MENTAL RETARDATION, MEGALOBLASTIC ANAEMIA, METHYLIMALONIC ACIDURIA AND ABNORMAL HOMOCYSTEINE METABOLISM DUE TO AN ERROR IN VITAMIN $B_{12}$ METABOLISM


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SUMMARY

1. The case is described of a child with retarded physical and mental development, recurrent megaloblastic anaemia, methylmalonic aciduria and abnormal homocysteine metabolism resulting from an inborn error in the metabolism of cobalamin. She died at the age of 7 years. At autopsy there was pulmonary fibrosis and the brain showed lesions typical of those seen in subacute combined degeneration of the cord.

2. A metabolic abnormality was present which resulted in an inability to maintain normal tissue concentrations of the two coenzyme forms of vitamin $B_{12}$, methylcobalamin and adenosylcobalamin. Lack of methylcobalamin led to deficient activity of $N^5$-methyltetrahydrofolate-homocysteine methyltransferase with reduced ability to methylate homocysteine, and lack of adenosylcobalamin to deficient activity of methylmalonyl-CoA mutase, which accounted for the methylimalonic aciduria.
3. Analyses of total vitamin B$_{12}$ and of individual cobalamins by a chromato-
biaautographic technique showed that in organs sampled at autopsy, the content of
total vitamin B$_{12}$ and of methylcobalamin, adenosylcobalamin and hydroxocobalamin
were all greatly reduced. The plasma had a high normal total vitamin B$_{12}$ content, but
showed a gross abnormality in the distribution of individual cobalamins, methyl-
cobalamin being decreased and adenosylcobalamin and hydroxocobalamin increased.
The erythrocytes showed a reduction in cobalamins resembling that in the solid
organs, though less severe.

4. The underlying abnormality in this patient appeared to be either a defect in
cellular uptake of vitamin B$_{12}$, or a defect in a metabolic pathway leading to the
formation of a common precursor of methylcobalamin and adenosylcobalamin.
Abnormalities in transcobalamins I and II, plasma factors involved in plasma trans-
port and cellular uptake of vitamin B$_{12}$, were excluded.

5. The clinical and biochemical findings in the patient are compared with those
described in three patients previously reported, who had methylmalonic aciduria and
homocystinuria, and who were in some respects similar to this patient. The present
case is unusual in that previous examples of errors in cobalamin metabolism have
not had megaloblastosis or neurological changes typical of vitamin B$_{12}$ deficiency.
It is also the first case in which direct estimations of individual cobalamins have been
made.

Key words: cobalamins, error in vitamin B$_{12}$ metabolism, methylmalonic aciduria,
megaloblastic anaemia, homocysteine metabolism, mental deficiency, fibroblasts.

In recent years several patients have been described who excrete excessive amounts of both
homocystine and methylmalonic acid in their urine (Mudd, Levy & Abeles, 1969; Levy, Mudd,
Schulman, Dreyfus & Abeles, 1970; Goodman, Moe, Hammond, Mudd & Uhlendorf,
1970). Investigations have revealed that the activities of at least two enzymes are affected in
these patients: (1) A decrease of $N^5$-methyltetrahydrofolate–homocysteine methyltransferase
activity leads to abnormalities of the metabolism of sulphur-containing amino acids, including
a tendency to accumulate and excrete excessive amounts of homocyst(e)ine and cystathionine,
and an inability to maintain normal concentrations of methionine in plasma and tissues. (2)
A decrease in the activity of methylmalonyl-CoA mutase (methylmalonyl-CoA CoA-carbonyl-
mutase, EC 5.4.99.2) leads to excessive urinary excretion of methylmalonic acid. The two
enzymes in question are the only ones in mammals known to require vitamin B$_{12}$ derivatives
for catalytic activity. The evidence further indicates that the decreases in these enzyme activities
are chiefly due not to lack of the apoenzyme proteins, but rather to lack of the required co-
enzymically active vitamin B$_{12}$ derivatives, methylcobalamin and adenosylcobalamin (Mudd
et al., 1969; Goodman et al., 1970; Mudd, Levy & Morrow, 1970a; Mudd, Uhlendorf, Hinds
& Levy, 1970b; Mahoney, Rosenberg, Mudd & Uhlendorf, 1971). These findings are most
readily explained by postulation of a defect in the cellular uptake or metabolism of vitamin
B$_{12}$ which renders the mutant cells unable to accumulate normal concentrations of either
methylcobalamin or adenosylcobalamin.

In this paper we report the case of a mentally retarded child with biochemical findings
similar to those described above. The patient differed from previous patients in that she had
recurrent episodes of megaloblastic anaemia. The existence of an error in the metabolism of vitamin B$_{12}$ was first indicated by the finding of methylmalonic aciduric acid, and the demonstration that though the total plasma vitamin B$_{12}$ was normal, proportions of individual forms of vitamin B$_{12}$ (cobalamins) in the plasma were highly abnormal. The patient died at the age of 7 years, and at post mortem was shown to have lesions in the central nervous system closely resembling those of subacute combined degeneration of the cord. The application of recently developed methods for chromato-bioautographic separation and quantification of individual cobalamins enabled us to show by direct estimation that amounts of methylcobalamin and adenosylcobalamin were abnormally low in several organs, and to extend the studies previously carried out in patients with similar defects in vitamin B$_{12}$ metabolism.

The following abbreviations will be used: adenosylcobalamin (5'-deoxyadenosylcobalamin), Ado-B$_{12}$; methylcobalamin, Me-B$_{12}$; hydroxocobalamin, OH-B$_{12}$; cyanocobalamin, CN-B$_{12}$.

CASE REPORT

The patient (M.M.), born 13 August 1964, was the first child of unrelated parents. Her younger brother was born with an anomaly of the chest wall which required a rib graft but is otherwise well at age 4 years.

History

Age 2 months. She was admitted to hospital because of feeding difficulties, vomiting and failure to thrive. Evidence of urinary infection was found but her symptoms persisted despite treatment.

