Pediat. Res. *8:* 857–859 (1974)

Letters to the Editor

L. CATHELINEAU, J. M. SAUDUBRAY, C. CHARPENTIER, AND C. POLONOVSKI[11]

Hôpital Trousseau, Paris, France

The presence (in blood or urine), in high quantities, of the homoanalogues of substrates of the urea cycle in the presence of argininosuccinate synthetase deficiency gives rise to two different interpretations. Scott-Emuakpor *et al.* (10) postulate the existence of a late developing urea cycle for homoanalogues. Levin *et al.* (4) reject this possibility and tend rather towards the hypothesis of a competitive inhibition of lysine metabolism by citrulline.

Our point of view is different from the two preceding ones. We believe that lysine and homocitrulline accumulate because they are, respectively, substrates of ordinary ornithine carbamyltransferase (O.C.T.) and of ordinary argininosuccinate synthetase (A.S.S.).

With regard to O.C.T., we base our argument on two lines of reasoning. (*1*) Whenever we have observed OCT deficiencies, we have encountered hyperlysinemia as a constant factor. Before our patients were placed on a diet, the lysine level was always 2 or 3 times normal: 52 μmoles for the first patient (8), 72 for the second, 41.3 for third (9), 71 for the fourth (1); normal limits are between 11.4 and 26.9 μmol/100 ml serum. In addition, one of our patients underwent a lysine load and the peak of lysine was much higher than that of the control subject (1). On the other hand, two other studies (3, 4) have shown, utilizing purified bovine hepatic OCT, that lysine is a substrate of OCT with an affinity about 50 times less that for ornithine. Thus, in OCT deficiency the hyperlysinemia would be a consequence of a block on a normal metabolic pathway, however minimal.

One cannot be certain with regard to homocitrulline as a substrate of A.S.S., for this enzyme has not yet been completely purified (7). However, recently, Ratner (6) has obtained 10% of the activity with homocitrulline as substrate of bovine A.S.S.

Nevertheless, the accumulation of lysine and homocitrulline in A.S.S. deficiency is an indirect argument in favor of this hypothesis.

The accumulation of homocitrulline in persistent hyperlysinemias (2) would arise from the same mechanism: lysine not being catabolized by the main pathway which is blocked would shift to the urea cycle, in which the first step is the formation of homocitrulline from lysine by the action of O.C.T.

REFERENCES AND NOTES

1. Cathelineau, L., Navarro, J., Aymard, P., Baudon, J. J., Mondet, Y., Polonovski, C., and Laplane, R.: Hyperammoniémie héréditaire par anomalie qualitative de l'ornithine carbamyl transférase hépatique et intestinale. Arch Franc. Pédiat., *29:* 713 (1972).
2. Ghadimi, H.: The hyperlysinemias. In: J. B. Stanbury, J. B. Wyngaarden, and D. S. Fredrickson: The Metabolic Basis of Inherited Disease, Ed. 3, p. 397 (McGraw-Hill, New York, 1972).
3. Jasiorowska, B., and Kleczkowski, K.: Lysine as substrate of ornithine carbamyl-transferase. Bull. Acad. Polon. Sci. Ser. Sci. Biol., *18:* 373 (1970).
4. Levin, B., Oberholzer, V. G., and Palmer, T.: Citrullinemia and an alternative urea cycle. Pediat. Res., *7:* 728 (1973).
5. Marshall, M., Cohen, P. P.: Ornithine transcarbamylase from streptococcus faecalis and bovine liver. J. Biol. Chem., *247:* 1954 (1972).
6. Ratner, S.: Enzymes of arginine and urea synthesis. Advan. Enzymol. *39:* 13 (1973).
7. Rochovansky, O., and Ratner, S.: Biosynthesis of urea. XII. Further studies on arginino succinate synthetase: Substrate affinity and mechanism of action. J. Biol. Chem., *242:* 3839 (1967).
8. Saudubray, J. M., Cathelineau, L., Charpentier, C., Boisse, J., Allaneau, C., Le Bont, H., and Lesage, B.: Déficit héréditaire en ornithine carbamyl-transférase avec anomalie enzymatique qualitative. Arch. Franc. Pédiat. *30:* 15 (1973).
9. Saudubray, J. M., Cathelineau, L., *et al.:* In preparation.
10. Scott-Emuakupor, A., Higgins, J. V., and Kohrman, A. F.: Citrullinemia: A new case, with implications concerning adaptation to defective urea synthesis. Pediat. Res., *6:* 626 (1972).
11. Requests for reprints should be addressed to: C. Polonovski, M.D., Hôpital Trousseau, 26 Avenue du Dr. Arnold-Netter, 75571 Paris Cedex 12, France.
12. Accepted for publication March 11, 1974.

