Plasma Amino Acid Disturbance in Infancy
I: Hypermethioninaemia and Transient Tyrosinaemia

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For 3 years a prospective screening survey for neonatal aminoacidopathies has been carried out by this Unit, covering five boroughs in the neighbourhood of the Royal Manchester Children's Hospital, representing about 5000 births per year. The finding during this survey of transient tyrosinaemia in 49 newborn infants has already been reported (Wong, Lambert, and Komrower, 1967); with one exception the tyrosine level reverted to normal within 4 weeks of the initial test, during which time vitamin C (50 mg. daily) was given. The exception was a boy who showed consistent plasma tyrosine increases for 93 days in spite of vitamin C therapy. It is interesting to note that of 8 tyrosinaemic children investigated in detail by Wong et al. (1967), 7 showed in addition some increase in plasma methionine in samples taken within 10 days of the first blood test, though plasma tyrosine levels were increased to a greater degree than the methionine levels. In only one was the plasma methionine greater than 1·0 mg./100 ml. (0·07 μmole/ml.).

This communication reports observations on 3 newborn infants, with a brief description of a fourth, who revealed hypermethioninaemia on further testing after an initial detection of tyrosinaemia.

Material and Methods

Paper chromatography of the plasma of centrifuged capillary blood samples was carried out according to the method of Scriver, Davies, and Cullen (1964). Visual comparison of the methionine, phenylalanine, and tyrosine spots with control plasma specimens to which these three amino acids had been added individually and at graded levels, afforded a semiquantitative estimate of plasma levels. With this technique, the detection of plasma levels of phenylalanine and tyrosine greater than 4 mg./100 ml. was possible.

Plasma methionine levels were assessed using the same technique but with one modification. Two plasma specimens were applied to the chromatography paper; one was treated with a drop of hydrogen peroxide (100 vol., 30% w/v) and allowed to air dry before development with the chromatographic solvent. Under these conditions, methionine is oxidized to ninhydrin-positive products which differ in their solvent mobility from the parent amino acid. Methionine tends to run close to valine in the solvent used, and thus comparison of the colour intensity at the methionine position between the oxidized and unoxidized specimens enabled a semiquantitative assessment of plasma methionine levels greater than 2·5 mg./100 ml. Plasma methionine levels greater than 5 mg./100 ml. are, however, readily detected without using the oxidation technique. This, together with the previously indicated capability of this system for phenylalanine and tyrosine estimation, appears to justify its further trial in community screening surveys for phenylketonuria at present under much discussion (Medical Research Council, 1968), since a single specimen of capillary blood enables detection of several aminoacidopathies at one pass, e.g. phenylketonuria, tyrosinosis, homocystinuria, maple syrup urine disease, histidinaemia, and prolinaemia.

For column chromatography, fasting venous blood was collected in heparinized tubes. The plasma was deproteinized using sulphosalicylic acid after the method of Hamilton (1962). The protein-free supernatant was analysed on a Technicon amino acid analyser using either the 21-hour system (Technicon Instrument Co.) or the 4·5 hour rapid system of Evans et al. (1966). Internal standardization with norleucine was employed.

Other analytical methods are described by Wong et al. (1967).

Case Reports

Case 1. Born at 37 weeks; birthweight 2·9 kg.; twin, second child stillborn. Tyrosinaemia detected at 18 days. Vitamin C, 50 mg. daily, advised. At 8 weeks, plasma tyrosine normal, methionine raised. At 11 weeks, weight 5·8 kg.; general condition good, liver palpable 3 cm. below right costal margin, spleen not felt; plasma methionine raised, 2·9 mg./100 ml. (0·19 μmole/ml.), but the other amino acid levels were within normal limits. Urine amino acid pattern
normal: Blood count; Hb 7g./100 ml. WBC 7000 per cu.mm. (P10, L80, M7, E3%). Serum albumin 4.3 g./100 ml., globulin 1.6 g./100 ml., bilirubin < 1 mg./100 ml. Thymol turbidity 1 unit. Alkaline phosphatase 67 KA units. SGOT 108 RF units/ml. SGPT 72 RF units/ml. No clinical or radiological evidence of rickets. At this time the baby was admitted to hospital for observation. The hypermethioninaemia persisted (3.1 mg./100 ml.; 0.21 μ mole/ml.), and the serum iron was 30 μg./100 ml. Intramuscular iron (100 mg.) was given, and at 5 months Hb was 12 g./100 ml., alkaline phosphatase 33 KA units, and plasma methionine normal. Weight was 8-0 kg. At 10 months motor and general physical development was excellent. Weight 10-0 kg., plasma methionine normal. The liver edge was not palpable. Serum iron 100 μg./100 ml., with a saturation of 21%. Hb 14 g./100 ml. WBC, 121.500. (P31, L60, M5, E4%). Alkaline phosphatase 34 KA units/100 ml.