Age 6 months. She was first admitted to the Hospital for Sick Children under the care of Dr G. H. Newns. With the exception of a red scaly rash on her face and trunk, no abnormalities were found on examination. Haematological investigation showed macrocytosis and a megaloblastic bone marrow (Fig. 1). Haemoglobin was 1.6 mmol/l (10.3 g/100 ml). Serum total vitamin B$_{12}$ (Euglena gracilis, z strain) was 738 pmol/l (1000 pg/ml) and serum folate (Lactobacillus casei) 35 nmol/l (15.6 ng/ml). Serum iron was 10 µmol/l (56 µg/100 ml) and total iron-binding capacity 65 µmol/l (360 µg/100 ml). Evidence of persistent urinary infection was found but no radiological abnormality of her renal tract could be demonstrated. Amino-aciduria was also found (see the Results section).

She was treated with a variety of antibiotics and her vomiting became less frequent, but 6 weeks after admission she developed pneumonia. Although her condition improved with further antibiotic treatment, chest X-rays showed persistent patchy opacities in both lungs and after blood transfusion (80 ml of packed cells), open lung biopsy was carried out. This showed the changes of resolving pneumonia. Before transfusion, haemoglobin was 1.13 mmol/l (7.3 g/100 ml). Transfusion was followed by a 3-month course of ferrous sulphate and ascorbic acid. At the end of this, haemoglobin had returned to normal. In view of the serum vitamin B$_{12}$ and folate concentrations, neither vitamin B$_{12}$ nor folic acid was given.

After discharge from hospital she made slow progress and during the next few years it became clear that considerable mental retardation was present. She was found to be functioning at the 18-month and 15-month levels when assessed at 3 years and 4½ years respectively, suggesting intellectual deterioration. She remained difficult to feed and for most of her life her diet consisted largely of milk and Twiglets (a cocktail biscuit containing yeast extract),
with supplements of vitamins A, C and D, thiamin, riboflavin, pyridoxine and nicotinamide. An approximate estimate of folate intake can be made. Between the age of 5 and 7 years she ate 200–300 g of Twiglets and drank about 1 l of milk per day. The milk contained at least 0-11 μmol (50 μg) of folate (Ford & Scott, 1968). The folate contents of Twiglets, estimated according to Chanarin (1969a), were: free folate 0-54 nmol/g (0.24 μg/g) and total folate 1-24 nmol/g (0.55 μg/g), so that her folate intake from this source was 0.25–0.37 μmol and her total daily intake of folate 0.36–0.49 μmol (equivalent to 160–215 μg of folic acid). Her vitamin B₁₂ intake, derived entirely from the milk was probably about 6·8 nmol (3 μg)/day (Chanarin, 1969b).

After measles at the age of 4 years 8 months she became more apathetic, with persistent cough and a transient scaly rash on her trunk.

Age 5 years. She was readmitted to hospital with signs of pneumonia. Her height, weight and head circumference were all below the third centile for her age and she showed evidence of considerable mental retardation. There were frequent blinking movements of her eyes which were thought to be minor convulsions and her ECG showed excess slow-wave activity with frequent irregular discharges. At this time her muscle tone and tendon reflexes were considered normal. Megaloblastic anaemia (haemoglobin 1·10 mmol/l; 7·1 g/100 ml) was again present (Fig. 1). The reticulocyte count was 4%, and a slightly raised reticulocyte count was found

![Graph](image-url)
Error in vitamin $B_{12}$ metabolism

on several other occasions (Fig. 1). Serum vitamin $B_{12}$ was 700 pmol/l (950 pg/ml) and serum folate was 26 nmol/l (11-5 ng/ml). She was treated with blood transfusion (300 ml of packed cells) and an intravenous dose of folic acid (34 $\mu$mol; 15 mg) in addition to antibiotics and digoxin. Although her general condition slowly improved, she had frequent episodes of central cyanosis. These episodes, which persisted for the rest of her life, were not associated with hypercapnia and could be rapidly reversed by giving oxygen.

Three weeks after the blood transfusion, she was treated with cyanocobalamin, 0.74 $\mu$mol (1000 $\mu$g) daily for 5 days. There appears to have been a haematological response to this (Fig. 1). Her parents considered that she became hyperactive and more difficult to manage after this treatment. Serum immunoglobulins estimated at the age of 5 years were normal.

**Age 5 years 6 months.** She was readmitted for further investigation. Apart from the appearance of a few well-defined erythematous patches on her trunk, there had been no significant change in her condition. During this admission she was found to be excreting large amounts of methylmalonic acid in her urine (see the Results section). Though haemoglobin was 2.30 mmol/l (14.8 g/100 ml), the bone marrow was megaloblastic. Total serum vitamin $B_{12}$ remained in the higher part of the normal range, but analysis of individual plasma cobalamins showed a grossly abnormal pattern with a large reduction in Me-$B_{12}$ (see the Results section, Table 1). This finding, in conjunction with the methylmalonic aciduria, first suggested that M.M. had an abnormality of cobalamin metabolism. Serum folate was greater than 73 nmol/l (32 ng/ml). A Figlu test was carried out, with an oral load of 19.4 mmol of L-histidine (3 g), and 0.13 mmol (22 mg) of Figlu was excreted in 8 h. The upper limit of normal for the method was 0.10 mmol (18 mg), but it was thought that an excretion of 0.13 mmol was not sufficiently elevated to be definitely abnormal. Repeated blood gas and electrolyte analysis showed no evidence of acidosis. There was no appreciable change in the methylmalonic aciduria after a single intramuscular injection of 0.63 $\mu$mol (1000 $\mu$g) of Ado-$B_{12}$.

After discharge from hospital there was little change in her condition until she developed a rubella-like illness at the age of 6 years 9 months. This was followed by gradual deterioration with unsteadiness, which progressed until she was unable to walk. Her rash recurred, her tongue became bright red and her vulva was inflamed.

