THE FORMATION OF GLYCOCYAMINE IN MAN AND ITS URINARY EXCRETION

By HENRY BORSOOK, JACOB W. DUBNOFF, JOHN C. LILLY, AND WILLIAM MARRIOTT

(From the William G. Kerckhoff Laboratories of the Biological Sciences, California Institute of Technology, Pasadena)

(Received for publication, September 18, 1940)

Glycocyamine was first isolated from human and dog urine and identified by Weber (1–3). He supported the view that glycocyamine is a normal precursor of creatine and that its appearance in urine (2) is "an overflow phenomenon of an intermediate metabolic product . . ." He expressed no views on the mechanism of its formation.

Glycocyamine is formed by the interaction of arginine and glycine in the kidney (and probably only in that organ) of the cat, dog, guinea pig, rabbit, and rat (4). In order to ascertain whether this reaction occurs in man we have studied the effect of arginine and glycine, ingested separately and together, on the glycocyamine content of urine and blood.

The subjects were seven normal men. The methods used for the determination of glycocyamine in urine and in blood have been described (5).

The first experiments consisted in comparing the urinary excretion of glycocyamine during 24, later only 12, hours in which no protein was ingested with that on a day in which gelatin was ingested. Gelatin consists of about 25 per cent glycine and 9 per cent arginine (6). Each experiment ran for 2 days. On the evening preceding the 1st day the subject had a normal meal at 6 p.m. and 300 ml. of water in the course of the evening. During the following day he drank 1700 ml. of orange juice and an additional 300 ml. of water. This was the "non-protein" day in Fig. 1. Next day the subject drank 875 ml. of orange juice, and 1000 ml. of water containing 275 gm. of sucrose. He ingested

65 gm. of gelatin between 8 and 9 a.m. The gelatin contained 16 gm. of glycine and 5.8 gm. of arginine. This was the "gelatin" day.

A typical result is given in Fig. 1. It shows the rapid rise in the urinary excretion of glycocyamine after the ingestion of gela-

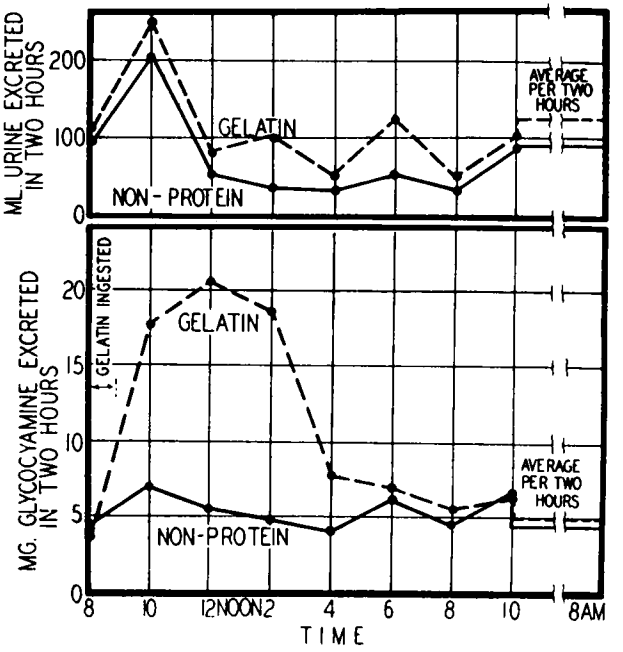

FIG. 1. Glycocyamine excretion in urine. The ordinates represent the glycocyamine excreted in the urine in the 2 hour period ending at the time indicated; the abscissae, the time at which the urine was voided. 65 gm. of gelatin were ingested between 8 and 9 a.m. The horizontal right-hand ends of both curves represent the total excretion in the night urine (10 p.m. to 8 a.m.) divided by 5.

tin. The maximum was attained between 10 a.m. and 12 noon. By 4 p.m. this rate had subsided near to the value on the non-protein day, and remained so during the following 14 hours. This rapid rise and fall in the rate of glycocyamine excretion occurred both when the arginine and glycine were taken in the form of gelatin and as the pure amino acids (see Figs. 1 and 2).

