PURINE METABOLISM.

II. THE EFFECT OF THE INGESTION OF GLYCINE ON THE EXCRETION OF ENDOGENOUS URIC ACID.

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It has been generally accepted that the ingestion of high protein diets leads to an increased excretion of endogenous uric acid. In his review on purine metabolism, Rose (1) has summarized the important contributions on this phase of the subject and has also discussed the various theories which have been formulated to explain the observed phenomena. Lewis, Dunn, and Doisy (2) have made a careful study of the hourly elimination of uric acid by fasting individuals following the ingestion of proteins and amino acids. Definite increases in uric acid excretion were observed in all cases with a maximum effect 3 to 4 hours after the ingestion of protein and 2 to 3 hours after the ingestion of amino acids. The increased uric acid elimination was considered by these authors to be due to an increased production of uric acid, as a result of the stimulating action of the absorbed amino acids. The authors have cited the specific dynamic action of proteins and protein derivatives as a parallel phenomenon. In the experiments just cited the hourly elimination of creatinine was practically constant. Zwarenstein (3) was of the opinion that if there was a general increase in cellular metabolism, there should also be an increase in creatinine elimination coincident with the increase in uric acid. Zwarenstein, therefore, repeated the work of Lewis, Dunn, and Doisy. His results indicated that the hourly excretion of uric acid by fasting individuals was not affected by the ingestion of proteins and amino acids.

Inasmuch as these results are at variance with all preceding work since 1905, it was thought advisable to make a study of the ex-

perimental procedures employed by Zwarenstein. The only significant point of difference was in the method used for the determination of uric acid. Lewis, Dunn, and Doisy (2) as well as other workers (4, 5) on this problem have precipitated the uric acid from the urine as an insoluble salt prior to its determination by a colorimetric procedure. Zwarenstein (3) on the other hand has used the more recent direct colorimetric method of Benedict and Franke (6). In this method the urine is treated with suitable reagents for the development of the uric acid color without the preliminary precipitation.

TABLE I.

Effect of Presence of Amino Acids on Determination of Uric Acid by the Benedict-Franke Direct Method.

Uric acid present.	Glycine present.*	Amino acid N present.	Uric acid found.
mg.	*mg.*	*mg.*	*mg.*
0.2	0	0	0.2
0.2	0.25	0.046	0.174
0.2	0.50	0.093	0.152
0.2	1.00	0.187	0.136
0.2	2.00	0.373	0.121

* The amounts of glycine indicated in the table were added directly to the flasks containing the standard amount of uric acid, prior to the development of the color.

It is very probable that there would be an increase in the amino acid content of the urine following the ingestion of proteins and amino acids in the amounts employed by Lewis, Dunn, and Doisy (2) and Zwarenstein (3). This might be expected particularly for the urines collected during the first 2 or 3 hours after the ingestion of the proteins and amino acids. These, it will be recalled, were the periods in which Lewis, Dunn, and Doisy observed an increased uric acid excretion. Therefore as a preliminary step, it was thought advisable to study the effect of added amino acids upon the direct determination of uric acid by the Benedict-Franke method. Accordingly, equal amounts of a sta dard uric acid solution (0.2 mg. of uric acid) were measured into a series of 50 cc. volumetric flasks. One flask was retained as a standard, while to each of the other flasks a definite amount of a glycine solution

was added. The contents of the flasks were all made up to the same volume (11 cc.) prior to the addition of the reagents, which develop the color. The determination was completed exactly as described by Benedict and Franke (6). It was obvious, even before the final solutions were compared in the colorimeter, that the presence of the added amino acid had prevented the full development of color of the uric acid. This experiment was repeated several times and while the results were not identical they are of the same order. The results of a typical experiment are presented in Table I. It is apparent from this table that the presence of even small amounts of amino acid nitrogen (0.046 mg.) leads to considerable error in the uric acid determination by the method of Benedict and Franke (6).