**Age 7 years 3 months.** She was readmitted to hospital. At this time there was marked clubbing of her fingers and toes, her tongue was red and atrophic and there was extensive pigmentation over her vulva and buttocks. She showed little interest in her surroundings and had almost continual blinking movements of her eyes. Her tendon reflexes were exaggerated and the plantar responses extensor. Although her muscles were, in general, hypotonic, there was tightness of the adductor muscles of the thighs. Haemoglobin was 1.78 mmol/l (11.5 g/100 ml). The blood film showed macrocytosis.

Large amounts of methylmalonic acid were again present in her urine and she was treated with a further course of Ado-$B_{12}$, 0.44 $\mu$mol (700 $\mu$g) given intramuscularly daily for 8 days. Her clinical condition precluded investigation of the effect of this treatment on her methylmalonic aciduria but she continued to deteriorate with signs of pneumonia. She died a month after admission, aged 7 years 4 months.

**Autopsy findings**

The main pertinent abnormal findings were as follows. The brain was small (860 g) with frontal and parietal atrophy. It showed histological changes typically associated with sub-
M. J. Dillon et al.

Acute combined degeneration of the cord (Barrett, 1913; Woltman, 1918). The bone marrow was hyperactive. The lungs had numerous red or brown nodules on the cut surfaces, and showed fibrosis and the changes of recent pneumonia. The autopsy findings are reported in detail by Dayan & Ramsay (1974).

SPECIAL INVESTIGATIONS: METHODS

Estimation of plasma and tissue cobalamins

Total plasma vitamin B₁₂ was estimated by radioisotopic assay (Matthews, Gunasegaram & Linnell, 1967). Total vitamin B₁₂ in tissues was estimated by radioisotopic assay after homogenization and extraction with a 0.2 mol/l sodium acetate buffer, pH 4.5, containing 0.6 mmol/l KCN. Samples for estimation of individual cobalamins were handled with precautions against exposure to light. Cobalamins were extracted with hot ethanol and individual cobalamins were separated and estimated by one- or two-dimensional thin-layer chromatography and bioautography as previously described (Linnell, Hussein & Matthews, 1970; Linnell, Hoffbrand, Peters & Matthews, 1971; Linnell, Hoffbrand, Hussein, Wise & Matthews, 1974). With the two-dimensional method, four cobalamins could be estimated: Me-B₁₂, CN-B₁₂, Ado-B₁₂ and OH-B₁₂. In samples analysed by one-dimensional chromatography only, the last two compounds could not be separated and were estimated together as Ado-B₁₂ + OH-B₁₂.

Estimation of urine methylmalonic acid

This was measured in 24 h urine collections by a quantitative gas-chromatographic procedure (Gompertz, 1968). Its identity was confirmed by gas-chromatography–mass spectrometry (Gompertz & Draffan, 1972).

Methylmalonyl-CoA mutase activity

This was measured in leucocyte homogenates by using saturating concentrations of substrate and Ado-B₁₂. The assay was linear with time and the amount of leucocyte protein added. The synthesis of D,L-[methyl-¹⁴C]methylmalonyl-CoA, the incubation conditions and the gas radiochromatographic assay of products are described in detail elsewhere (Goodey & Gompertz, 1972).

The measurement of the conversion of methylmalonyl-CoA into succinate in unsupplemented liver homogenates was performed so that the concentration of endogenous Ado-B₁₂ and not methylmalonyl-CoA mutase apoenzyme might be rate-limiting. Assays in which additional Ado-B₁₂ was added were also performed. Under these conditions, however, the reaction did not follow linear kinetics. The liver, removed within 5 h of death, was prepared by the method of Morrow, Barness, Cardinale, Abeles & Flaks (1969). The incubation mixture and the measurement of the conversion of the D,L-[methyl-¹⁴C]methylmalonyl-CoA into succinate were as described for the leucocyte assay (Goodey & Gompertz, 1972); 0.5–1.0 mg of protein was used per assay.

Ion-exchange chromatography of amino acids

Quantitative estimation of amino acids in plasma and urine was carried out by ion-exchange chromatography by the method of Spackman, Stein & Moore (1958).
Identification of urine homocystine

To achieve unequivocal identification of urine homocystine, a sample of the compound was isolated from the urine by preparative column chromatography. The isolated material was subjected to (a) oxidation with performic acid, (b) reduction with dithiothreitol, and conversion into the N-ethylmaleimide derivatives, and (c) hydrolysis with 6 mol/l HCl in an evacuated, sealed flask at 105°C for 18 h. The ninhydrin-reactive product(s) formed had the same properties during column chromatography as respectively homocysteic acid, the N-ethylmaleimide derivatives of homocysteine and the material formed by treatment of authentic homocystine with 6 mol/l HCl under the specified conditions. The last-named product eluted between valine and cystine, but was not further identified.

Culture of fibroblasts

Fibroblasts were cultured from a skin biopsy taken at the age of 7 years. They were grown in the dark and those to be used for cobalamin analysis were harvested under dark-room conditions. They were grown in Eagle's (1959) minimal essential medium, which also contained the non-essential amino acids and 10% dialysed serum.

Enzyme activities in fibroblast extracts

Cystathionine synthase activity. The activity of this enzyme was estimated in fibroblast extracts as described by Mudd, Finkelstein, Irreverre & Laster (1965).

N5-Methyltetrahydrofolate-homocysteine methyltransferase activity. This was estimated in gel-filtered fibroblast extracts as described by Mudd et al. (1970b).

Investigation of transcobalamin

Serum (0.5 ml) was mixed with [57Co]CN-B12 (0.07 pmol) and allowed to equilibrate for 1 h at room temperature. The sample was then fractionated on a Sephadex G-100 column (150 cm long x 2.2 cm diameter) with 0.04 mol/l phosphate buffer, pH 7.4, in 0.96 mol/l sodium chloride. Fractions (5 ml) were collected and the radioactivity in each was measured.