It is improbable that the increased urinary excretion of glyco-

cyamine observed after the ingestion of gelatin represents glycocyamine "washed out" of the tissues. If that had been the case, the excretion during the last 14 hours of the gelatin day probably would have fallen below that during the same interval on the non-protein day; and the total amount of glycocyamine excreted over the 24 hour period would have been nearly the same on these 2 days. The 24 hour excretion of glycocyamine on the non-protein day was 65 mg. and on the gelatin day 117 mg. The lack of any relation in Fig. 1 between urine volume and the amount of glycocyamine excreted is in accord with this interpretation.

Table I is a summary of the findings in experiments similar to that in Fig. 1 in five other subjects. The data for the period from 6 a.m. to 6 p.m. only are given.

TABLE I

Effect of Ingestion of 65 Gm. of Gelatin on Urinary Glycocyamine

Subject No.	Non-protein day		Gelatin day	
	Urine volume	Glycocyamine	Urine volume	Glycocyamine
	ml.	*mg.*	*ml.*	*mg.*
1	475	43.7	890	87.5
2	1715	34.3	1785	114.0
3	370	34.0	600	60.0
4	1220	32.9	1520	88.2
5	470	24.4	1350	81.6

The figures for glycocyamine excretion on the non-protein days in Table I indicate that over 24 hours they would have been of the order of magnitude of 40 to 60 mg. Weber (3) reported average figures for 24 hours of 39.5 mg. for males and 75.1 mg. for females. Weber's values are lower, probably because the figures obtained with his analytical method represent only 75 per cent or less of the glycocyamine present.

A series of experiments was carried out on two subjects in which arginine and glycine, alone and together, were administered in the form of the pure amino acids. The two subjects remained on the identical diet for the 9 days of the experiment. Breakfast and luncheon consisted of orange juice and coffee; dinner was a normal meal in which the meat course was a weighed amount of Hamburger steak. During the day 200 ml. of water were drunk

every 2 hours. Urine was collected every 2 hours from 6 a.m. to 6 p.m. On the 3rd day of this régime the urine voided every 2 hours during the day was analyzed; the values obtained gave the "normal" graph in Fig. 2. On the 5th day the subjects ingested 16 gm. of glycine between 8.30 and 9.30 a.m.; the glyco-

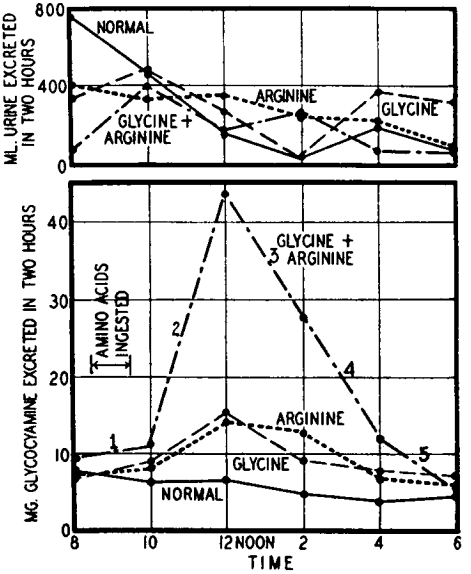

FIG. 2. Glycocyamine excretion in urine. The ordinates represent the glycocyamine excreted in the urine in the 2 hour period ending at the time indicated; the abscissae, the time at which the urine was voided. The amino acids were ingested, on each of the 4 days, in four portions between 8.30 and 9.30 a.m. The blood concentrations, at successive 2 hour intervals after the beginning of the experiment, on a day on which glycine and arginine were ingested together were *1*, 0.6, *2*, 0.4, *3*, 0.7, *4*, 0.8, and *5*, 1.0 mg. per cent.

cyamine excretion on this day is designated in Fig. 2 as "glycine." On the 7th day between 8.30 and 9.30 a.m. the subjects consumed 16 gm. of glycine and 4.8 gm. of arginine, and on the 9th day 4.8 gm. of arginine. The glycocyamine excretion on these days shown in Fig. 2 is designated "glycine + arginine" and "arginine."