B. LEVIN, V. G. OBERHOLZER, AND T. PALMER

Queen Elizabeth Hospital for Children, London, England

We have read with great interest the letter written by Dr. Cathelineau *et al.* They agree with us that the high levels of lysine, homocitrulline, and homoarginine found in argininosuccinate synthetase deficiency do not necessarily indicate the existence of an alternative urea cycle, but they further conclude that the increases follow directly from the defective urea cycle enzyme and are not due to inhibition of lysine catabolism by citrulline. In support of their conclusion they report finding high levels of lysine in untreated patients with ornithine transcarbamylase deficiency. Although we have always found lysine levels to be normal in patients with this disease investigated after treatment has started, we have also observed an elevated level of lysine in plasma on a single occasion in an untreated patient (2). However, we have interpreted this finding differently. Ammonia has been reported to inhibit the catabolism of lysine via saccharopine (1) and we believe that inhibition by ammonia is the most likely explanation of the high lysine levels in untreated ornithine transcarbamylase deficiency. After treatment by low protein intake, levels of ammonia in blood are markedly reduced so there would be less inhibition of lysine catabolism as well as a reduced intake of lysine, and this would explain the normal lysine levels found in treated patients. There may be some inhibition of lysine catabolism by ammonia in argininosuccinate synthetase deficiency, in addition to the proposed inhibition by citrulline. The hypothesis that the raised levels of lysine in patients with urea cycle defects are caused by inhibition of a major lysine catabolic pathway seems more plausible than that they result directly from defects in a pathway which by all available evidence is of little or no importance for lysine metabolism. The significance of a metabolic pathway may best be judged by the build-up of metabolites when a defect occurs in that pathway; furthermore, the levels of homocitrulline in argininosuccinate

synthetase deficiency are extremely small compared with the levels of citrulline, even if it is assumed their elevation is a direct consequence of the enzyme defect. Also homoargininosuccinic acid has yet to be reported as present in argininosuccinate lyase deficiency.

Finally, we would like to point out a printer's error in our original letter, which may have confused some readers. Saccharopine is similar in structure to argininosuccinic acid, and not as stated.

REFERENCES AND NOTES

1. Fellowes, F. C. I.: Biosynthesis and degradation of saccharopine, an intermediate of lysine metabolism. Biochem. J., *136:* 321 (1973).
2. Palmer, T., Oberholzer, V. G., and Levin, B.: Amino acid levels in patients with hyperammonaemia and argininosuccinic aciduria. Clin. Chim. Acta, in press.
3. The present address of Dr. B. Levin is: East Ham Memorial Hospital, London E7 8QR, England. To whom correspondence and requests for reprints should be addressed.
4. Accepted for publication April 1, 1974.

On a Late Developing Urea Cycle

A. B. SCOTT-EMUAKPOR

Human Genetics Laboratory, Department of Zoology, University of Ibadan, Ibadan, Nigeria

The interpretation given by Cathelineau, Saudubray, Charpentier, and Polonovski (1) to the presence of high quantities of the homoanalogues of substrates of the urea cycle in the presence of argininosuccinate synthetase deficiency is very interesting. It, in fact, confirms our published work (6) on lysine transcarbamylation. In that publication, we showed (*1*) that the conversion of lysine to homocitrulline is similar to the conversion of ornithine to citrulline, (*2*) that in the conversion of either substrate, the same enzyme is involved, because of evidence of some competition of both substrates for the same enzyme, and (*3*) that in the reaction involving either lysine or ornithine, carbamylphosphate is required, which implies that these are transcarbamylation reactions and also suggests that ornithinecarbamyltransferase (OCT) is the enzyme involved.

We have also implied that the affinity of the enzyme for lysine is much less than that for ornithine (an affinity of 50 times less will be a fair approximation). In this regard, therefore, we are in agreement with the authors.