To determine whether an increased concentration of amino acids in the urine would influence the determination of uric acid by the Benedict-Franke method, urines were analyzed before and after the addition of glycine. The same urines were then reanalyzed for uric acid by the method of Morris and Macleod (7). In the latter method the uric acid is separated from the urine as the zinc salt, prior to its colorimetric estimation. The results of several experiments clearly indicated that if the analysis for uric acid was made by the direct method of Benedict and Franke, the presence of 1 mg. of added glycine led to results which were from 20 to 25 per cent too low. The effect of the added amino acids was negligible, however, if the analysis was made by the method of Morris and Macleod. The results of two experiments are given to illustrate this point. The analysis of a urine indicated a concentration of 0.43 mg. of uric acid per cc. of urine by the method of Benedict and Franke and 0.46 mg. per cc. by the method of Morris and Macleod. Glycine was then added to a portion of the urine so that the amount of urine used for a second analysis contained 1 mg. of added glycine (0.187 mg. of amino nitrogen). For the second analysis, a value of 0.34 mg. of uric acid per cc. was obtained by the method of Benedict and Franke and 0.45 mg. by the method of Morris and Macleod. A second urine on analysis gave a value of 0.40 mg. of uric acid per cc. by both methods. In the presence of 1 mg. of added glycine, the value for uric acid by the Benedict-Franke method dropped to 0.29 mg. per cc.,

and the value obtained by the method of Morris and Macleod was 0.38 mg. per cc.

These preliminary experiments suggest that Zwarenstein's failure to find an increase in uric acid elimination following the ingestion of proteins and amino acids might be explained by analytical difficulties. In the experiments reported by Zwarenstein, Subject A ingested 10 gm. of glycine in the first experiment and 20 gm. of alanine in the second experiment. Subject B consumed 250 gm. of boiled egg white in one experiment and 200 gm. of Cheddar cheese in a later experiment. As suggested previously in this paper, it is probable that the ingestion of such large amounts of proteins and amino acids would lead to an increased excretion of amino acids in the urine. A small increase in the amino acid content as shown would cause considerable error in the uric acid determination by the Benedict-Franke method. No data are available in the literature, however, relative to the hourly excretion of amino nitrogen under the conditions of Zwarenstein's experiments. The authors of this paper, therefore, decided to repeat part of the work of Zwarenstein, analyzing the hourly samples of urine for amino acid nitrogen as well as uric acid and creatinine.

In two experiments the urines were analyzed for uric acid by the method of Benedict and Franke (6) and by the method of Morris and Macleod (7). In a third experiment the uric acid was determined by the above methods, and also by the method of Folin and Denis (8) and the method of Benedict and Hitchcock (9). Thus, in this experiment, the uric acid was determined by one direct method and by three indirect methods, each of which employs a different method for the precipitation of the uric acid prior to its colorimetric estimation. Creatinine was determined by Folin's microcolorimetric method and amino acid nitrogen by the colorimetric procedure of Folin (10).

The experiments were carried out in the manner first described by Lewis, Dunn, and Doisy (2). The subject of the experiments was a healthy male, 65 kilos in weight, who lived on his usual diet on the days preceding the experiment. The usual evening meal was eaten at 6 p.m. on the day preceding the experiment and then no further food was eaten until the experiment was completed. On arising on the experimental day, the bladder was emptied, and

the collection of hourly samples began. 200 cc. of water were ingested at the beginning of each hour to facilitate the collection of hourly samples of urine and to insure reasonably large volumes. After the collection of two control samples, 10 gm. of glycine were ingested and the hourly collection continued for the following 5

TABLE II.

Effect of Ingestion of Glycine on Uric Acid Excretion.

