THE ABILITY OF NEPHRITIC PATIENTS TO DEAMINIZE AND FORM UREA FROM INGESTED GLYCINE

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The regular finding in uremic coma of a marked increase in the amino nitrogen concentration of plasma and the frequent occurrence of a moderate elevation during the terminal stage of Bright's disease have recently been reported (Kirk, 1933).

The present study was undertaken to obtain further information about the amino acid metabolism in nephritis, especially during the terminal stage of the disease and in uremia.[1] For this purpose determinations were made of the rate of deaminization of ingested amino acids, and of urea formation following the ingestion.

Animal experiments of Van Slyke, Cullen and McLean (1915) and Bollman, Mann and Magath (1924), confirmed by results of Krebs and Henseleit (1932) with the tissue technique of Warburg, have led to the conclusion that the liver is the only important site of urea formation in the body. The experiments of Bollman, Mann and Magath (1926) indicated also that, in the dog at least, the liver is responsible for the deaminization of amino acids which precedes urea formation, as amino acids injected into a liverless dog could be recovered quantitatively from the tissues and urine of the animal many hours after the injection. The technique of experiments with dogs does not, however, yield results sufficiently exact to exclude the possibility of a minor amount of deaminization in organs other than the liver, because estimation of the amino nitrogen content of the entire animal at the beginning and end of an experiment can be only approximate. By *in vitro* experiments Krebs (1933) found that kidney as well as liver tissue could deaminize amino acids; it therefore appears possible that a minor part of the deaminization that occurs *in vivo* may be located in the kidneys.

Several attempts have been made by previous investigators to devise liver function tests based on observations of blood amino nitrogen or urea curves after ingestion of proteins or amino acids. Such curves might be expected to indicate the efficiency of the deaminizing and urea forming functions, respectively. In general such tests, applied almost exclusively to patients with suspected or obvious liver lesions, have given results of doubtful significance. This may be due to the technique employed. Von Falkenhausen (1924) and Witts (1929) estimated the amino nitrogen concentration of plasma at regular intervals following amino acid ingestion. The amino nitrogen determinations were, however, made by the colorimetric method of Folin (1922), which has later been found to be unsatisfactory for quantitative analysis of blood samples of increased amino nitrogen content (Van Slyke and Kirk, 1933).

A series of investigators (Witts, (1929), Cohen and Levin (1927), Theis (1928)) have attempted to detect abnormal urea formation by studying the blood urea curve following ingestion of proteins or amino acids. However, estimation of the urea excretion in the urine was omitted, and it does not appear that the blood urea curves alone could yield accurate estimates of the rate of urea formation. That normal persons after a protein meal sometimes show no increase in blood urea, but even a decrease, due to the fact that a diuresis may set in and wash out the urea from the body more rapidly than it is formed, was observed by Peters and Van Slyke (1931). Urea formation from ingested protein or amino acids can be quantitatively determined only by estimation of both the amount of urea retained in the body and the amount excreted in the urine.

This is well illustrated by the following figures obtained in a normal person and in a nephritic patient with 30 per cent of kidney function. The urea formation was calculated in a 6-hour period after ingestion of 60 grams of gelatine by the method described in this paper. Although the total urea formation is practically the same in the two individuals the values for retained and excreted urea differ greatly.

[1] The nomenclature for the different types and stages of Bright's disease used in this paper is the same as employed in previous publications from this Hospital (Van Slyke, Stillman, Möller, *et al.*, (1930); Kirk, (1933)).

	Normal individual	Nephritic patient with 30 per cent of kidney function
	mgm.	*mgm.*
Urea nitrogen excreted in the urine	2758	1472
Urea nitrogen retained in the body	1433	2774
Total urea nitrogen formation	4191	4246

PROCEDURE FOR ESTIMATING RATES OF DEAMINIZATION AND UREA FORMATION

For study of the rate of deaminization and calculation of the urea formation from ingested amino acids the following test was applied to 5 normal persons, to 3 uremic patients and to 4 patients with marked nephrotic symptoms accompanying the chronic active stage of hemorrhagic Bright's disease.

