Ammonia Intoxication and Intermediary Metabolism (36693)

A. J. CLIFFORD,[1] R. L. PRIOR, H. F. HINTZ, P. R. BROWN,[2] AND W. J. VISEK
Department of Animal Science, Cornell University, Ithaca, New York 14850

Ammonia intoxication is associated with a depletion of α-ketoglutarate (α-Kg) and oxalacetate (1); an impaired decarboxylation of pyruvate and α-Kg (2) an inhibition of isocitric dehydrogenase (3) and a depletion of liver reduced pyridine nucleotides (3, 4). Although the primary biochemical dysfunction in ammona toxicity is not known, all of the above proposed mechansims have a direct bearing upon intermediary carbohydrate metabolism.

In the present report ammonia toxicity was produced in rats and horses. Changes in concentrations of metabolic intermediates were followed in blood as a function of time. A preliminary report of a portion of this work has been presented (5).

Materials. Male Sprague-Dawley rats (200 g) were injected intraperitoneally with 11 units[3] of crystalline jackbean urease. Control rats were injected with saline in equal volume. Animals were killed by exsanguination every 30 min for 3 hr after injection. Aliquots of blood were treated immediately for determinations of ammonia (6), glucose (7) and pyruvate (8). For α-ketoglutarate (α-kg) in blood, the method of Bergmeyer and Bernt (9) was followed except that 5 ml of 2.4 N $HClO_4$ was used for deproteinization of 5 ml of blood. The concentrations of glucose, pyruvate and α-kg were correlated with the concentration of blood ammonia obtained on the same sample. Procedures outlined by Steele and Torrie (10) were followed for statistical analyses.

Control and intoxicated rats were anesthetized with a mixture of ether and oxygen (11). Their livers were exposed and frozen *in situ* by immersion in liquid nitrogen. Free nucleotides from 3 g of liver were extracted with 3 vol of 0.6 N $HClO_4$, neutralized to pH 6–7 with 3 N KOH. The neutralized extract was lyophylized, dissolved in 1 ml of distilled water and nucleotides were separated by high pressure ion exchange chromatography (12). Free nucleotides were also determined in 3 ml of blood obtained at decapitation. Identical procedures were used to extract nucleotides from blood and liver.

Studies of blood metabolite concentrations following release of ammonia from urea were conducted in 200–300 kg ponies of unknown ancestry. A review of the literature had revealed an apparent lack of information on ammonia intoxication in horses in which the predominant site of urea hydrolysis appears to be the cecum. For these studies the horse offered the additional advantage of a sufficient blood volume for serial sampling. Feed was withheld but water remained available for 16 hr prior to the administration of 2 liters of a solution containing 454 g of urea. Thirty milliliter samples of jugular blood were drawn immediately prior to this administration and at frequent intervals thereafter until death. Aliquots of blood were processed immediately for glucose, pyruvate, α-Kg and ammonia. These metabolites were measured using techniques identical to those used for rats. Determinations of pH were made immediately after the blood was drawn. Metabolite concentrations in blood drawn before urea was ad-

[1] Present address: Department of Nutrition, University of California, Davis, CA.

[2] Section of Biochemical Pharmacology, Department of Biological and Medical Sciences, Brown University, Providence, RI 02912.

[3] One unit = enzyme activity that liberates 1 mg NH_3–N from a urea phosphate buffer in 5 min at 20° and pH 7.0. One unit of crystalline urease represents about 11 μg protein.

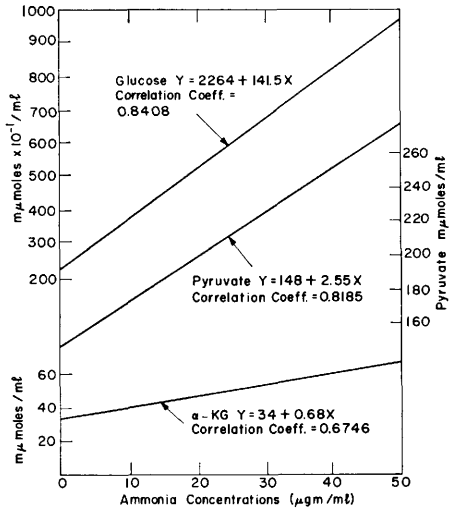

FIG. 1. Glucose, pyruvate and α-ketoglutarate in blood of ammonia intoxicated rats plotted as a function of blood ammonia concentrations. Animals were killed at intervals of 20 min for 3 hr from the time of jackbean urease injection.

ministered served as control values for each animal.

Results. Blood glucose, pyruvate and α-Kg concentrations in ammonia intoxicated rats increased and correlated positively with ammonia (Fig. 1). The respective correlation coefficients were 0.84, 0.82 and 0.67 indicating that 71, 67 and 45% of the variation in glucose, pyruvate and α-Kg, respectively, was attributable to variation in ammonia concentrations.

Ammonia intoxication had no effect on ATP, AMP, GTP, GDP and GMP concentrations in blood or liver (Table I).