SPECIAL INVESTIGATIONS: RESULTS

Plasma and erythrocyte cobalamin

The results of estimation of plasma cobalamins in M.M. and two other patients are shown in Table 1, together with normal values. The first plasma sample from M.M. was examined in May 1970 (aged 5½ years) after the demonstration of methylmalonic aciduria. The total plasma vitamin B12 was at the upper limit of normal. The distribution of individual cobalamin was grossly abnormal. Concentrations of Ado-B12 and OH-B12 were both raised. The Me-B12 concentration was slightly subnormal in absolute terms, and markedly subnormal when expressed as a percentage of total vitamin B12, Me-B12 making up only 10% of the total vitamin B12 whereas in normal plasma Me-B12 constitutes more than half the total. Analysis of a second sample 6 days later gave almost identical results. Four hours after a test dose of 15.8 nmol (25 μg) of Ado-B12, given intramuscularly, Me-B12 increased from 87 to 494 pmol/l. After 28 h, plasma Me-B12 had fallen to 268 pmol/l, but was still within the normal range. Over a year later (July 1971) the plasma Me-B12 concentration (235 pmol/l) remained within
TABLE 1. Plasma cobalamins in patient M.M. and two other patients with methylmalonic aciduria

Patient J.R. showed a disturbance similar to that of M.M. Patient S.H. had a deficiency of methylmalonyl-CoA mutase. i.m., Intramuscular injection.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Total vitamin B$_{12}$ (pmol/l)$^{(1)}$</th>
<th>Me-B$_{12}$ (pmol/l)</th>
<th>CN-B$_{12}$ (pmol/l)</th>
<th>Ado-B$_{12}$ (pmol/l)</th>
<th>OH-B$<em>{12}$ + OH-B$</em>{12}$ (pmol/l)$^{(2)}$</th>
<th>Ratio Me-B$<em>{12}$: (Ado + OH-B$</em>{12}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M.M.</td>
<td>5$^{6/12}$</td>
<td>804 (1090) 87 0 330 387 717 0.12</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>6 days pretreatment</td>
<td></td>
<td>812 (1100) 87 0 334 391 725 0.12</td>
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<tr>
<td></td>
<td>Immediately predose</td>
<td></td>
<td>1476 (2000) 494 0 — — 982 0.50</td>
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<tr>
<td></td>
<td>4 h after 15.8 μmol of Ado-B$_{12}$ i.m.</td>
<td></td>
<td>830 (1125) 268 0 — — 562 0.48</td>
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<tr>
<td></td>
<td>28 h after 15.8 μmol of Ado-B$_{12}$ i.m.</td>
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</tr>
<tr>
<td>M.M.</td>
<td>6$^{11/12}$</td>
<td>609 (825) 235 40 — — 334 0.70</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>J.R.</td>
<td>14</td>
<td>531 (720) 106 0 304 121 425 0.25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S.H.</td>
<td>10</td>
<td>675 (915) 622 0 — — 53 11.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal values$^{(3)}$ (mean and range)</td>
<td>&amp; - -</td>
<td>385 (522) 276 2.4 60 47 107 2.9</td>
<td>&amp; - -</td>
<td>&amp; - -</td>
<td>&amp; - -</td>
<td>&amp; - -</td>
<td></td>
</tr>
</tbody>
</table>

$^{(1)}$ Calculated as CN-B$_{12}$. $^{(2)}$ Calculated as Ado-B$_{12}$. $^{(3)}$ Linnell et al. (1971).
the normal range, though it still formed an abnormally low proportion of the total vitamin B\textsubscript{12}.

Analyses of two additional plasma samples are included for comparison. (1) J.R. (Goodman et al., 1970) has a similar disturbance to that found in M.M. Plasma from this patient shows changes similar to those in plasma of M.M., Me-\textsubscript{B12} being subnormal and Ado-\textsubscript{B12} raised, with the total vitamin B\textsubscript{12} within normal limits (Table 1). (2) S.H. This patient showed a deficiency of methylmalonyl-CoA mutase activity (Oberholzer, Levin, Burgess & Young, 1967). Plasma from S.H. had a cobalamin pattern entirely different from that observed with plasma from M.M. or J.R. Me-\textsubscript{B12} was slightly elevated and Ado-\textsubscript{B12 + OH-B12} were within normal limits. The results of the plasma analyses in M.M. and J.R. emphasize that normal or slightly raised total plasma vitamin B\textsubscript{12} concentrations may mask gross alterations in the concentrations of individual cobalamins.

**Tissue cobalamins**

Table 2 shows analyses of organs obtained from M.M. at autopsy, together with control values similarly obtained from children without evidence of vitamin B\textsubscript{12} deficiency or disturbance of vitamin B\textsubscript{12} metabolism. The major abnormality in organs from M.M. was a great decrease in all forms of vitamin B\textsubscript{12}. Total vitamin B\textsubscript{12} in liver was particularly low, being less than 2\% of control values. There was a moderate increase in the proportion of OH-B\textsubscript{12} in all tissues, accompanied by a decrease in the proportion of Ado-B\textsubscript{12}. In brain, the proportion of Me-\textsubscript{B12} was markedly depressed. Table 2 also gives values for erythrocyte cobalamins obtained during life. The decrease in erythrocyte cobalamins was less severe than in the tissues taken at autopsy, values for Me-\textsubscript{B12} and Ado + OH-\textsubscript{B12} falling in the lower part of the normal range.

**Cobalamins in cultured fibroblasts**

Studies of cultured fibroblasts (to be reported) showed that accumulation of Me-\textsubscript{B12} and Ado-\textsubscript{B12} was abnormally decreased in cells from M.M. Accumulation of OH-\textsubscript{B12} was also reduced, in agreement with the results of analyses of tissues obtained at post-mortem examination.