Twenty-five grams of glycine, dissolved in 500 cc. of water, were given by mouth at 9 a.m. to the subject, who had had nothing to eat since supper on the previous evening. Blood samples were drawn immediately before the glycine ingestion, and at 10 a.m., 11 a.m., 12 noon, 1 p.m., and 3 p.m. The plasma amino nitrogen concentration of all the samples was determined. The specimens drawn at 9 a.m., 12 noon and 3 p.m., further served for urea nitrogen analyses of whole blood. The urine was collected in two 3-hour periods (9 a.m. to 12 noon, and 12 noon to 3 p.m.), the volumes were measured, and the urea nitrogen, ammonia nitrogen and amino nitrogen concentrations were determined. From the urea analyses of blood and urine the urea formation was calculated as the sum of the urea excreted in the urine and the increase of the urea content of the body. The latter was estimated from the blood urea increase, because urea is so rapidly diffusible that its concentration in all the tissues of the body per kilo water present remains approximately equal to that of the blood (Marshall and Davis (1914), Bollman, Mann and Magath (1924)). Hence approximately: Total body urea increase = increase per liter blood × body weight in kilos × 0.8. The factor 0.8 represents approximately the ratio of water content per kilo of whole body to water content per liter of blood.

From the total urea nitrogen values obtained must be subtracted the endogenous urea nitrogen formation. This is determined on a subsequent day under similar conditions, but without ingestion of glycine. In all calculations of urea formation the urine ammonia nitrogen values were added to the urea nitrogen values.

ANALYTICAL METHODS

Plasma amino nitrogen: Manometric method of Van Slyke (1929) after destruction of the urea with urease. *Urine amino nitrogen:* Northrop's (1926) formaldehyde titration method as applied to urine by Van Slyke and Kirk (1933). *Blood urea nitrogen:* Gasometric urease method, procedure B, of Van Slyke (1927). *Urine urea nitrogen:* Gasometric urease method of Van Slyke (1927) for urine analyses. *Urine ammonia nitrogen:* Aeration and titration method of Van Slyke and Cullen (1916).

RESULTS

The *plasma amino nitrogen curves* obtained in 4 normal persons and in 3 uremic patients are presented in Figure 1. The hatched area in Figure 1 indicates the normal range of plasma amino nitrogen following ingestion of 25 grams of glycine. The individual normal amino nitrogen curves after absorption of the glycine showed great similarity, the maximum value being usually observed one hour after the ingestion. In the uremic patients, however, a much greater rise occurred,[3] even in instances where the fasting amino nitrogen values were normal, probably indicating a retardation of the deaminization rate of the absorbed amino acids. Frequently the amino nitrogen values remained considerably elevated for several hours in the blood of the nephritic patients.

The degree of this metabolic dysfunction is illustrated by the following figures: The *increase* in plasma amino nitrogen in the normal individuals after ingestion of 25 grams of glycine was 5 to 7 mgm. per 100 cc., and averaged 5.8 mgm. The highest concentration attained was 13.9 mgm. In the uremic patients, on the other hand, the rise was 2 to 4 times as great as in the

[3] In one uremic patient, a child of 11 years, no change in the amino nitrogen concentration of plasma and no measurable urea formation occurred after ingestion of 20 grams of glycine. It may be supposed that in this case the glycine was not absorbed from the intestine.

