XL. STUDIES IN CREATININE AND URIC ACID METABOLISM.

II. INGESTION OF PROTEINS AND AMINO-ACIDS AND THE HOURLY ELIMINATION OF CREATININE AND URIC ACID.

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It is almost universally accepted that urinary creatinine is independent of the amount of protein ingested but that protein ingestion exerts a marked influence on the excretion of uric acid. No attempt has yet been made to explain the marked difference shown by these two endogenous catabolites.

According to Mares [1888] the increased uric acid excretion on protein ingestion is due to the nuclear disintegration occasioned by the work of digestion. Another theory ascribes the increase to stimulation of purine synthesis. Graham and Poulton [1913] suggest that part of the endogenous uric acid arises by synthesis from protein and carbohydrate and part from the metabolism of cell nuclei. The latter, they state, "represents the general wear and tear in the tissues and is not affected by the diet given."

The work of Lewis *et al.* [1918, 1923] and Gibson and Doisy [1923] indicates that the influence exerted by protein is due to a general stimulation of cellular metabolism by the amino-acids. They have also suggested that the effect of amino-acids is a parallel phenomenon to their specific dynamic action.

There seems to be no reason, however, why there should not be an increase in creatinine excretion as well as in the uric acid excretion if protein or amino-acid ingestion causes a general stimulation of the cellular metabolism. The work reported in this paper was initially undertaken with the intention of investigating the effect of amino-acids on both the creatinine and uric acid excretions and thus to repeat the experiments of Lewis and Doisy as their results seemed rather remarkable when looked at from the point of view indicated above in regard to a possible effect on creatinine elimination. The first experiments with amino-acids showed results so at variance with the results of the American workers that it was thought advisable to repeat also the experiments on the influence of protein ingestion.

It was shown by Mares and others that during the increased nitrogen excretion which follows a meal the increase in uric acid has a briefer duration than the increase in urea and occurs characteristically in the earlier hours of the hyperexcretory period, and further, that while the urea is maintained above the level of the abstinence period, the uric acid falls to its abstinence value or even below this. The urea excretion may be regarded as the index of amino-acid assimilation and consequently on the basis of the general stimulation theory one should not expect such a large discrepancy in the time relations of the excretions of urea and uric acid.

Various observers have recorded no effect on the hourly elimination of uric acid as the result of protein feeding [cf. Hammarsten, 1922].

Siven [1901] found that extractives of meat caused an increase in uric acid excretion while protein had no influence. Part of the excreted uric acid comes from the ingested purine bases which are directly oxidised to uric acid, and part originates from the body. Burian and Schur [1900], Kaufmann and Mohr [1902], Pfeil [1903] and others found that purine-free food had no influence on the excretion of uric acid.

Hopkins and Hope [1898] found that after ingestion of 410 g. of egg-white and one pint of milk some increase in uric acid was obtained, but it was very small compared with that after a meat diet of approximately equal nitrogen value.

In passing, it may be mentioned that the results of Hopkins and Hope have been quoted in several text-books of Physiological Chemistry in such a way as to create a totally erroneous impression. It is stated that after a meal of bread and potatoes a marked increase in the hourly uric acid excretion is obtained, whereas an examination of this experiment in the paper of Hopkins and Hope shows that this effect was obtained on ingesting 200 to 300 g. of meat and small quantities of bread and potatoes.

EXPERIMENTAL.

The subjects of these experiments were normal young men. For three or four days before the experimental day the diet was free from creatine, creatinine and purines. After the evening meal on the day before the experiment no food was taken until the end of the experiment except for the amino-acid or protein whose influence was being investigated. As a basis for comparison experiments in which no foodstuff was ingested were made and taken as fasting normals. 200 cc. of water were drunk every hour to ensure complete emptying of the bladder, and the creatinine and uric acid were estimated directly after collection.

Creatinine was determined by the Folin colorimetric method and uric acid by the colorimetric method of Benedict and Franke [1922]. For the uric acid estimation the urine was in every case so diluted that the reading for the "unknown" was always between 14 and 26; in the majority of cases it was between 17 and 23, the standard being set at 20 mm. Identical results were obtained for all subjects and except for the experiment with egg-white all the

results below are those for subject *A* who found great difficulty in ingesting a sufficient amount of egg-white.

RESULTS AND DISCUSSION.

