

Early Life Nutritional Deprivation: Persistent Alteration of Blood and Urine Amino Acids in Mice (37504)

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Protein-calorie malnutrition is a widely prevalent condition affecting over one half of the world's children (1). It has been strongly implicated as a cause of mental retardation, although a direct causative effect is difficult to substantiate (2, 3) since a child who is undernourished during gestation and early infancy invariably suffers from other environmental consequences of poverty. The short-term effects of early life nutritional deprivation have been studied extensively in animals under controlled conditions (4, 5). This report describes persistent changes in amino acid levels in adult mice subjected to early life undernutrition.

Materials and Methods. Inbred DBA/2J and C57BL/6J mice were bred locally and raised in a temperature, light and sound controlled environment. Mature females were fed an 8% casein diet (Nutritional Biochemicals Corp., Cleveland) either beginning at or 2 wk prior to mating and continuing until the young were weaned to a normal 27% casein diet (Nutritional Biochemicals Corp., Cleveland) at 21 days of age. Control breeders were fed the normal diet throughout. Marked taste aversion to the low protein diet was documented resulting in approximately 60% reduction in total intake of food. This led to severe protein-calorie undernutrition.

Blood samples were obtained from the infra-orbital venous sinus of adult progeny and were transferred to blood collecting cards (6). Urine specimens, collected in metabolic cages, were preserved with thymol and stored frozen as necessary.

Chemical determinations were based on micro-thin-layer electrophoresis and chromatography on 5 × 5 cm cellulose layers (7-9). Ethanollic extracts of the equivalent of 1.6

μl whole blood were applied. Because of the high proteinuria in mice, urine samples were also extracted with ethanol, with the equivalent of 0.3-2.0 μl being spotted. The chromatograms were evaluated by visual comparison with each other and with quantitative standard patterns. The amino acids were subjectively categorized as "high," "moderately high," "moderate" and "low."

Results. The results are summarized in Table I. The normal blood amino acid pattern seen in adult progeny of females on the control diet showed prominently alanine, valine, the (unresolved) basic amino acids, glutamine, serine, threonine, glutamate and the (unresolved) leucines. DBA/2J mice from mothers fed the experimental diet beginning 2 wk before mating had a markedly reduced glutamine level in the blood. This difference did not occur in the progeny of mothers fed the low protein diet only from the time of mating nor in any of the control animals.

Amino acid patterns of urines of control progeny showed a large amount of what appeared to be polypeptides; taurine, leucine and/or isoleucine were generally found at a high level. One or more of the amino acids phenylalanine, valine and tyrosine were frequently seen on the DBA/2J chromatograms, but were never observed on those from the C57BL/6J mice. Urinary amino acids in progeny of DBA/2J mothers fed the low protein diet beginning at mating showed a marked general decrease in taurine while the rest of the amino acids appeared to remain the same. In urines from normal male C57BL/6J breeders who had been fed the low protein diet for 1 to 2 wk immediately preceding urine collection, taurine was also noticeably lower than in controls.

TABLE I. Blood and Urine Amino Acid Patterns of Adult Mice.

Strain	Diet	Age (days)	Sex	N	Variations in blood amino acid patterns			
					Urine taurine levels			
					High	Mod-high	Mod	Low
C57BL/6J	Control	105	M	10	Normal			
DBA/2J	Control	92	M	10	Normal			
DBA/2J	Experimental ^a	129-167	M	5	Normal			
DBA/2J	Experimental ^b	182-216	M	5	Glutamine low in 4/5			
					High	Mod-high	Mod	Low
C57BL/6J	Control	81-140	M	6	5	6	4	1
			F	10				
DBA/2J	Control	70-111	M	9	8	5	2	1
			F	7				
DBA/2J	Experimental ^a	40-200	M	4	0	1	1	6
			F	4				
DBA/2J	Experimental ^c	>70	M	5	0	0	1	4

^a Maternal diet from mating to weaning.

^b Maternal diet from 14 days pre-mating to weaning.

^c Fed the diet for 7-14 days preceding sampling.