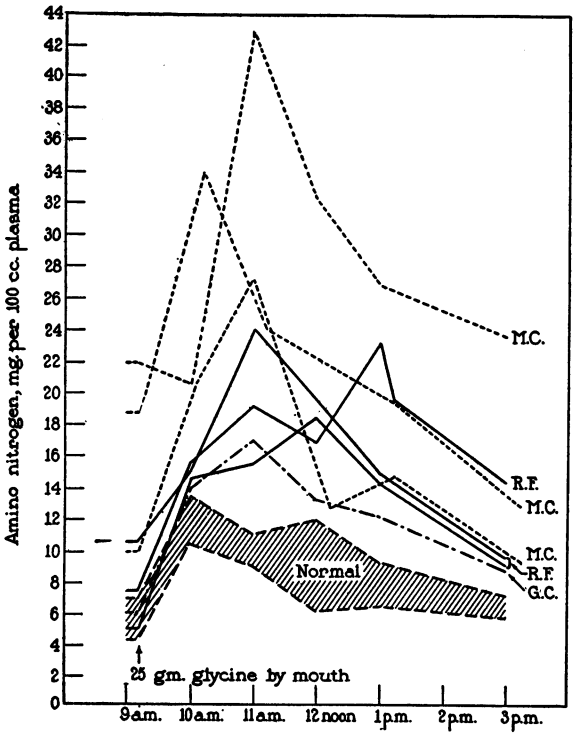

FIG. 1. PLASMA AMINO NITROGEN CURVES AFTER INGESTION OF 25 GRAMS OF GLYCINE.

Four normal subjects and three uremic patients. There are 3 curves each for patients M. C. and R. F., and one curve for G. C.

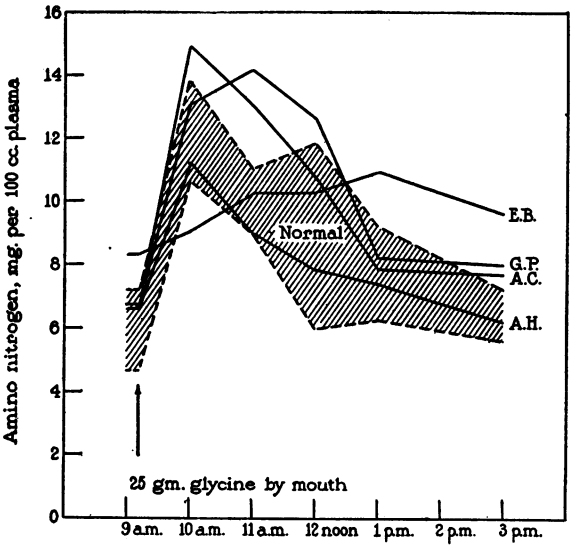

FIG. 2. PLASMA AMINO NITROGEN CURVES AFTER INGESTION OF 25 GRAMS OF GLYCINE.

Four patients in the chronic active stage of hemorrhagic nephritis, with the nephrosis syndrome predominating.

normal persons, and the plasma amino nitrogen reached values as high as 43 mgm. per 100 cc. The cause of this marked increase does not appear to be impaired excretion of the amino acids in the urine, which, under the conditions used, is only a minor factor in removing amino acids from the circulation. Even in the normal individuals the total excretion of amino nitrogen in the 6-hour period of the test constituted less than 5 per cent of the ingested amount. It is possible that the tissues of uremic patients are more nearly saturated with amino acids than the tissues of normal individuals, and that for this reason the glycine passes less rapidly and completely from blood to tissues in the uremic subject. Such pre-saturation of the tissues would not occur, however, if the deaminizing function were previously normal. Therefore, even if the direct cause of the high and prolonged blood amino nitrogen curve were previous saturation of the tissues with amino acids, the primary cause would be retarded deamination.

The plasma amino nitrogen curves obtained in 4 patients with marked nephrotic syndrome accompanying the chronic active stage of hemorrhagic Bright's disease are illustrated in Figure 2. These patients still had urea clearances above 20 per cent of normal, and hence would not be classed as terminal (Van Slyke, Stillman, *et al.*, 1930). The postabsorptive plasma amino nitrogen curves fall nearly within the area which includes the curves obtained in the normal individuals.

The *rates of urea nitrogen formation,* following the ingestion of 25 grams of glycine by the same normal and nephritic subjects, are presented in Figure 3.

Whereas the normal persons in the first 3 hours following the glycine feeding transformed an average of 48 per cent of the ingested 4670 mgm. of amino nitrogen into urea nitrogen, a much smaller urea formation (9.8 and 24 per cent) was found in two of the three *uremic* patients in the terminal stage.

On the other hand, the patients in the chronic active stage, with symptoms chiefly nephrotic, formed urea at the same rate as normal subjects.