The creatinine excretion was not affected by either protein or amino-acid ingestion, which is in agreement with the well-established fact that urinary creatinine is independent of the amount of protein in the diet. We can thus confine ourselves almost entirely to an examination and discussion of the uric acid figures, which are quite contrary to fairly widely accepted conclusions.

Examination of Tables III and IV shows very definitely that the ingestion of 10 or 20 g. of glycine or alanine had no effect on the hourly level of uric acid elimination, the glycine and alanine curves being almost identical with the fasting normal. The normal fasting uric acid level shows a decline during the afternoon hours, which is in accordance with the findings of numerous observers.

Table I. *Fasting normal. Subject A. Wt. 53.6 kg.*

Time	Vol. of urine	Creatinine	Uric acid	Remarks
Nov. 14th				
8-9 a.m.	73 cc.	69.7 mg.	19.7 mg.	200 cc. water every hour (and in all experiments)
9-10	396	68.0	18.6	
10-11	576	65.5	21.4	
11-12	352	61.2	16.5	
12-1 p.m.	170	62.1	16.2	
1-2	158	66.8	14.7	
2-3	246	68.0	14.8	
3-4	180	69.0	12.0	
4-5	228	66.1	12.0	
Nov. 29th				
8-9 a.m.	35 cc.	70.1 mg.	17.0 mg.	
9-10	268	69.0	17.6	
10-11	184	68.9	16.1	
11-12	188	73.7	16.6	
12-1 p.m.	152	72.6	16.9	
1-2	212	69.7	17.3	
2-3	254	67.8	17.2	
3-4	240	69.0	16.8	

Table II. *Nov. 16th. Ingestion of glycine. Subject A.*

Time	Vol. of urine	Creatinine	Uric acid	Remarks
8-9 a.m.	132 cc.	73.8 mg.	19.2 mg.	
9-10	438	72.0	19.1	
10-11	142	73.1	18.9	10 g. glycine at 10 a.m.
11-12	465	76.4	18.2	
12-1 p.m.	265	69.4	18.2	
1-2	75	71.6	16.0	
2-3	196	69.1	14.7	
3-4	200	74.0	14.4	

Table III. *Nov. 25th. Ingestion of alanine. Subject A.*

Time	Vol. of urine	Creatinine	Uric acid	Remarks
8-9 a.m.	32 cc.	75.7 mg.	18.5 mg.	
9-10	49	72.5	18.8	
10-11	79	75.4	17.2	20 g. alanine at 10 a.m.
11-12	240	74.9	16.7	
12-1 p.m.	422	78.0	15.3	
1-2	112	76.9	12.1	
2-3	138	75.5	13.0	
3-4	172	70.0	13.1	

Table IV. *Nov. 1st. Fasting normal. Subject B. Wt. 54.3 kg.*

Time	Vol. of urine	Creatinine	Uric acid	Remarks
7- 8 a.m.	58 cc.	68.7 mg.	18.6 mg.	200 cc. water every hour
8- 9	155	73.4	20.0	
9-10	244	67.0	21.3	
10-11	82	66.0	20.0	
11-12	235	68.8	18.7	
12- 1 p.m.	145	70.4	18.6	
1- 2	170	70.0	18.6	
2- 3	186	70.6	18.0	

Table V. *Nov. 8th. Ingestion of egg-white. Subject B.*

Time	Vol. of urine	Creatinine	Uric acid	Remarks
7- 8 a.m.	24 cc.	68.8 mg.	15.3 mg.	
8- 9	27	67.1	20.0	
9-10	137	72.4	18.7	
10-11	32	65.8	18.3	250 g. boiled egg-white =4.3 g. N.
11-12	32	69.6	21.6	
12- 1 p.m.	40	74.0	20.8	
1- 2	116	70.9	16.0	
2- 3	196	74.8	16.0	
3- 4	348	70.0	15.3	
4- 5	68	65.0	14.7	

Table VI. *Nov. 22nd. Ingestion of cheese. Subject A.*

Time	Vol. of urine	Creatinine	Uric acid	Urea	Remarks
8- 9 a.m.	22 cc.	68.5 mg.	16.3 mg.		
9-10	144	68.2	18.0	—	
10-11	178	72.7	16.8	—	200 g. Cheddar cheese, 10-10.20=7.52 g. N.
11-12	220	73.2	16.6	1.10 g.	
12- 1 p.m.	66	72.7	15.5	1.06	
1- 2	118	77.6	18.4	1.53	
2- 3	220	72.3	17.6	1.76	
3- 4	378	72.2	15.9	1.13	
4- 5	158	74.2	17.0	1.58	
5- 6	124	67.6	12.4	1.00	