The clinical description of ammonia intoxication in the ponies of this study has been presented elsewhere (13). Blood glucose, pyruvate, α-Kg and ammonia concentrations varied somewhat in pattern and rate of change depending upon the length of time before the onset of convulsions. In the horse that survived for 5.5 hr (Fig. 2) ammonia increased from about 1.0 to 2.5 μg/ml at 2 hr to about 15 μg/ml at 4 hr. At 40 min after urea was administered, α-ketoglutarate was well below the control value and increased markedly by hour 5. Blood pyruvate remained approximately at the control level for the first 2 hr and then increased sharply to about 14 times the control value after 4 hr. In the horse that lived for 12 hr (Fig. 3), blood ammonia increased from a control concentration of 2.5 to 20 μg/ml by hour 11. The concentration of α-ketoglutarate was depressed at 30 min and was markedly elevated at 2 hr. Pyruvate concentrations declined and did not return to control concentrations until hour 5 when it increased sharply to 420 nmoles/ml at 9 hr. Blood pH in all animals remained within the range of 7.2–7.4. The horse that survived for only 3 hr (Fig. 4) showed similar increases in ammonia, glu-

TABLE I. Purine Nucleotide Concentrations[a] in Blood and Liver of Rats Intoxicated with Ammonia.

	Liver		Blood	
	Control (4)	Intoxicated (5)	Control (4)	Intoxicated (5)
AMP	189 ± 18	158 ± 30	41 ± 37	60 ± 23
ADP	798 ± 59	894 ± 51	165 ± 17	207 ± 16
ATP	3299 ± 83	3053 ± 245	1143 ± 116	1362 ± 36
Total adenine	4286 ± 1117	4106 ± 255	1329 ± 130	1612 ± 34
GMP	35 ± 3	41 ± 3	29 ± 3	18 ± 4
GDP	211 ± 20	228 ± 29	70 ± 31	47 ± 15
GTP	760 ± 21	594 ± 81	161 ± 43	146 ± 15
Total guanine	1007 ± 39	864 ± 102	260 ± 58	224 ± 13

[a] Means ± standard error of mean (μmoles/kg tissue). Numbers in parentheses represent the number of animals per treatment.

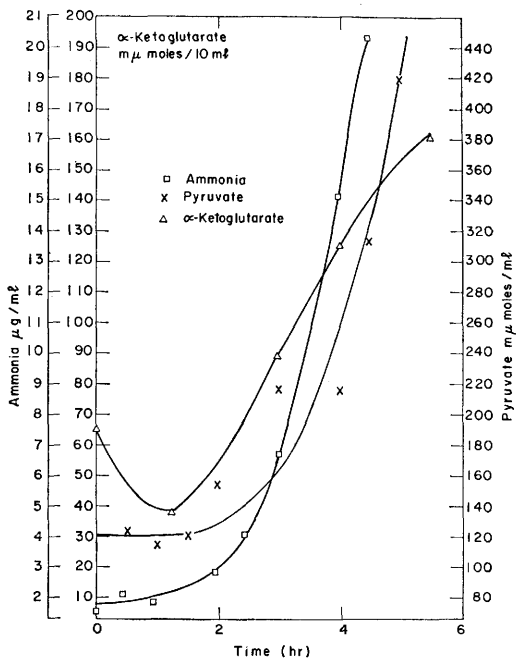

FIG. 2. Blood ammonia, glucose, pyruvate and α-ketoglutarate determined in a horse that died 5.5 hr after oral administration of 454 g of urea.

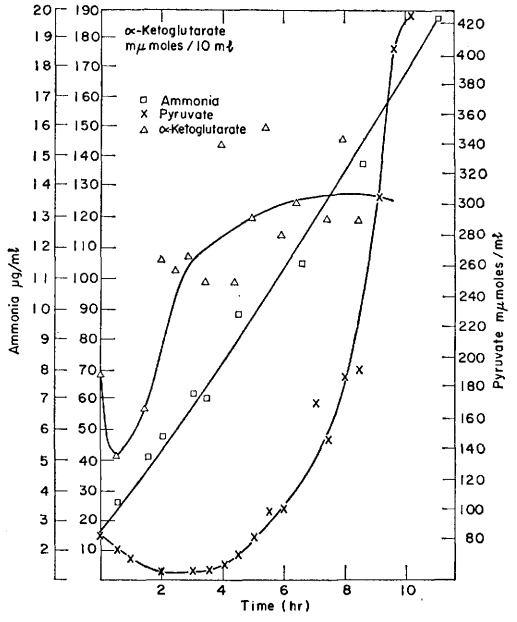

FIG. 3. Blood ammonia, glucose, pyruvate and α-ketoglutarate determined in a horse that died 12 hr after oral administration of 454 g of urea.

cose, pyruvate and α-kg. However, in this animal the changes occurred much more rapidly and the early decrease in α-Kg was not observed.