**Methylmalonic acid excretion**

Quantitative results revealed that during a 25 day period at age 5\frac{1}{2} years the urinary methylmalonic acid excretion varied from 4-0 to 6-2 mmol/day (470–730 mg/day). The excretion of methylmalonic acid was not significantly affected by the administration of single intramuscular doses of either 15-8 or 633 nmol (25 or 1000 \textmu g) of Ado-\textsubscript{B12}.

**Methylmalonyl-CoA mutase in extracts of leucocytes and liver**

Methylmalonyl-CoA mutase activity in leucocytes from M.M. was measured on two occasions: at age 5\frac{1}{2} years and during her terminal admission. The findings on the two occasions were essentially the same. The assays were performed with saturating concentrations of substrate and Ado-\textsubscript{B12} and thus reflect the sum of holo- and apo-enzyme activities present. Under the conditions used, leucocytes from M.M. showed no diminution in the rate at which they converted methylmalonyl-CoA into succinate. In the assay performed during the terminal admission, 27-4 nmol of succinate was formed h\textsuperscript{-1} mg of protein\textsuperscript{-1}. The mean value for eight
<table>
<thead>
<tr>
<th>Tissue</th>
<th>Total B₁₂</th>
<th>Me-B₁₂</th>
<th>CN-B₁₂</th>
<th>Ado-B₁₂</th>
<th>OH-B₁₂</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nmol/l</td>
<td>ng/g</td>
<td>nmol/l</td>
<td>%</td>
<td>nmol/l</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M.M.</td>
<td>10.0</td>
<td>13.6</td>
<td>0.9</td>
<td>9.0</td>
<td>0.2</td>
</tr>
<tr>
<td>Controls (6) (1)</td>
<td>615</td>
<td>834</td>
<td>68</td>
<td>11</td>
<td>24</td>
</tr>
<tr>
<td>±122</td>
<td>±166</td>
<td></td>
<td>±25</td>
<td>±2.9</td>
<td>±8.8</td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M.M.</td>
<td>14.2</td>
<td>19.2</td>
<td>1.8</td>
<td>12.6</td>
<td>0.2</td>
</tr>
<tr>
<td>Controls (5)</td>
<td>169</td>
<td>229</td>
<td>30</td>
<td>18</td>
<td>1.0</td>
</tr>
<tr>
<td>±30</td>
<td>±41</td>
<td></td>
<td>±6.2</td>
<td>±2.7</td>
<td>±0.7</td>
</tr>
<tr>
<td>Spleen</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>M.M.</td>
<td>6.1</td>
<td>8.3</td>
<td>1.5</td>
<td>24</td>
<td>0</td>
</tr>
<tr>
<td>Controls (3)</td>
<td>23.6</td>
<td>32</td>
<td>6.1</td>
<td>26</td>
<td>0.2</td>
</tr>
<tr>
<td>±2.8</td>
<td>±3.8</td>
<td></td>
<td>±1.7</td>
<td>±8.2</td>
<td>±0.1</td>
</tr>
<tr>
<td>Brain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M.M.</td>
<td>6.7</td>
<td>9.1</td>
<td>0.2</td>
<td>3.0</td>
<td>0.1</td>
</tr>
<tr>
<td>Controls (6)</td>
<td>38.3</td>
<td>52</td>
<td>5.0</td>
<td>13</td>
<td>0.2</td>
</tr>
<tr>
<td>±7.4</td>
<td>±10</td>
<td></td>
<td>±1.0</td>
<td>±3.1</td>
<td>±0.1</td>
</tr>
</tbody>
</table>

| Erythrocytes |        |      |        |    |        |    |        |    |        |    |        |    |
| M.M. (2)     | 81     | 110  | 8.4    | 10 | 4.2    | 5.2| 68     | 84|        |    |
| Controls (12) (3) | 149  | 202  | 26     | 19| 8.9    | 6.0| 114    | 74|        |    |
| ±19          | ±25    |      | ±3.4   | ±3.4| ±2.1   | ±1.2| ±18    | ±3.0|        |    |

(1) Number of subjects (age range 1-15 years).
(2) At the age of 7 ½ years, 6 weeks before death.
(3) Normal adults (Linnell et al., 1974).
(4) Calculated as Ado-B₁₂.
adult control subjects was $18.6 \pm \text{SEM} 1.5 \text{ nmol h}^{-1} \text{ mg of protein}^{-1}$. In contrast, the value for R.H., a child with a type of methylmalonic aciduria which did not respond to treatment with vitamin $\text{B}_12$ and was not accompanied by homocystinuria, was $1.1 \text{ nmol h}^{-1} \text{ mg of protein}^{-1}$.

The results of measurements of the capacity of undialysed liver extracts from M.M. and a control patient to convert $\text{DL-methylmalonyl-CoA}$ to succinate are presented in Table 3. The extent to which extracts from the liver of M.M. carried out this conversion was markedly decreased in the absence of added $\text{Ado-B}_12$.

**Plasma and urine amino acids**

At the age of 6 months, analysis of the urine by qualitative paper chromatography showed an excess of lysine, histidine, glycine, alanine, serine, threonine, glutamine and asparagine. At 5 years, a similar analysis showed an excess of glycine, threonine, serine and alanine. On both occasions qualitative chromatography of plasma amino acids showed no obvious abnormality.