Time.	Volume.	Creatinine.	Uric acid.		Amino acid N.	Amino acid N in sample analyzed.*
			Morris-Macleod method.	Benedict-Franke method.		
Experiment 1; March 7.						
	cc.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
7–8 a.m.	39	72	23.6	17.2	5.7	0.07
8–9 "	40	71	21.2	17.4	5.2	0.07
9–10 "†	60	76	27.6	18.7	27.9	0.23
10–11 "	139	71	28.8	15.0	60.5	0.87
11 a.m.–12 m.	133	68	27.0	15.6	24.2	0.36
12 m.–1 p.m.	125	61	18.4	13.8	10.6	0.17
1–2 p.m.	116	73	18.1	14.3	8.4	0.14
Experiment 2; March 14.						
7–8 a.m.	34	80	19.5	15.6	6.8	0.10
8–9 "	29	63	15.7	12.9	5.0	0.09
9–10 "†	50	67	27.0	11.5	27.5	0.55
10–11 "	62	64	28.0	12.5	34.7	0.56
11 a.m.–12 m.	50	68	21.0	10.3	15.5	0.31
12 m.–1 p.m.	64	68	14.7	11.9	10.6	0.17
1–2 p.m.	122	61	12.2	11.1	12.2	0.20

* By amino acid N in sample analyzed is meant the amount of amino acid nitrogen contained in the volume of urine used for the determination of uric acid by the Benedict-Franke method.

† 10 gm. of glycine ingested at 9 a.m.

hours. It is to be noted that this study is limited to the effect of the ingestion of glycine upon uric acid elimination. Our justification for this will be discussed later in the paper. The results obtained are presented in Tables II and III. The values for uric acid recorded in these tables have been carefully checked and rechecked by two observers. The amounts of urine used

for the determinations of uric acid were such that the colorimetric readings fell between 15 and 25 with the standard set at 20 mm. Zwarenstein calls attention to the fact that this precaution was observed in his work. Unfortunately, this precaution does not prevent considerable error in the uric acid determination by the Benedict-Franke method, if the urines contain appreciable amounts of amino acid nitrogen. This will be discussed in detail later in the paper.

TABLE III.

Effect of Glycine Ingestion on Uric Acid Excretion.
Experiment 3; March 19.

Time.	Volume.	Creatinine.	Uric acid.				Amino acid N.	Amino acid N in sample analyzed.*
			Morris-Macleod method.	Benedict-Franke method.	Folin-Denis method.	Benedict-Hitchcock method.		
	cc.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
8–9 a.m.	29	71	23.9	20.1	22.6	17.4	4.8	0.04
9–10 "	28	69	23.1	19.0	18.5	15.4	4.4	0.041
10–11 "†	50	71	32.8	19.0	30.8	32.0	27.5	0.27
11 a.m.–12 m.	48	66	32.6	18.7	34.6	33.6	27.8	0.29
12 m.–1 p.m.	47	67	25.4	16.9	26.3	25.4	12.9	0.14
1–2 p.m.	43	73	20.6	15.7	16.8	21.5	6.5	0.08
2–3 "	45	82	16.2	14.9	12.6	19.8	4.5	0.05

* See foot-note to Table II.

† 10 gm. of glycine ingested at 10 a.m.

An examination of Tables II and III reveals the following points. (1) If the analysis for uric acid is made by an indirect method (Morris and Macleod, Folin and Denis, or Benedict and Hitchcock), there is a marked increase in the hourly excretion of uric acid for the three periods following the ingestion of glycine. (2) If the analysis for uric acid is made on the same urines by the direct method of Benedict, no increase over the control periods is observed. The values would indeed indicate a decrease for these periods. (3) For the first two or three periods following the ingestion of 10 gm. of glycine, the amount of amino acid nitrogen

excreted per hour is 5 to 6 times greater than that of the control periods.

In the last column of Tables II and III is given the amount of amino acid nitrogen present in the volume of urine which is used for the determination of uric acid by the Benedict-Franke method. A comparison of these values with the amounts of amino acid nitrogen which were shown to produce considerable error in the determination of uric acid by this method (see Table I) will explain why the uric acid values by the Benedict-Franke method are low for the experimental periods. Our results then are in accordance with the work of Lewis, Dunn, and Doisy; that is, the ingestion of glycine produces a distinct rise in the uric acid excretion. It was not considered necessary for the purpose of this study to repeat the work with other amino acids or proteins since these results with glycine clearly indicate that Zwarenstein's results are at variance with those of earlier investigators because of the method he employed for uric acid. Experiments have been carried out with alanine and glutamic acid similar to the experiment with glycine reported in Table I. When equivalent amounts of these amino acids (glycine, alanine, glutamic acid) are present, the effect on the uric acid estimation by the Benedict-Franke method is much the same.