Discussion. The data from rats demonstrate that blood glucose, pyruvate and α-Kg increase as blood ammonia rises during acute ammonia intoxication. It is also apparent from the studies in horses that the concentration of glucose, pyruvate and α-Kg in blood may vary significantly within the same animal, depending upon the time of sampling in relation to the onset of toxicity. Based upon these results and those of others (1), it is

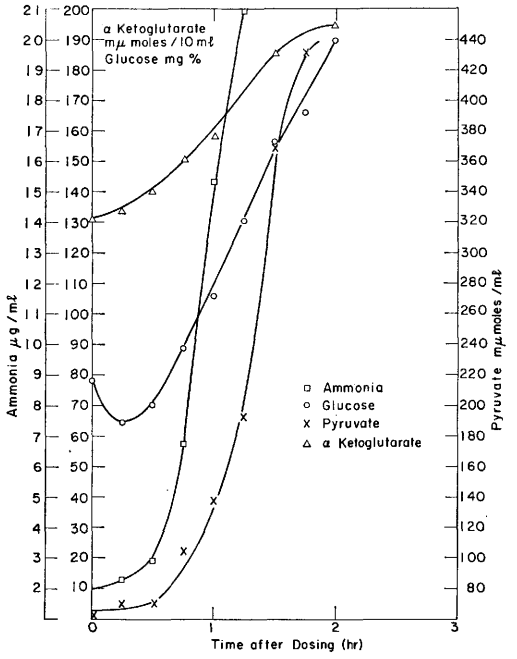

FIG. 4. Blood ammonia, glucose, pyruvate and α-ketoglutarate determined in a horse that died 3 hr after oral administration of 454 g of urea.

apparent that experiments in animals given large doses of ammonium salts could lead to the conclusion that there is a depletion of α-Kg if the sampling of blood was stopped before α-Kg begins to rise.

Bessman and Bessman (1) reported that the increase in blood pyruvate may be a consequence of a deficiency of 4 carbon metabolites such as oxalacetate needed to metabolize pyruvate rather than an inhibition of

pyruvate decarboxylation. However, more recent studies in our laboratory have shown that succinate and malate concentrations also increase during ammonia intoxication. Addition of succinate increased O_2 uptake but did not affect pyruvate utilization in brain tissue (2) which argues against an actual deficiency of 4 carbon metabolites. Furthermore, blood glucose increased before pyruvate which suggests also that the increase in blood pyruvate may be due to stimulation of glycolysis early in the course of intoxication. These findings in addition to the absolute depletion of reduced pyridine nucleotides previously reported (4) provide clear evidence that intermediary metabolism is altered by ammonia intoxication. All the data support the conclusion that hyperammonia initiates a chain of events in which carbohydrate metabolism is altered to cause an accumulation of glucose, pyruvate and α-Kg in blood.

Summary. Ammonia intoxication of rats markedly increased blood glucose, pyruvate, α-ketoglutarate and ammonia concentrations. Ammonia intoxication of horses produced similar changes in the blood metabolites and the sequence of these events was as follows: blood ammonia increased and α-ketoglutarate decreased during the first 2 hr, but then increased very rapidly. These changes were followed by increases in blood glucose and pyruvate concentrations. Ammonia intoxication did not affect liver mono-, di- or triphosphates of adenine and guanine. These findings indicate that a primary lesion in ammonia intoxication is a derangement of intermediary carbohydrate metabolism.

This research was financially supported by a grant from The Nutrition Foundation and U.S. Public Health Service, Grant No. GM-16538-01. The authors acknowledge the assistance of Mabel Goetchius, John Milner and Gary Gibson.

1. Bessman, S. P., and Bessman, A. N., J. Clin. Invest. **34**, 622 (1955).

2. McKhann, G. M., and Tower, D. B., Amer. J. Physiol. **200**, 420 (1961).

3. Katunuma, N., Okada, M., and Nishii, Y., *in* "Advances in Enzyme Regulation" (G. Weber, ed.), p. 317. Macmillan Co., New York (1966).

4. Clifford, A. J., Prior, R. L., and Visek, W. J., Amer. J. Physiol. **217**, 1269 (1969).

5. Clifford, A. J., and Prior, R. L., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **29**, 428 (1970) (Abstr.).

6. Chaney, A. L., and Marbach, E. P., Clin. Chem. **8**, 130 (1962).

7. Saloman, L. L., and Johnson, J. E., Anal. Chem. **31**, 453 (1959).

8. Bucher, T., Czok, R., Lamprecht, W., and Lotzka, E., *in* "Methods of Enzymatic Analysis" (H. Bergmeyer, ed.), p. 253. Academic Press, New York (1965).

9. Bergmeyer, H. U., and Bernt, E., *in* "Methods of Enzymatic Analysis" (H. Bergmeyer, ed.), p. 324. Academic Press, New York (1965).

10. Steele, R. G. P., and Torrie, J. H., "Principles and Procedures of Statistics," pp. 52, 277. McGraw-Hill, New York (1960).

11. Bucher, N. L. R., and Swaffield, M. N., Biochim. Biophys. Acta **129**, 445 (1966).

12. Brown, P. R., J. Chromatogr. **52**, 257 (1970).

13. Hintz, H. F., Lowe, J. E., Clifford, A. J., and Visek, W. J., J. Amer. Vet. Med. Ass. **157**, 963 (1970).

Received May 1, 1972. P.S.E.B.M., 1972, Vol. 140.