<table>
<thead>
<tr>
<th>TABLE 3. Conversion of methylmalonyl-CoA into succinate by liver extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td>The figures show percentage conversion of methylmalonyl-CoA into succinate in 30 min, and, in parentheses, nmol of succinate formed 30 min$^{-1}$ mg of protein$^{-1}$.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Endogenous Ado-$\text{B}_{12}$</th>
<th>Saturating Ado-$\text{B}_{12}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal child</td>
<td>48% (30)</td>
<td>68% (44)</td>
</tr>
<tr>
<td>M.M.</td>
<td>0.7% (1)</td>
<td>72% (106)</td>
</tr>
</tbody>
</table>

At 7 years (May 1970), a 24 h sample of urine was analysed by ion-exchange chromatography. Compared with control values (Levy et al., 1970) this showed a decrease in excretion of methionine (13 $\mu$mol/24 h) and an increase in excretion of glycine (2000 $\mu$mol/24 h). Homocystine, which is normally absent from the urine, was present (23 $\mu$mol/24 h). On another occasion in the same month, urinary excretion of homocystine was 47 $\mu$mol/24 h. The urine also contained material giving a ninhydrin-positive peak eluting in the position of cystathionine. Calculated as cystathionine, this amounted to 29 $\mu$mol/24 h, although insufficient urine was available to permit identification of this material. Since a chronic urinary tract infection in M.M. almost certainly gave rise to some bacteriuria, the possibility that some or all of the homocystine detected in her urine resulted from bacterial metabolism of cystathionine (Levy & Mudd, 1973) has not been excluded. Thus M.M. excreted one or more abnormal sulphur-containing amino acids, but the true balance between homocystinuria and cystathioninuria cannot be established.

The cause of the increased excretion of non-sulphur-containing amino acids was not clear. Generalized amino aciduria associated with raised plasma concentrations of many amino acids has been described in pernicious anaemia (Heathcote, Davies & Mooney, 1971). A fasting sample of plasma could not be obtained. Analysis of a random plasma sample showed moderate elevations in valine (340 $\mu$mol/l), histidine (197 $\mu$mol/l) and isoleucine (103 $\mu$mol/l) when compared with normal fasting values, but these elevations are unlikely to be of any
significance in a non-fasting sample. Methionine (18.2 μmol/l) and other plasma amino acids were within normal limits (Levy et al., 1970). Homocystine was not detected, but the sample had been stored frozen before deproteinization, a procedure known to result in disappearance of plasma homocystine (Carson, Cusworth, Dent, Field, Neill & Westall, 1963) so that this finding does not exclude significant homocystinaemia.

**TABLE 4. Growth of fibroblasts in media supplemented with methionine or homocystine in M.M., control subjects, a patient with methylenetetrahydrofolate reductase deficiency and a patient with aberrant vitamin B₁₂ metabolism**

Methionine and choline were omitted from the culture medium and replaced by the supplements shown. Methionine or homocystine was added to a final concentration of 0.1 or 0.05 mmol/l respectively. Several replicate flasks were inoculated with approximately 250 × 10⁵ cells of each fibroblast line, and then incubated for 1 day with unsupplemented medium. The cells in one flask for each cell line were then harvested to yield ‘zero-time’ measurements range: 210–258 × 10⁵ cells. The cells in the remaining replicate flasks were then re-fed with media supplemented as shown. Re-feeding was repeated on day 2 after ‘zero time’ and all cells were harvested on day 6. J.S. was the unaffected sibling of a child with Nieman-Pick disease. See Mudd et al. (1972) for discussion of the case of B.M. and Goodman et al. (1970) for discussion of J.R.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Clinical state</th>
<th>Increase in cell number after ‘zero time’ (%)</th>
<th>Relative increase (homocystine/methionine)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Supplement to medium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M.M.</td>
<td>Present case</td>
<td>None</td>
<td>Methionine</td>
</tr>
<tr>
<td>J.M.</td>
<td>Normal volunteer</td>
<td>20</td>
<td>900</td>
</tr>
<tr>
<td>J.S.</td>
<td>Control subject</td>
<td>30</td>
<td>950</td>
</tr>
<tr>
<td>B.M.</td>
<td>Methylenetetrahydrofolate reductase deficiency</td>
<td>20</td>
<td>1000</td>
</tr>
<tr>
<td>J.R.</td>
<td>Aberrant vitamin B₁₂ metabolism</td>
<td>0</td>
<td>740</td>
</tr>
</tbody>
</table>

*Cystathionine synthase activity*

The activity of this enzyme in an extract of fibroblasts cultured from M.M. was 16.7 nmol of cystathionine formed 135 min⁻¹ mg of protein⁻¹. This value is within the control range (3.7–70), a finding which is strongly against the possibility that the excessive homocystine excretion was due to cystathionine synthase deficiency (Uhlendorf & Mudd, 1968; Uhlendorf, Conerly & Mudd, 1973).

*Methylation of homocysteine*

Several experiments were carried out to evaluate the capacity of fibroblasts from M.M. to methylate homocysteine. As shown in Table 4, control human fibroblasts are able to grow in media in which methionine has been replaced by homocysteine. Previous experiments have demonstrated that under these conditions the methionine required for growth is generated through the action of N⁵-methyltetrahydrofolate–homocysteine methyltransferase. Cells for any reason deficient in this reaction will not grow (Mudd et al., 1970b; Mudd, Uhlendorf, Freeman, Finkelstein & Shih, 1972). This observation was confirmed in the present experi-
A direct demonstration of the localization of this block was supplied by assays of activities of \( N^5 \)-methyltetrahydrofolate–homocysteine methyltransferase in gel-filtered fibroblast extracts, with and without supplementation with Me-B\( \text{B}_{12} \), the cobalamin derivative which is a cofactor for the reaction in question (Table 5). Under our standard conditions, extracts from control fibroblasts catalyse formation of 2.9–7.3 nmoles of methionine 60 min\(^{-1}\) mg of protein\(^{-1}\) from homocysteine and \( N^5 \)-methyltetrahydrofolate in the absence of exogenous Me-B\( \text{B}_{12} \). Addition of Me-B\( \text{B}_{12} \) stimulates these rates approximately two-fold, showing that the methyltransferase in such extracts is present roughly half as apoenzyme, half as holoenzyme. Growth of control cells in the presence of OH-B\( \text{B}_{12} \) leads to the accumulation of more methyltransferase, which is then predominantly in the holoenzyme form. The extracts of fibroblasts grown from M.M. differed from control extracts in several respects. There was little, if any, \( N^5 \)-methyltetrahydrofolate–homocysteine methyltransferase activity if cells were grown in the basal medium and the extracts were assayed without added Me-B\( \text{B}_{12} \). Addition of Me-B\( \text{B}_{12} \) to the reaction mixture stimulated activity to a value only a little below the control range. Growth of M.M. fibroblasts in the presence of OH-B\( \text{B}_{12} \) resulted in accumulation of a measurable amount of methyltransferase holoenzyme, but both holoenzyme and total methyltransferase remained far below the amounts found in control extracts produced under similar conditions. In all these aspects the results with cells from M.M. were similar to those observed with cells from J.R. and B.M., each of whom is blocked at a different site in the pathway in question (Goodman et al., 1970; Mudd et al., 1972). The fibroblasts from M.M. failed to grow upon the medium in which homocysteine had replaced methionine, suggesting this patient, too, is blocked in the \( N^5 \)-methyltetrahydrofolate-dependent conversion of homocysteine into methionine.

