animal was kept in a metabolism cage and fed on dog biscuits, porridge and milk. The urine for 48-hour periods was collected in bottles containing a few cc. of toluene.

At the end of each 2-day period the sides and funnel-shaped bottom of the cage were washed with water, the washings added to the urine and the whole made up to 2 litres. The clear filtrate from 200–300 cc. of the diluted urine was used for the estimation of creatinine and allantoin.

Allantoin was estimated by the Wiechowski method as described by Hunter and Givens [1911].

INGESTION OF URIC ACID IN MAN.

As soon as the daily elimination of uric acid and creatinine had reached a fairly steady level, 2–5 g. uric acid suspended in water were taken by mouth in two or three doses during the course of the day. An increased output of uric acid over the previous steady level indicated that absorption of uric acid had taken place. This increased excretion of uric acid after ingestion did not always take place and this effect varied from individual to individual and also in the same individual at various times.

In every case, however, in which the uric acid had been absorbed a large increase in creatinine excretion resulted.

Subject A. R. (Fig. 1) took 3 g. of uric acid on the 7th day. On the following day the excretion of uric acid rose from an average value for the preceding 7 days of 0.38 g. to 0.634 g., an increase of 0.254 g. The creatinine excretion for the same day rose to 2.49 g., an increase of 0.63 g.

A second dose of 3 g. was taken on the 14th day and in this case the uric acid seemed to have been absorbed more rapidly as there was an increase both in the uric acid and creatinine outputs for that day.

A similar result was obtained with G. L. B. (Fig. 2). This subject showed rather a peculiar fall in creatinine excretion on Sundays. (This low value was always present on this day. 1st and 8th periods, Fig. 2.) A subsequent

experiment on the same subject showed exactly similar low outputs of creatinine on the 1st and 8th periods.

Fig. 1. Subject A. R.

Fig. 2. Subject G. L. B.

Two experiments, one on G. L. B. (Fig. 2) and one on R. W. (Fig. 3), were run concurrently and show rather strikingly the difference whether uric acid is absorbed or not. In the former case after taking 4 g. uric acid the amount

excreted in the urine rose from an average value of 0.55 g. in the fore-period to 0.72 g. and the creatinine increased from 1.80 to 2.25 g. In the second case no change occurred in the uric acid and the creatinine also remained at the previous steady level. On taking a second dose of 4 g. some days afterwards the same result was obtained. Two experiments on subject A. R. also illustrate this point. The first dose of uric acid (3 g. on the 5th day) caused a rise both in the creatinine and uric acid, while the second dose (4 g. on the 14th day) caused no change in either.

Fig. 3. Subject R. W.

Similar though less well-marked results were obtained in the case of subject H. Z.

Table I summarises some of the results obtained.

Table I.

Subject	Amount of uric acid ingested (g.)	Increase in uric acid (g.)	Increase in creatinine (g.)
A. R.	3	0.23	0.63
A. R.	3	0.16	0.35
A. R.	4	—	—
G. L. B.	4	0.17	0.42
R. W.	4	—	—
H. Z.	4	—	—
H. Z.	2	0.06	0.18

From the above results we may thus conclude that ingestion of uric acid when followed by absorption causes in all cases an increase in the excretion of creatinine.

An explanation of the increased creatinine excretion after uric acid ingestion may be found in a consideration of the different results obtained when purine-rich material is given and when uric acid is ingested as such. In the latter case an increase in creatinine is obtained, while with purine-rich food no such effect is shown. Both after thymus and pancreas ingestion the uric acid output rose while the creatinine remained at its previous steady level. Taylor and Rose [1913] obtained the same result on feeding sweetbreads: the increase in uric acid was not accompanied by an increase in creatinine excretion.

The ingestion of uric acid as such may cause its appearance in the blood in a much more insoluble and less easily excreted form than when it appears as the result of purine-rich food. The kidney normally gets rid of uric acid less readily than creatinine or urea and transformation of this less easily excreted form of uric acid to creatinine and urea may be an additional means by which the body gets rid of the excess of uric acid. When purine-rich food is given, the uric acid may appear in blood in the normal more easily excreted form and hence there is no need for a transformation to creatinine.

The above interpretation although not improbable can by no means be regarded as conclusive until we know more about the form in which uric acid is present in blood.

INGESTION OF URIC ACID IN THE DOG.

On reference to Table II it will be seen that in the dog the amount of creatinine excreted daily is less than the daily allantoin excretion. In the human subject we find just the reverse relation between the amounts of the two endogenous catabolites in the urine.

Fig. 4. Subject dog I.

The uric acid, 5 g. on the 5th day and 10 g. on the 10th day, was given mixed with milk and porridge. The average 48-hour period allantoin output for this dog was 1.23 g. The first dose of uric acid caused an increased excretion of allantoin of 1.35 g. and the second an increase of 1.88 g. (see Fig. 4). In both cases there was a secondary rise in the allantoin excretion a day or two after the first rise—0.41 g. and 0.97 g. respectively. So that 5 g. uric acid caused in all an increased elimination of 1.76 g. allantoin and 10 g. gave rise to an increased output of 2.85 g., *i.e.* from 30 % to 35 % of the uric acid ingested appeared as allantoin. Only traces of uric acid were found in the urine. Throughout this experiment the creatinine output remained fairly constant. This is in marked contrast to the effect on creatinine excretion of uric acid

ingestion in man. Now, the dog's organism contains the enzyme uricase and thus possesses an excellent means whereby thoroughly and rapidly to get rid of any uric acid which may be introduced. In the dog therefore there is this natural path for the speedy removal of uric acid, whereas this means of dealing with uric acid is lacking in the human organism which, if the theory put forward above is correct, is forced to a certain extent to turn the less easily excreted uric acid into the more easily excreted creatinine and urea.