Fig. 2 shows that after the ingestion of glycine alone more glycocyamine appeared in the urine. These increases were small,

however, compared to those obtained when the two amino acids were taken together. Thus on the normal day the glycocyamine excretion in 12 hours was 29.0 mg., on the glycine day 54.3 mg., on the arginine day 55.4 mg., and on the glycine + arginine day 99.0 mg. The sum of the increases over the normal day with glycine and arginine when these were ingested separately was 51.7 mg.; when ingested together it was 70 mg.

In the second subject the glycocyamine excretion on the normal day was 31.2 mg., on the glycine day 30.7 mg., on the arginine day 28.4 mg., and on the glycine + arginine day 61.8 mg.

The increase in glycocyamine in the urine after the ingestion of arginine and glycine was of the same order of magnitude as when the same amounts of these amino acids were ingested in the form of gelatin. The increase in urinary glycocyamine after the ingestion of gelatin may be ascribed to the arginine and glycine it contained.

These findings taken in conjunction with those obtained with surviving slices of kidney and with kidney extracts (4) may be considered as establishing the formation of glycocyamine by transamidination in man.

Blood was analyzed every 2 hours on the days on which the subjects ingested the glycine and arginine together. In the subject of Fig. 2 the concentrations were, in mg. per cent, 0.6, 0.4, 0.7, 0.8, and 1.0. The peak of the urinary excretion of glycocyamine occurred between the second and third of these values. There was no discernible relation between the glycocyamine concentration in the blood and its increased rate of excretion in the urine. The findings were essentially the same in the other subject.

We have made some preliminary observations on the excretion of glycocyamine by human subjects with kidney disease. On an ordinary diet they excreted less glycocyamine than normal individuals and the increase after the ingestion of arginine and glycine was less also. Thus in one subject who was diagnosed as having subacute glomerulonephritis the excretion of glycocyamine in the urine in an experiment similar to that of Fig. 1 was on the non-protein day 1.5, 3.0, 2.5, 2.6, 2.1, and 1.0 mg. in successive 2 hour intervals between 6 a.m. and 6 p.m.; and on the gelatin day 1.0, 3.1, 5.3, 2.4, 1.9, and 2.8 mg.

SUMMARY

1. When arginine and glycine are ingested together by human subjects, there is a rapid rise in the amount of glycocyamine excreted in the urine. This increase is greater than the sum of the increases which may occur when the same amounts of amino acids are taken separately.

2. A similar increase in glycocyamine excretion is observed after the ingestion of gelatin, which is rich in glycine and arginine. The order of magnitude of this increase is the same as that given by the quantity of arginine and glycine contained in the gelatin when these are administered as pure amino acids.

3. These findings indicate that in man glycocyamine is formed by transamidination.

BIBLIOGRAPHY

1. Weber, C. J., *Proc. Soc. Exp. Biol. and Med.*, **32**, 172 (1934).
2. Weber, C. J., *Proc. Am. Soc. Biol. Chem.*, *J. Biol. Chem.*, **109**, p. xcvi (1935).
3. Weber, C. J., *Proc. Am. Soc. Biol. Chem.*, *J. Biol. Chem.*, **114**, p. cvii (1936).
4. Borsook, H., and Dubnoff, J. W., *J. Biol. Chem.*, **138**, 389 (1941).
5. Dubnoff, J. W., and Borsook, H., *J. Biol. Chem.*, **138**, 381 (1941).
6. Mitchell, H. H., and Hamilton, T. S., The biochemistry of the amino acids, American Chemical Society monograph series, New York (1929).