It is evident that the processes by which absorbed amino acids are removed from the blood stream and transformed into urea proceed at a

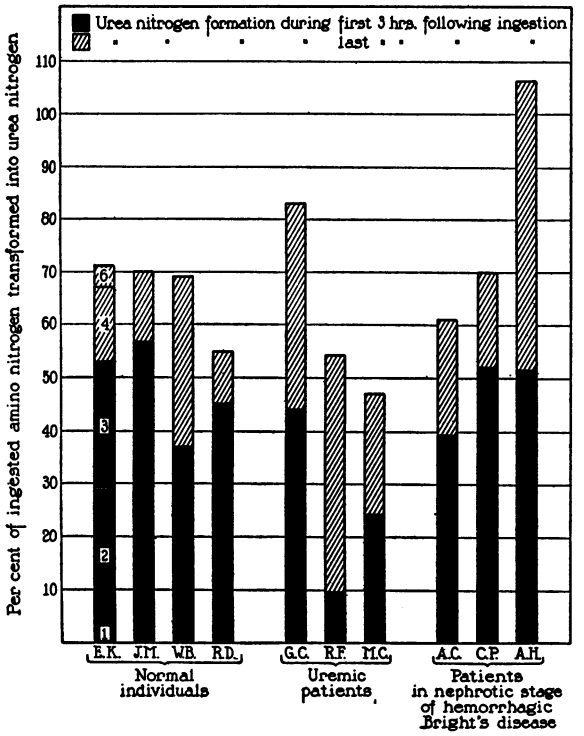

FIG. 3. UREA FORMATION FROM 25 GRAMS OF INGESTED GLYCINE.

In the first column the numbers indicate successive hours after the ingestion.

normal rate until the terminal stage of nephritis is reached: then retardation of these processes frequently occurs.

Urea synthesis from ammonia

The retarded urea formation observed in the terminal stage of nephritis might be a consequence of delay in either deaminization of the ingested glycine, or in the synthesis of urea from the nitrogen split off by deaminization. Considerable urea formation during the second 3-hour period in Case R. F. presented in Figure 3 would suggest that the retarded initial formation of urea was dependent on delayed deaminization.

In order to obtain more direct data on the efficiency of urea synthesis in uremia, experiments were undertaken in which material for the urea synthesis was supplied directly as ammonia nitrogen instead of in the form of amino acid nitrogen. The investigation included a study of the blood ammonia curve in normal and uremic individuals following ingestion of ammonium citrate, and calculation of the urea formation. Such tests were performed on two of the uremic patients, who had been found to show abnormally high and prolonged rises of plasma amino nitrogen after feeding of glycine (G. C. and R. F.), and in one of whom (R. F.) the urea formation after amino acid feeding was markedly reduced.

At 9 a.m. 13.3 grams of ammonium citrate (containing 2950 mgm. of ammonia nitrogen) were given dissolved in water to the patient, who had been fasting since supper on the previous evening.[4] Blood samples for ammonia determination were drawn immediately before the ingestion and at 9:30 a.m., 10 a.m., and 12 noon. The ammonia analyses were made by the method of Van Slyke and Hiller (1933), the analyses being started less than 3 minutes after the blood samples were drawn. Calculation of the urea formation in the 3-hour period was performed as described above under the glycine test.

The results are presented in Table I. No difference was observed between the 2 normal individuals and the 2 uremic patients either in the fasting blood ammonia values or in the values obtained after feeding of the ammonia salt. The urea formation following the ammonia ingestion was not retarded in the uremic patients. These results demonstrate that the ability to transform

TABLE I

Blood ammonia values and urea nitrogen formation in two normal individuals, and two uremic patients, following ingestion of 13.3 grams of ammonium citrate (2950 mgm. ammonia nitrogen)

Case	Blood ammonia values				Urea nitrogen formed in 3 hours
	Fasting	30 minutes after ingestion	60 minutes after ingestion	3 hours after ingestion	
	mgm. per cent	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm.*
E. K. normal..	0.029	0.045	0.060	0.049	1920
J. M. normal..	0.033	0.031	0.030	0.031	1847
R. F. uremic..	0.040	0.059	0.047	0.033	2453
G. C. uremic..	0.016	0.022	0.018	0.016	1750

[4] Tests of the same character, but not including calculation of the urea formation, have been employed by Burchi (1926, 1927) and Fuld (1933), in the study of liver patients. Burchi's figures for fasting blood ammonia in normal persons are much higher than results obtained by reliable techniques of analysis.

ammonia into urea is unimpaired in uremia. They give support to the assumption that the delay in urea formation, observed after glycine ingestion in uremic patients, is caused by impairment in the body mechanism which deaminizes amino acids, rather than in the mechanism which forms urea out of the ammonia yielded by deaminization.