The feed throughout this period was a full-cream dried milk, and the protein intake approximately 5g./kg. body weight per day.

Case 2. Born at term, weight 3.2 kg. Tyrosin- aemia was evident at 10 days of age and vitamin C (50 mg. daily) was advised. At 6 weeks a significantly raised plasma methionine level (greater than 5 mg./100 ml.) was detected, although tyrosine was not raised. At 7 weeks, weight 4.7 kg., she was developing normally; urine showed a normal amino acid pattern. Capillary blood showed persisting hypermethioninaemia (5 mg./100 ml.). At 10 weeks, normal development had been maintained, weight 5.7 kg., both urine and capillary blood were found to be within normal limits on amino acid chromatography. However, there was some degree of normochromic anaemia (Hb 9.2 g./100 ml.), erythrocytes showing anisocytosis. The white cell count was 13,800/cu.mm., with a relative lymphocytosis (56%). During this period the feed was a full-cream dried milk, protein intake being 4-4.5 g./kg. body weight per day.

Case 3. Born at 31 weeks after 16 years of marriage. Lower segment caesarean section. Birthweight 1.7 kg. At 6 weeks, plasma tyrosine 15 mg./100 ml. Vitamin C (50 mg. daily) advised. At 12 weeks, both plasma tyrosine and methionine levels were raised. At 13 weeks (weight 4.4 kg.), general condition good; column chromatography showed marked increase in plasma methionine (11.9 mg./100 ml.; 0.8 μ mole/ml.) and tyrosine (5.6 mg./100 ml.; 0.3 μ mole/ml.). Other amino acid levels were normal or slightly raised. Serum albumin 5.0 g./100 ml., globulin 1.9 g./100 ml. Apparent reduction of γ-globulin. SGOT 84 units/ml., SGPT 52 units/ml. Alkaline phosphatase 35 KA units/100 ml. Urine amino acid chromatogram normal. At 15 weeks, there was little change. Weight 4.8 kg. Liver 1 cm. palpable below right costal margin, methioninaemia persisting. Immunoelectrophoresis IgG 250 mg./100 ml.; IgA 25 mg./100 ml.; IgM < 5 mg./100 ml.

The protein intake during this time had been 6 g./kg. per day, with a daily methionine intake of 700 mg. (140 mg./kg.). This was reduced to 360 mg. daily (70 mg./kg.) the diet based on Trufood Low Phenylalanine and Tyrosine (without methionine), with an allowance of cows' milk. At 18 weeks, weight 5.6 kg. Liver as above. Plasma methionine 0.7 mg./100 ml. (0.05 μ mole/ml.); the other amino acid levels were normal. Serum alkaline phosphatase 28 KA units/100 ml. Hb 8.6 g./100 ml., with relative lymphocytosis (82%). Serum globulin 1.1 mg./100 ml.; IgG 260 mg./100 ml.; IgA 10 mg./100 ml.; IgM 13 mg./100 ml.

At 5 months the boy's condition was excellent, with good physical and mental development. Weight 6.9 kg. Methionine intake 250 mg. daily (65 mg./kg. per day).

Case 4. Born at term, weight 3.8 kg., given artificial milk feed (4 g. protein/kg. per day). At 10 days hypermethioninaemia (greater than 5 mg./100 ml. plasma). This persisted at 6 weeks (between 2.5 and 5.0 mg./100 ml.). At 7 weeks, general condition good, plasma amino acid levels now normal; urine chromatogram normal; Hb 9.4 g./100 ml.