Table VII. *Nov. 18th. Ingestion of meat. Subject A. Wt. 53.6 kg.*

Time	Vol. of urine	Creatinine	Uric acid	Remarks
8- 9 a.m.	37 cc.	73.0 mg.	18.5 mg.	
9-10	112	69.1	19.7	
10-11	190	73.0	20.1	165 g. fried beef- steak, 35 g. white bread = } 10- 4.85 g. N. } 10.20
11-12	270	84.0	27.5	
12- 1 p.m.	150	88.8	34.9	
1- 2	226	86.9	40.0	
2- 3	240	80.0	31.3	
3- 4	216	77.0	22.7	
4- 5	258	76.4	20.1	
5- 6	204	72.7	17.0	
6- 7	226	70.3	19.7	

The ingestion of 200 g. of Cheddar cheese (Table VI) or of 250 g. boiled egg-white (Table V) had also no influence on the uric acid excretion. The increased excretion of urea (Table VI) indicates that assimilation of large amounts of amino-acids had occurred and reached its maximum $3\frac{1}{2}$ hours after ingestion.

After meat ingestion, however, a large increase occurred both in the creatinine and uric acid excretions (Table VII). The uric acid rose from 19 mg.

to 40 mg. and the creatinine from 72 to 80 mg. This result is in marked contrast to that obtained on ingesting large amounts of purine-free protein, and is in essential agreement with the results of Hopkins and Hope [1898], Burns and Orr [1916] and numerous other investigators.

We cannot escape from the conclusion that a purine-free diet has, under the conditions of these experiments, no influence on the hourly elimination of uric acid or creatinine, and that the increase after the ingestion of meat can be ascribed to the presence of creatinine and uric acid or their immediate precursors in the ingesta.

The above conclusions in regard to the effect of a purine-free diet taken in conjunction with the fact that assimilation of a large amount of amino-acids takes place on ingestion of purine-free protein seems definitely to dispose of any theory of general cellular stimulation by amino-acids, and of Mares's theory that an increase in uric acid excretion after protein ingestion can be due to the increased work of digestion, and consequently disposes also of the necessity for formulating any theory in regard to the effect of purine-free protein on the excretion of uric acid.

The absence of any influence on uric acid and creatinine of a purine-free protein diet is in line with the results obtained after ingestion of amino-acids.

We thus come to the general conclusion that the elimination of both uric acid and creatinine is independent of the amount of purine- and creatine-creatinine-free protein in the diet. It was shown in a previous paper [1926] that the daily variations in urinary creatinine and uric acid on such a diet follow one another closely. The results in this paper indicate that these variations are not due to variations in the amount of protein ingested.

On the whole the results, which indicate a further general correlation between uric acid and creatinine, are more in accordance with our conception of endogenous metabolism than the results hitherto published, which show such an unaccountable discrepancy in the reactions of these two endogenous catabolites to the nature of the diet.

Amongst others, Höst [1919] has shown that the excretion of uric acid varies within limits with increase or decrease in the calorific value of the food. Some of Rose's [1921] experiments also indicate that the calorie content of the food is the important factor.

In cases of starvation or low carbohydrate diets or diabetes the amounts of both creatinine and uric acid in the urine decrease, so that the excretion of creatinine seems to be subject to the same influences as the excretion of uric acid. One of the factors in causing variations in uric acid and creatinine excretions may thus be the effect of differences in the calorie value of the diet on tissue metabolism which is of course quite a different conception from that of specific dynamic action.

SUMMARY.

1. Ingestion of glycine or alanine is without effect on the hourly output of uric acid or creatinine.
2. A purine-free protein meal (egg-white and cheese) is similarly without effect on the hourly excretion of uric acid or creatinine.
3. Ingestion of a meal rich in uric acid or creatinine precursors causes a large increase in the excretion of these two catabolites in the urine.
4. Both urinary uric acid and creatinine are thus independent of the creatine-creatinine- and purine-free protein content of a diet.
5. It is suggested that the calorific value of the diet may be an important factor affecting both uric acid and creatinine metabolism.

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