DISCUSSION

The experimental data presented above have revealed a distinct metabolic dysfunction in patients suffering from uremia and have thereby confirmed the results reported in a previous paper (Kirk, (1933)). In addition it is shown that putting the deaminizing function under strain by the ingestion of 25 grams of glycine may reveal impairment of this function at a time when the fasting plasma amino nitrogen is still within the normal limits.[5] No definite conclusion can be drawn from this investigation about the site of this impaired function, but it should be mentioned in this connection that all the uremic patients studied showed a normal liver function when subjected to the bromsulphalein test of Rosenthal and White (1925). Normal elimination of the dye was found even in a semicomatose patient, who on the day of the test had a fasting plasma amino nitrogen value of 38 mgm. per cent.

From clinical experience in this hospital it appears doubtful that demonstration, by the glycine feeding test, of a retarded rate of deaminization in a nephritic patient indicates the necessity for a low protein diet. The plasma amino nitrogen concentration usually returns to initial values within the 6-hour period of the test, which means that the deaminization, even of the rather large amounts of amino acid taken, is completed during the usual interval between meals. A review of the observed practical advantages of a normal protein intake in even the later stages of nephritis, based on the experiences obtained in this clinic, has recently been published by Alving (1934).

An illustration of the beneficial effect of free protein intake in face of nearly constantly elevated plasma amino nitrogen is seen in the clinical course of the disease of a nephritic patient, presented in Figure 1 of a previous paper (Kirk, (1933)). Amino nitrogen analyses of plasma were made daily for 3 months after recovery from an acute attack of uremia. Usually the fasting amino nitrogen values were found considerably elevated. In spite of these laboratory findings the patient was allowed an unlimited protein intake and on this regime gained weight and strength and was able to leave the hospital at the end of the period of observation with nearly completely restored working ability.

[5] For detection of impaired deaminization one amino nitrogen determination performed 3 hours after the ingestion of 25 grams of glycine suffices.

SUMMARY

1. A test of the deaminizing and urea-forming functions is described in which the plasma amino nitrogen curve and the amount of urea formed are observed during 3 hours after ingestion of 25 grams of glycine.

2. In uremic patients the increase in plasma amino nitrogen was 2 to 4 times greater than in normal individuals, and the return to pre-ingestion level was slower.

3. Normal persons transformed an average of 48 per cent of the ingested amino nitrogen into urea nitrogen during the first three hours following the glycine. In 2 out of the 3 uremic patients studied the urea formation in this period was reduced to 10 and 24 per cent respectively of the ingested nitrogen. However, during the period 3 to 6 hours after ingestion the uremic subjects formed as much urea as the normal subjects or more.

4. The same patients who showed high and prolonged curves for blood amino nitrogen and initially retarded urea formation after ingestion of glycine, were able to form urea from ingested ammonium citrate as rapidly as the normal individuals. The curve for blood ammonia following ingestion of 13.3 grams of ammonium citrate likewise showed no difference from the curve obtained in normal subjects. The conversion of ammonia into urea is therefore unimpaired in uremia. The retarded formation of urea observed after feeding of glycine appears to be attributable rather to delay in deaminization, than to delay in the subsequent transformation of the ammonia into urea.

5. In patients in the intermediate, chronic active stage of Bright's disease, no abnormality in

the curve for plasma amino nitrogen or in urea formation was found after feeding of glycine.

6. The finding of abnormally high curves for plasma amino nitrogen after ingestion of glycine, or even of moderately elevated content of plasma amino nitrogen in the fasting condition, does not apparently suffice to indicate the necessity for restricting protein intake.

BIBLIOGRAPHY

Alving, A. S., The diet in chronic Bright's disease. M. Clin. North America, 1934, **17**, 1195.