ALLANTOIN-CREATININE RATIO IN VARIOUS ANIMALS.

The animals were all kept on a diet free from creatinine and purine. In the case of the rats and guinea-pigs, four animals were kept in a small metabolism cage for 6 days, at the end of which period 200–400 cc. urine had been collected. The dogs and rabbits and cat were kept in a large metabolism cage for from 2–4 days according to the size of the animal and amount of urine excreted. In each case the urine was made up to 1 or 2 litres with water according to amount of urine collected. 200 cc. were filtered and the filtrate used for determinations.

Table II.

Animal	Weight (kg.)	Allantoin (mg.)	Creatinine		Allantoin Total creatinine
			Total (mg.)	Preformed (mg.)	
Rat I	0.25	20	4.6	2.6	4.35
Guinea-pig	0.31	76	17.5	9	4.34
Dog IV	1.6	273	59	39	4.63
Rabbit	2	221	—	86	2.57
Dog II	6.3	286	—	111	2.58
Dog I	8.5	615	—	261	2.35
Dog III	18.5	1278	—	538	2.37
Cat	1.9	209	—	51	4.01
Coyote	7	870	—	270	3.2

The figures for the coyote are taken from Hunter and Given's paper [1911]. In this case the creatinine and allantoin were determined when the animal was on a meat diet. It is interesting to find that in this animal also the amount of allantoin excreted is more than the amount of creatinine. Total creatinine determinations (creatine + creatinine) were carried out on the urines of rats, guinea-pigs and dog IV. The amount of creatine in the urine of the other animals was small.

From Table II it will be seen that the smaller animals all show a ratio of allantoin to total creatinine of about 4.4 to 1, while the ratios of allantoin to preformed creatinine in all the other animals except the cat are in the neighbourhood of 2.4 to 1. The rats and guinea-pigs show ratios which are nearly the same. The weight of dog III was a little more than double that of dog I, yet the allantoin-creatinine ratios are practically identical.

RESULTS AND DISCUSSION.

Before proceeding to a discussion of the results of ingestion of uric acid some interesting points in regard to the normal daily outputs of creatinine and uric acid must be dealt with.

It will be noticed (see figures) that the daily creatinine excretion, although more constant on the whole than the daily uric acid excretion, shows marked variations. This was found to be the case in each of the four subjects of the experiments. There were individual differences in regard to the extent of these variations, subject R. W. showing a more constant daily creatinine output than the other three, but taking into account the whole series of experiments it is evident that the creatinine excretion from day to day is not so remarkably constant as had been supposed. It is thus analogous to the daily endogenous uric acid elimination which was also found to be not so constant in the same individual as was first put forward by Burian and Schur [1905]. Cathcart comes to the same conclusion [1912].

The quantity and kind of purine-free food is of importance in determining the endogenous uric acid output and a similar relation may hold in the case of creatine- and creatinine-free food and the elimination of endogenous creatinine.

Folin's figures [1905] illustrate the same point: the daily variations in the excretion of creatinine are well marked.

A further point of interest is that these daily variations in the creatinine follow fairly closely the variations in the excretion of uric acid. This was the case in the majority of urines analysed. Leathes [1907] studied the hourly output of uric acid and found that on a purine-free diet the excretion of uric acid is most active in the early waking hours and much less active at night, in spite of the fact that the kidneys are more active during sleep than during waking hours of the day, and, even on a low nitrogen diet, are almost at maximum activity at night. The hourly creatinine output shows variations similar to the hourly excretion of uric acid which are of interest in connection with results discussed above. Leathes concludes from his experiments that the output of uric acid and creatinine during the night must be the expression of a diminished functional activity in some other organ or organs than the kidney.

The results obtained all point to the fact that creatinine and uric acid are correlated in some way. It is not improbable that nuclein (*i.e.* histidine-containing protein plus nucleic acid) is a common precursor of creatinine and uric acid.

On the other hand, if we assume that creatinine does arise from creatine, the above results may be explained if we accept Burian's theory of the origin of uric acid [Burian and Schur, 1905]. According to Burian only a small part of the endogenous uric acid is derived from nucleoprotein of cellular origin. He looks upon the hypoxanthine of muscle as the most important source

of uric acid. Hypoxanthine is formed in muscle principally during stimulation, and conversion of hypoxanthine to uric acid goes on continually both while muscle is at rest and during stimulation. Furthermore, Burian states that hypoxanthine formation is analogous to creatine formation in muscle: "Nach meiner Auffassung entstammen das Hypoxanthin und das Kreatin ein und demselben Grundprozesse." Thus if creatinine arises from creatine and uric acid from hypoxanthine we have an explanation of the correlation in the excretions of uric acid and creatinine.

From a more general point of view, this correlation is probably simply an expression of the fact that they are both end-products of endogenous metabolisms.

SUMMARY.

1. The daily excretion of creatinine was found to undergo marked variations and is not so constant as was supposed. The daily variations in the creatinine and uric acid excretions on a diet free from creatine-creatinine and purine followed one another closely.

2. The result of uric acid ingestion in man was that an increase in creatinine excretion was obtained whenever absorption of uric acid had occurred. This probably points to a transformation of the iminazole nucleus in uric acid to creatinine.

3. Ingestion of nuclear-rich material (thymus and pancreas) caused an increase in the uric acid output but did not affect the creatinine.

4. 5-10 g. of uric acid given to a dog *per os* caused a large increase in the allantoin excretion, the creatinine however remaining at the previous fairly steady level.

5. In man the ratio of uric acid to creatinine is about 1 to 4. On the other hand, in dogs the ratio of allantoin to creatinine is about 2.4 to 1. Ratios similar to the latter were found in other animals (cat, rat, guinea-pig, rabbit).

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