### Table 5. \( N^5 \)-Methyltetrahydrofolate–homocysteine methyltransferase activities in extracts of fibroblasts from patient M.M., control subjects and patients with aberrant vitamin B\( \text{B}_{12} \) metabolism

<table>
<thead>
<tr>
<th>Cells grown in:</th>
<th>Assay conditions:</th>
<th>Basal medium</th>
<th>Basal medium+OH-B( \text{B}_{12} ) (0.74 μmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No added B( \text{B}_{12} )</td>
<td>With Me-B( \text{B}_{12} )</td>
<td>No added B( \text{B}_{12} )</td>
</tr>
<tr>
<td>Control subjects(^{(1)})</td>
<td>2.9–7.3</td>
<td>6.1–11.3</td>
<td>11.5–18.7</td>
</tr>
<tr>
<td>Patients with homocystinuria and methylmalonic aciduria</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M.M.</td>
<td>0.06</td>
<td>5.2</td>
<td>1.8</td>
</tr>
<tr>
<td>E.M.(^{(1)})</td>
<td>0.03</td>
<td>4.5</td>
<td>0.9</td>
</tr>
<tr>
<td>J.R.(^{(2)})</td>
<td>0.60</td>
<td>3.0</td>
<td>1.6</td>
</tr>
<tr>
<td>M.R.(^{(2)})</td>
<td>0.40</td>
<td>2.7</td>
<td>1.3</td>
</tr>
</tbody>
</table>

\(^{(1)}\) Values reported by Mahoney et al. (1971).

\(^{(2)}\) Values reported by Goodman et al. (1970).
from E.M., J.R. or M.R. (i.e. the three other known patients with homocystinuria and methylmalonic aciduria), as is shown by comparison with the previously reported results, which are included in Table 5.

Investigation of transcobalamins

When the patient's serum was labelled with $[^{57}\text{Co}]\text{CN-B}_1$ the resulting binding pattern was normal. Approximately 20% of the applied radioactivity eluted with the TC I peak and 80% with TC II. Similar results have been reported by others after fractionating normal serum on Sephadex G-200 (Hom, Olesen & Lous, 1966).

**DISCUSSION**

Patient M.M. died at the age of 7 years after a history of chronic illness and poor physical and mental development. She had several episodes of megaloblastic anaemia, retarded bone age, persistent central cyanosis and a tendency to develop infections. Biochemical and enzymic investigations suggested that her methylmalonic aciduria was due to lack of Ado-B$_{12}$, and that she had difficulty in methylating homocysteine because of lack of Me-B$_{12}$. Cobalamin analysis provided extensive direct evidence in support of this. Liver, kidney, spleen and brain, obtained at post-mortem examination of M.M., each contained abnormally low concentrations of Me-B$_{12}$, Ado-B$_{12}$, OH-B$_{12}$ and total vitamin B$_{12}$. These deficiencies are particularly striking in view of the fact that 1 month before death she had received a total of 3.5 μmol (5600 μg) of Ado-B$_{12}$ parenterally. Calculations based on the data for M.M. in Tables 1 and 2 indicate that less than 22 nmol (30 μg) of total vitamin B$_{12}$ can be accounted for in the plasma and tissues which were analysed. It appears that she did not long retain the major proportion of the Ado-B$_{12}$ given to her. It is probable that M.M.'s total body vitamin B$_{12}$ was less than 74 nmol (100 μg), whereas the normal total in a child of this age might be 740–1480 nmol (1000–2000 μg).

Cobalamin analyses of plasma samples obtained before vitamin B$_{12}$ treatment present a pattern somewhat different from those for her tissues. Although the plasma concentration of Me-B$_{12}$ was abnormally low, the concentrations of Ado-B$_{12}$ and OH-B$_{12}$ were elevated. From a diagnostic point of view, it is particularly important that total plasma vitamin B$_{12}$ was not depressed. Thus an analysis making this estimation only, as in the routine clinical determination, would have failed to reveal the specific abnormality present in M.M.

The biochemical findings in the case of M.M. are very similar to those reported previously for three patients, each of whom showed methylmalonic aciduria and homocystinuria secondary to inability to accumulate normal cellular concentrations of either Ado-B$_{12}$ or Me-B$_{12}$ (Mudd et al., 1969, 1970a, b; Goodman et al., 1970; Mahoney et al., 1971). Clinically, two features of M.M. are especially noteworthy and stand in contrast to those present in the patients previously studied. (a) Megaloblastic anaemia was first noted in M.M. at 6 months of age. This anaemia underwent an unexplained series of changes in severity, disappearing at about 1 year, recurring at 5 years, and being less marked during hospitalizations at 5 years 6 months and 7 years 3 months. (b) M.M. showed progressive dementia with severe mental retardation, together with brief convulsions and neurological signs compatible with a diagnosis of subacute combined degeneration of the cord. The neuropathological changes in the brain were typical of this condition. Patients with a defect in cellular uptake of vitamin B$_{12}$, or in synthesis
Error in vitamin $B_{12}$ metabolism