Bollman, J. L., Mann, F. C., and Magath, T. B., Studies on the physiology of the liver. VIII. Effect of total removal of the liver on the formation of urea. Am. J. Physiol., 1924, **69**, 371.

Bollman, J. L., Mann, F. C., and Magath, T. B., Studies on the physiology of the liver. XV. Effect of total removal of the liver on deaminization. Am. J. Physiol., 1926, **78**, 258.

Burchi, R., Saggio della funzionalità epatica con lo studio della ammoniemia spontanea e provocata. Folia clin. chim. et micr., 1926, **1**, 3.

Burchi, R., I saggi della funzionalità epatica e la prova dell'ammoniemia spontanea e provocata studio della sufficienza funzionale del fegato. Folia clin. chim. et micr., 1927, **2**, 5.

Cohen, P., and Levin, S. J., The protein test for urea formation function of the liver. Arch. Int. Med., 1927, **39**, 787.

Folin, O., A system of blood analysis. Supplement III. A new colorimetric method for the determination of the amino-acid nitrogen in blood. J. Biol. Chem., 1922, **51**, 377.

Fuld, H., Über die diagnostische Verwertbarkeit von Ammoniakbestimmungen im Blut. Klin. Wchnschr., 1933, **12**, 1364.

Kirk, E., Amino nitrogen changes of the blood in nephritis. J. Clin. Invest., 1933, **12**, 1091.

Krebs, H. A., Untersuchungen über den Stoffwechsel der Aminosäuren im Tierkörper. Ztschr. f. physiol. Chem., 1933, **217**, 191.

Krebs, H. A., and Henseleit, K., Untersuchungen über die Harnstoffbildung im Tierkörper. Ztschr. f. physiol. Chem., 1932, **210**, 33.

Marshall, E. K., Jr., and Davis, D. M., Urea; its distribution in and elimination from the body. J. Biol. Chem., 1914, **18**, 53.

Northrop, J. H., A convenient method for the formol titration. J. Gen. Physiol., 1926, **9**, 767.

Peters, J. P., and Van Slyke, D. D., Quantitative Clinical Chemistry. Vol. I, Interpretations. Williams and Wilkins, Baltimore, 1931, p. 344.

Rosenthal, S. M., and White, E. C., Clinical application of the bromsulphalein test for hepatic function. J. A. M. A., 1925, **84**, 1112.

Theis, E. H., The protein test for urea formation function of the liver. California and West. Med. J., 1928, **29**, 83.

Van Slyke, D. D., Determination of urea by gasometric measurement of the carbon dioxide formed by the action of urease. J. Biol. Chem., 1927, **73**, 695.

Van Slyke, D. D., Manometric determination of primary amino nitrogen and its application to blood analysis. J. Biol. Chem., 1929, **83**, 425.

Van Slyke, D. D., and Cullen, G. E., The determination of urea by the urease method. J. Biol. Chem., 1916, **24**, 117.

Van Slyke, D. D., Cullen, G. E., and McLean, F. C., The formation of urea in the liver. Proc. Soc. Exper. Biol. and Med., 1915, **12**, 93.

Van Slyke, D. D., and Hiller, A., Determination of ammonia in blood. J. Biol. Chem., 1933, **102**, 499.

Van Slyke, D. D., and Kirk, E., Comparison of gasometric, colorimetric, and titrimetric determinations of amino nitrogen in blood and urine. J. Biol. Chem., 1933, **102**, 651.

Van Slyke, D. D., Stillman, Edgar, Möller, E., Ehrich, W., McIntosh, J. F., Leiter, L., MacKay, E. M., Hannon, R. R., Moore, N. S., and Johnston, C., Observations on the courses of different types of Bright's disease, and on the resultant changes in renal anatomy. Medicine, 1930, **9**, 257.

Von Falkenhausen, M. F., Über den Aminosäurengehalt des Blutes und seine Bedeutung für die Beurteilung der Leberfunktion. Arch. f. exper. Path. u. Pharmakol., 1924, **103**, 322.

Witts, L., Observations on the metabolism of amino acids in health and disease. Quart. J. Med., 1929, **22**, 477.