A few statements should be made regarding the uric acid values obtained by the Benedict-Franke method in Experiments 1 and 2 (Table II). The amount of urine used for each determination was such that the colorimetric readings were in most cases between 16 and 20 mm. with the standard set at 20 mm. In the light of results obtained in Experiment 3 (Table III), it is now believed that, if still smaller amounts of these urines had been used, the reading would still have remained in the colorimetric range (less than 25 mm.) and correspondingly higher results for uric acid would have been obtained. The higher values for uric acid result from a dilution of the amino acids of the urine to a concentration which has less effect on the determination of uric acid by the Benedict-Franke method. This point is well illustrated by the data taken from the analysis of the urine for the 11 to 12 o'clock period (Experiment 3, Table III). In the first analysis of this urine by the direct Benedict-Franke method, 10 cc. of a 1:10 dilution were used. The reading of the unknown against the

standard (0.2 mg. of uric acid) was 16.7 mm. This on calculation yields a concentration of 0.24 mg. of uric acid per cc. of urine or 11.5 mg. for the hour period. The urine was then reanalyzed by the same method, 10 cc. of a 1:20 dilution being used. The colorimetric reading was 20.5 against the standard set at 20 mm. This corresponds to a concentration of 0.39 mg. of uric acid per cc. of urine, or a total of 18.72 mg. of uric acid for the period as compared with 11.52 when twice as much urine was used. A third analysis was made on 10 cc. of a 1:40 dilution of the urine, and the colorimetric reading was now 30.8 mm. against the 20 mm. standard. This, of course, is outside of the range recommended for accurate work. The amount of uric acid per period is 24.96 mg. if the calculation is made from the last determination. It is to be noted that higher values for uric acid were obtained as the concentration of the amino acids decreased. Even when 10 cc. of a 1:40 dilution of the urine were used, the amino acid nitrogen present (0.145 mg.) was sufficient to cause an error of 20 per cent as indicated by the preliminary experiments. The uric acid excretion for this period as determined by the method of Morris and Macleod was 32.6 mg., by the method of Folin and Denis 34.6 mg., and by that of Benedict and Hitchcock, 33.6 mg.

SUMMARY.

In confirmation of the work of Lewis, Dunn, and Doisy (2) these results show that the ingestion of glycine by a fasting man is followed by an increased hourly excretion of uric acid. This effect is noted during the 1st hour after the ingestion of the glycine and continues for the following 2 hours. It is believed that the failure of Zwarenstein (3) to obtain similar results under comparable experimental conditions was due to the use of the direct method of Benedict and Franke (6) for the determination of uric acid. Experiments are given in this report, which show that the concentration of amino acids in the urine for the periods immediately following the ingestion of glycine (10 gm.) is from 5 to 6 times that for the control periods. It has also been shown that the uric acid method of Benedict and Franke gives results which are much too low for the urines containing these higher concentrations of amino acids.

BIBLIOGRAPHY.

1. Rose, W. C., *Physiol. Rev.*, **3,** 544 (1923).
2. Lewis, H. B., Dunn, M. S., and Doisy, E. A., *J. Biol. Chem.*, **36,** 9 (1918).
3. Zwarenstein, H., *Biochem. J.*, **22,** 307 (1928).
4. Gibson, H. V., and Doisy, E. A., *J. Biol. Chem.*, **55,** 605 (1923).
5. Rose, W. C., *J. Biol. Chem.*, **48,** 575 (1921).
6. Benedict, S. R., and Franke, E., *J. Biol. Chem.*, **52,** 387 (1922).
7. Morris, J. L., and Macleod, A. G., *J. Biol. Chem.*, **50,** 55 (1922).
8. Folin, O., Laboratory manual of biological chemistry, New York and London, 3rd edition, 141 (1922).
9. Benedict, S. R., and Hitchcock, E. H., *J. Biol. Chem.*, **20,** 619 (1915).
10. Folin, O., *J. Biol. Chem.*, **51,** 393 (1922).