Discussion. The substantial decreases in blood glutamine and urinary taurine seen in adult mice subjected to severe early life undernutrition suggests that there was a persistent metabolic response to this treatment. While the studies of Lee and Chow (10, 11) and Hsueh, Agustin and Chow (12) are not directly comparable to the present investigation, they also found changes persisting into adult life in rats that had been subjected to nutritional deprivation in early life. Low serum glutamine levels have been observed in intellectually impaired patients with untreated phenylketonuria (PKU) (13-17), an inborn error in which serum phenylalanine is grossly elevated and the transport of other essential amino acids reduced due to competitive inhibition (18). In children with marasmic kwashiorkor, 2 mo after dietary treatment had been started (19) plasma glutamine levels remained much reduced. Valine, threonine, taurine and tyrosine levels were also still low, while the other amino acids were normal. Before treatment, these children also had *increased* taurine levels in intracellular and extracellular fluids (skeletal muscle, plasma, urine), thought to be due to its impaired conversion to isethionic acid

(19). Experiments on the effects of dietary manipulation on taurine levels in rats have yielded conflicting results (20-22). Aside from obvious differences of species and diet schedule, factors other than diet *per se* may be involved. Physical trauma or administration of ACTH or glucocorticoids can increase urinary taurine (23-25). There may also be a sex dependent difference in utilization (20), although there was no evidence for such an effect in our mice.

A persistent diminution of taurine excretion might result from an alteration in kidney function. This is not considered likely since there was no increase in blood taurine nor in the urinary output of any of the other *beta* amino acids in the mice.

A reduction in the excretion of taurine can be postulated as due to either decreased synthesis from cysteine (22) or increased formation of nitrogen-free isethionate (19), either of which would effectively result in conservation of amino nitrogen. Preferential conversion of cysteine to pyruvate would provide a means of saving the carbon chain as well as the amino group.

The glutamine effect is more difficult to interpret, but the importance of this sub-

stance in ammonia metabolism is also suggestive of an adaptive response to conserve whatever nitrogen is available.

Summary. Four of five adult DBA/2J mice subjected to severe protein-calorie malnutrition in early life showed a notable reduction of blood glutamine levels while adult mice who had suffered a less severe nutritional deprivation showed a marked diminution of urinary taurine. It is suggested that there is a persistent metabolic adjustment in response to the need for increased nitrogen retention.

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1. Béhar, M., in "Malnutrition, Learning and Behavior" (N. S. Scrimshaw and J. E. Gordon, eds.), p. 30. M. I. T. Press, Cambridge, MA (1968).
2. Cravioto, J., in "Deprivation in Psychobiological Development," p. 38. Pan-Amer. Health Organization, Washington, DC (1966).
3. Dobbing, J., in "Applied Neurochemistry" (A. N. Davison and J. Dobbing, eds.), p. 287. Davis, Philadelphia (1968).
4. Dickerson, J. W. T., Dobbing, J., and McCance, R. A. Proc. Roy. Soc. Ser. B **166**, 396 (1967).
5. Dobbing, J., and Widdowson, E. M., Brain **88**, 357 (1965).
6. Guthrie, R., and Susu, A., Pediatrics **32**, 338 (1963).
7. Samuels, S., and Veliz, G., Clin. Chem. **17**, 51 (1971).
8. Samuels, S., J. Chromatogr. **32**, 751 (1968).
9. Samuels, S., and Fisher, C., J. Chromatogr. **71**, 297 (1972).
10. Lee, C. J., and Chow, B. F., J. Nutr. **87**, 439 (1965).
11. Lee, C. J., and Chow, B. F., J. Nutr. **94**, 20 (1968).
12. Hsueh, A. H., Agustin, C. E., and Chow, B. F., J. Nutr. **91**, 195 (1967).
13. McKean, C. M., and Peterson, N. A., N. Engl. J. Med. **283**, 1364 (1970).
14. Perry, T. L., Hansen, S., Tischler, B., Bunting, R., and Diamond, S., N. Engl. J. Med. **282**, 761 (1970).
15. Colombo, J. P., Arch. Dis. Childhood **46**, 720 (1971).
16. Wong, P. W. K., Berman, J. L., Partington, M. W., O'Flynn, M. E., and Hsia, D. Y. Y., N. Engl. J. Med. **285**, 580 (1971).
17. Hill, A., Macaulay, J., and Zalewski, W. A., Clin. Biochem. **5**, 194 (1972).
18. Neame, K. D., Nature (London) **192**, 173 (1961).
19. Vis, H. L., in "Caloric Deficiencies and Protein Deficiencies" (R. A. McCance and E. M. Widdowson, eds.), p. 119. Little, Brown, Boston (1968).
20. Awapara, J., J. Biol. Chem. **218**, 571 (1956).
21. Wu, C., J. Biol. Chem. **207**, 775 (1954).
22. Portman, O. W., and Mann, G. V., J. Biol. Chem. **220**, 105 (1956).
23. Pentz, E. I., Moss, W. T., and Denko, C. W., J. Clin. Endocrinol. Metabol. **19**, 1126 (1959).
24. Williamson, M. B., and Passman, J. M., Clin. Chem. **6**, 140 (1960).
25. Turner, F. P., and Brum, V. C., J. Surg. Res. **4**, 423 (1964).

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