This fact notwithstanding, our postulate (5) of the existence of a late developing urea cycle still stands. We did not base our postulate solely on our observation of elevated homoanalogues of substrates of the urea cycle. It was also based on (*1*) the patient's ability to regulate his blood ammonia levels (although slowly) even on a high protein diet; and (*2*) the patient's ability to synthesize normal amounts of urea. If one does not accept our postulate, then homocitnilline and citrulline which are respectively the products of lysine and ornithine transcarbamylations will be "dead-end" products in argininosuccinate synthetase (A.S.S.) deficiency.

We felt, in reaching our conclusion, that the transcarbamylation of both ornithine and lysine to "dead-end" products cannot alone account for the normal levels of ammonia reported in our patient. We also felt that the transamidation of lysine to homoarginine reported by Ryan *et al.* (4) and the subsequent hydrolysis of the homoarginine to urea cannot alone account for the normal urea levels reported in our patient.

Ratner's work (3) has now provided a fresh evidence for the existence of our postulated cycle. He has obtained 10% bovine A.S.S. activity with homocitrulline as substrate.

Our postulated cycle is shown in Fig. 1. In the figure, *A* is the OCT-catalyzed lysine transcarbamylation shown by Scott-Emuakpor and Kohrman (6), suggested by Ryan *et al* (4), and now reported by Cathelineau *et al.* (1); *B* is the condensation of homocitrulline and aspartate by the enzyme A.S.S. to homoargininosuccinate (A.S.A.) recently shown by Ratner (3); *C* is the splitting of A.S.A. to homoarginine and fumarate by the enzyme argininosuccinase. The reverse of this reaction had been established by Strandholm *et al.* (8), which implies that the reaction indeed exists. *D* is the hydrolysis of homoarginine to urea by the enzyme arginase. This reaction has been known for some time (4), but had been dismissed as being of no practical consequence. Scott-Emuakpor (7) has shown that in this hydrolysis, lysine, as well as urea, is produced.

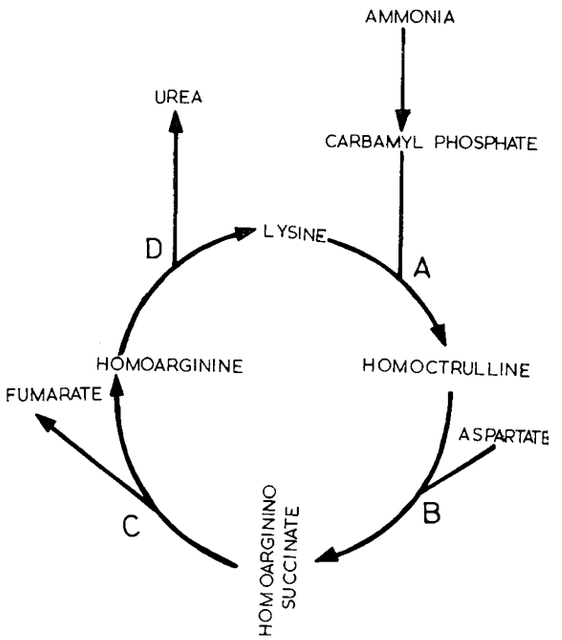

Fig. 1. Postulated lysine-urea cycle. For explanations of *A–D*, see the text.

It can be seen, therefore, that the recent work of Ratner (3) tied together the loose point of our postulated cycle. I do not think one can overlook the evidence before us.

It is for these same reasons that I find it difficult to accept the hypothesis of Levin *et al.* (2) that the elevated levels of homoanalogues of the urea cycle substrates can be accounted for by a competitive inhibition of lysine metabolism by citrulline.

REFERENCES AND NOTES

1. Cathelineau, L., Saudubray, J. M., Charpentier, C., and Polonovski, C.: Letters to the editor, Pediat. Res., *8:* 000 (1974).
2. Levin, B., Oberholzer, V. G., and Palmer, T.: Citrullinemia and an alternate urea cycle. Pediat. Res., *7:* 728 (1973).
3. Ratner, S.: Enzymes of arginine and urea synthesis. Advan. Enzymol., *39:*13 (1973).
4. Ryan, W. L., Barak, A. J., and Johnson, R. J.: Lysine, homocitrulline and homoarginine metabolism by the isolated perfused rat liver. Arch. Biochem. Biophys., *123:*294 (1968).
5. Scott-Emuakpor, A., Higgins, J. V., and Kohrman, A. F.: Citrullinemia: a new case with implications concerning adaptation to defective urea synthesis. Pediat. Res., *6:*626 (1972).