Discussion

In Cases 1, 2, and 3 reported in detail, there was a similar sequence of events, i.e. an initial tyrosin- aemia at 10 to 12 days was followed by hypermethioninaemia at 6 to 12 weeks, together with anaemia when tested at 10 to 18 weeks. Hypermethioninaemia has been described in 3 cases of hepatic cirrhosis (accompanied in the one case investigated in detail, by a gross aminoaciduria with an appreciable tyrosine and methionine excretion, as well as the keto acid derived from methionine (Perry et al., 1965)), and in 2 cases of 'neonatal hepatitis' of an acute nature (Cusworth, Dubowitz, and Harvey, 1966). Hypermethioninaemia also occurs as a secondary manifestation of severe liver damage in acute tyrosinosis (Gjessing and Halvorsen, 1965; Scrivener, Clow, and Silverberg, 1966) attributed primarily to severe loss of functioning hepatic tissue. This claim has been questioned by Gaul, Rassin, and Sturman (1968) who showed a loss of specific activity of methionine activating enzyme and cystathionine synthetase in two cases of acute tyrosinosis with cirrhosis and accompanying hypermethioninaemia, while the specific activity of cystathionase was normal. In the 3 cases reported here in detail there was no indication of severe liver disturbance, though an increase in serum alkaline phosphatase and transaminase levels was found in Cases 1 and 3, with hypo-γ-globulinaemia in the latter child. In no case was there an increased urinary excretion of tyrosine, methionine, or any other amino acid.
It is possible that in each case (including Case 4) the hypermethioninaemia was the result of an excessive intake of the amino acid, aggravated in Case 3 by prematurity, with an accompanying delay in the maturation of the transsulphuration enzyme systems. Studies on the development of the methionine-activating enzyme system in rat liver have revealed that the specific activity of this enzyme begins to rise in late fetal life, reaching a maximum 2 days after birth (Chase, Volpe, and Laster, 1968), though there are no such data for full-term and premature infants. An excessive intake of methionine could occur when a cows' milk diet is given, since it contains considerably more protein (and methionine) than does human milk. It is interesting that the 22 cases of hypermethioninaemia detected in the neonatal period by this Unit were all given artificial milk feeds.

After 15 weeks on artificial milk feeds Case 3 responded satisfactorily within 3 weeks to dietary regulation of methionine. Though the restriction was from 140 to 70 mg./kg. per day, the regimen still exceeded the methionine requirement of 45 mg./kg. per day suggested by Holt and Snyderman (1965) for infants. Snyderman et al. (1968) have reported an increase in plasma methionine in normal infants fed a high intake of milk protein (9 g./kg. per day). This observation was also made by Levy et al. (1968) in the course of a screening survey similar to that carried out in this Unit; marked plasma methionine increases were detected in 3 infants, aged 5 to 9 weeks, who were given at least 11 g. protein/kg. per day. In each case reduction of protein intake to 3 g./kg. per day resulted in a return of plasma amino acid levels to normal.

One could explain the sequence of events as follows: as a result of a high protein intake the plasma methionine becomes raised, leading to a minor disturbance of hepatocellular tissue. This, in its turn, might be reflected in a slight increase of serum enzymes of hepatic origin.

The cause of anaemia in these children with hypermethioninaemia remains obscure. The results of dietary experiments with animals suggest a possible explanation. Klavins, Kinney, and Kaufman (1963) and Klavins and Johansen (1965) fed rats a dietary excess of methionine, which resulted in a redistribution of body iron, with iron deposited in the spleen and, to a lesser extent, in the liver. The rats developed a microcytic, hypochromic anaemia, erythroid hyperplasia of the bone-marrow, and some increase in circulating leucocytes, though there were no significant differences in the differential counts. Thus, a relation seems to exist between methionine and the haemopoietic system. Klavins et al. (1963) suggest that since excess methionine has been shown to impair the absorption of histidine by chicken intestine (Taylor, Newman, and Paine, 1959), and histidine is claimed to be the limiting amino acid in the biosynthesis in vitro of rabbit reticulocyte proteins (Borsook, Fischer, and Keighley, 1957), these two factors contribute to the anaemia.

In 20 of the 22 cases, the hypermethioninaemia reverted to normal levels within 12 weeks; in 5 children within 4 weeks of birth. Four infants were examined in the hospital because the raised amino acid level persisted beyond 7 weeks: the general condition of each baby was good and the examination was negative apart from a significant degree of anaemia.

A diet with some reduction of methionine was introduced in one case only (Case 3) because of the duration of the hypermethioninaemia, and this seems to be the only indication for a deliberate lowering of the methionine intake per se. In the main a slight and temporary adjustment of the daily protein consumption should suffice.

We are not able to explain the reason for the initially raised alkaline phosphatase levels—an observation made by Levy et al. (1968)—which are somewhat higher than our own normal values for the first year of life. However, the later levels in each instance, though appreciably lower, were still raised, and could be explained by the rapid growth of the infant at the time of the examination. This finding apart, there is no evidence at the present time to suggest that the temporary hypermethioninaemia has produced any permanent harm to the baby.

**Summary**

Transient hypermethioninaemia was detected in 22 infants during a prospective screening survey for aminoacidemias. All the infants were artificially fed, and where full information was available, the daily protein intake was in excess of 4 g./kg.

Three cases of more prolonged hypermethioninaemia are described, in each of which there was a significant degree of anaemia and a rise in alkaline phosphatase.

There is no evidence as yet that a temporary hypermethioninaemia produces any permanent harm.

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REFERENCES


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