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Microbiological Determination of Apparent Free Methionine in the Blood of Children.

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Few studies on the blood levels of methionine, one of the amino acids indispensable for man, have been reported. By the use of the microbiological method of assay, Harper, Kinsell, and Barton,¹ found that the mean fasting level of apparent free *L*-methionine in the plasma of 11 normal male adults, was 0.85 ± 0.09 † mg per 100 ml with a range from 0.46 to 1.48. However, there has been no published information concerning the values in the newborn or older child. The purpose of this communication is to present the results of studies on the blood of (1) hospitalized "normal" children; (2) hospitalized children with various diseases; and (3) on the blood of the umbilical cord at the time of delivery.

Method. All of the children were in a post-absorptive state. Blood samples from them were obtained from either the antecubital or jugular vein. The "normals" had been hospitalized for the correction of non-infectious orthopedic difficulties. In the case of the newborn infants, cord blood was taken from the maternal end of the umbilical cord as soon as possible after delivery. Heparin was used as the anticoagulant in each case.

Proteins were removed from the plasma by heating and adding 5% acetic acid.¹ *L*-methionine in the filtrates was determined microbiologically, with *Leuconostoc mesenteroides* P-60 as the assay organism.² One ml of the basal medium recommended by Harper *et al.*¹ was used in each determination; the

plasma filtrates were added at 3 different levels (0.4, 0.6, and 1.0 ml); and the final volume was adjusted to 2 ml. All tubes were incubated for 72 hours and acid production was determined by electrometric titration with NaOH.

Results. The mean concentration of apparent free *L*-methionine in the plasma of "normal" children was 0.29 ± 0.02 mg per 100 ml of plasma. The range was from 0.19 to 0.47. The results are plotted in Fig. 1A. The difference between these levels and those found by Harper *et al.* in normal adult males with the same method of assay is statistically significant. Thirty-three hospitalized children, who were suffering from various diseases, had plasma methionine levels which were in the same range as those of the "normal" children. The mean was 0.42 ± 0.05 mg per 100 ml of plasma with a high of 1.07 and a low of 0.08 (Fig. 1B). At the time of testing, many of the children were convalescing, but there was no significant difference between their levels and those of the acutely ill ones.

Of 30 umbilical cord bloods tested, the mean level was 0.86 ± 0.04 mg per 100 ml of plasma (Fig. 1C). These results were in the same range as those found in adult males and were more than 3 times as high as those in "normal" children or those who had been ill.

Discussion. The foregoing data indicate that the plasma levels of apparent free *L*-methionine in the blood in the umbilical cord at birth is approximately the same as the fasting level reported for adult males.

At least two factors may operate to account for the lower plasma levels found in the children: (1) The main need for protein in the adult is to repair body tissue. The growing child needs an additional supply for growth and for the formation of new tissue. The increased demands in the child may lead to a greater rate of disappearance of essential

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¹ Harper, H. A., Kinsell, L. W., and Barton, H. C., *Science*, 1947, **106**, 319.

† Throughout this paper the figures following the symbol " $\pm$ " refer to the standard error of the mean.

² Dunn, M. S., Camien, M. N., Shankman, S., and Block, H., *J. Biol. Chem.*, 1946, **163**, 577.

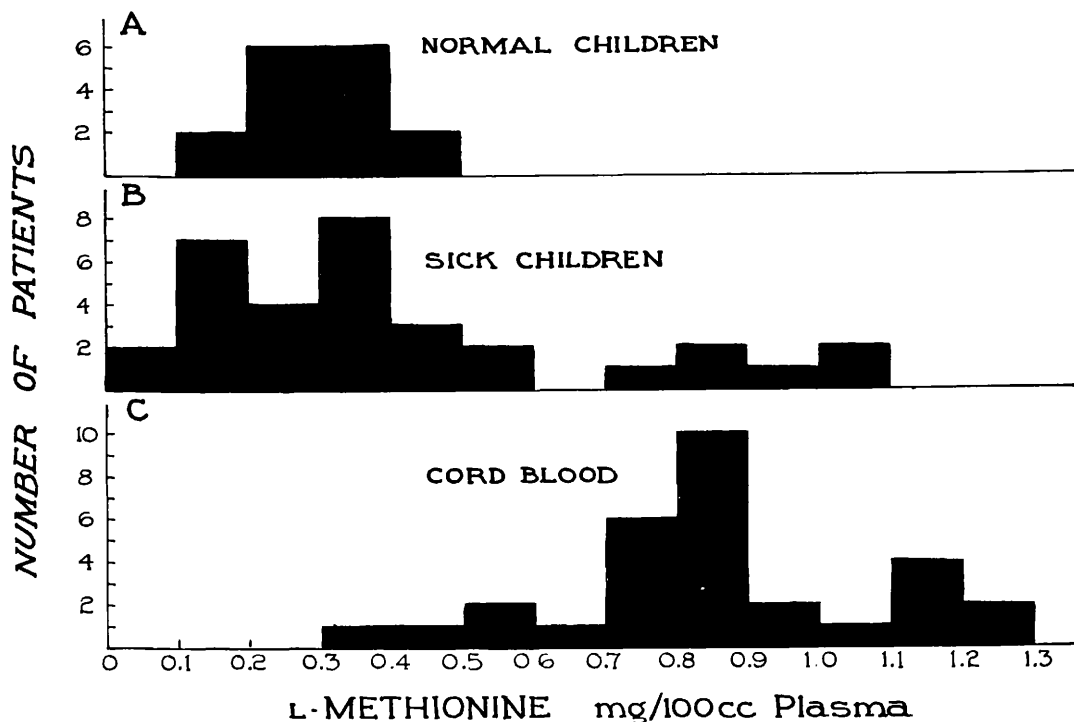


FIG. 1.

Apparent free *L*-methionine in plasma: (A) in hospitalized "normal" children; (B) in hospitalized children with various diseases; (C) in the blood in the umbilical cord at the time of delivery. The levels reported for normal adult males are 0.85 ± 0.09 mg per 100 ml of plasma.

amino acids from the blood. Hence, when an amino acid is determined in the plasma after a fasting period of 14-16 hours, less may be found in the child than in the adult. (2) A difference in the absorptive capacity of children and adults might play some part in making plasma levels of the former lower, but this seems unlikely. Preliminary tests in this laboratory, involving analysis of the blood of previously fasted subjects after the ingestion of 0.1 gm of methionine per kg of body weight, have shown that the amount of methionine absorbed in children approximates that found in adults.

Conclusion. The concentration of apparent free *L*-methionine in the plasma of children is significantly lower than has been recorded in the literature for adult male subjects. The plasma of the blood in the umbilical cord at the time of delivery contains, on the average, the same amount of *L*-methionine as is recorded for adult males, and more than three times as much as is present in the plasma of "normal" children.

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