of the two coenzyme forms of vitamin $B_{12}$ as postulated in M.M., might be expected to develop the haematological and neurological changes of classical vitamin $B_{12}$ deficiency syndromes, and the absence of such features in the previous patients with aberrant vitamin $B_{12}$ metabolism has been rather puzzling. The first of these patients, E.M. (Levy et al., 1970), died at 7 weeks after a progressive downhill course which included poor feeding, failure to gain weight, haematemesis, melaena, normocytic anaemia, azotaemia and terminal generalized convulsions. It was suggested that he might have developed megaloblastic anaemia had he lived longer. The other two patients (Goodman et al., 1970) are brothers who appear to be less severely affected than were either M.M. or E.M. The older, J.R., was well at age 14 years except for moderate mental retardation and mild non-specific neurological abnormalities. He had disproportionately long extremities, somewhat like a patient with Marfan’s syndrome. His younger brother, M.R., was well at the age of 2½ years. The homocystinuria may have been delayed in appearance since this amino acid was not present in the urine of M.R. at 1 year, but was detected at age 2 years.

An important question is whether the episodes of megaloblastic anaemia in M.M. might have resulted from folate deficiency due to an inadequate diet. A recommended dietary intake of folic acid for a child of 2–8 years is 0.45 µmol (200 µg)/day (Recommended Dietary Allowances, 1968) so that M.M.’s intake of 0.36–0.48 µmol (160–215 µg) was probably adequate. Unfortunately, neither erythrocyte nor liver folate was estimated. Since the normal or high serum folate values may not be a reliable index of folate status owing to the methylcobalamin deficiency, the possibility of folate deficiency cannot be positively excluded.

In considering the similarities and differences between M.M. and the other patients with combined homocystinuria and methylmalonic aciduria, it may be well to remember that the exact metabolic error has not been specifically defined in any case among this group of patients. The biochemical pathways by which mammals convert $OH-B_{12}$, or other forms of vitamin $B_{12}$, into coenzymically active derivatives have been little studied. Cellular uptake is sure to be a necessary step, and is likely to be followed by reduction of the cobalamin as a prelude to alkylation with either the methyl or adenosyl moieties to form the coenzymically active compounds (Fig. 2; see also review by Mahoney & Rosenberg, 1970). In the bacterium Clostridium tetanomorphum, two separate enzymes are thought to catalyse the stepwise reduction of $OH-B_{12}$ (cobalt in the $Co^{3+}$ valence state) to vitamin $B_{12r}$ ($Co^{2+}$) and then vitamin $B_{12s}$ ($Co^{+}$) (Walker, Murphy & Huennekens, 1969). A disruption at the level of cellular uptake, or at either of the two reductive steps, could potentially interfere with the formation of both Me-$B_{12}$ and Ado-$B_{12}$ and so, in theory, produce the syndrome of homocystinuria and methylmalonic aciduria. Further possibilities would include a specific enzymic destruction of, or failure to retain, alkylated forms of vitamin $B_{12}$.

The present results of cobalamin analyses in M.M. clearly demonstrate that she was deficient in both Me-$B_{12}$ and Ado-$B_{12}$. However, in our opinion, these results do not permit a decision between the several possible causes of the combined deficiency. On the one hand, a defect in cellular uptake would be expected to bring about a decrease in total cellular vitamin $B_{12}$ as was actually observed. It would not, however, account for the observed tendency for the proportions of Me-$B_{12}$ and Ado-$B_{12}$ to decrease unless it were assumed that the kinetic relationships between uptake and synthesis of the coenzyme forms were such that a decrease in uptake led to a disproportionate reduction in their synthesis. On the other hand, a defect in an enzyme catalysing the reduction of $OH-B_{12}$ or $B_{12r}$ would readily account for a decrease
in the proportions of Me-B\textsubscript{12} and Ado-B\textsubscript{12}, but not for the decrease in total vitamin B\textsubscript{12}, unless it were postulated that the cell were unable to retain the metabolite which would tend to accumulate as a result of the metabolic deficiency. Thus simple models involving defects in either the uptake or the early metabolic conversions of vitamin B\textsubscript{12} will not completely account for our observations unless further ad hoc assumptions be introduced. Another feature that cannot be satisfactorily accounted for in the present state of knowledge is the increase in both Ado-B\textsubscript{12} and OH-B\textsubscript{12} in the plasma, accompanied by a reduction in Me-B\textsubscript{12}.

Several lines of evidence argue that if any of the group of patients with combined homocystinuria and methylmalonic aciduria has a defect in cellular uptake of vitamin B\textsubscript{12}, this defect must be due to a lesion in some cellular factor and not to an abnormality of extra-cellular transport factor(s) normally present in the plasma. This conclusion is supported, first, by the persistence of the characteristic biochemical abnormalities in cells of M.M., E.M., J.R. and M.R. in tissue culture under conditions in which these cells, as well as control cells, were grown in a uniform extra-cellular milieu. Secondly, the clinical syndrome caused by abnormal or missing transcobalamin II, a plasma factor thought to be involved in some aspects of vitamin B\textsubscript{12} transport and cellular uptake, appears to be appreciably different from that manifested by any of the four patients with aberrant vitamin B\textsubscript{12} metabolism under discussion here. This is shown by recent reports of two patients with virtually no detectable transcobalamin II who presented with vitamin B\textsubscript{12}-responsive megaloblastic anaemia, but no abnormality of methylmalonate or sulphur-containing amino acid metabolism (Hakami, Neiman, Canellos & Lazerson, 1971; Scott, Hakami, Teng & Sager, 1972). Thirdly, in the case of M.M., the direct evidence reported here of normal transcobalamins I and II in the plasma adds additional support to the suggestion that any possible abnormality of uptake of vitamin B\textsubscript{12} by her cells must be due to a defective cellular factor.
Together, these considerations indicate that, as a group, the patients with combined homocystinuria and methylmalonic aciduria have cellular lesion(s) affecting vitamin $B_{12}$ uptake or metabolism and causing cellular deficits of Me-$B_{12}$, Ado-$B_{12}$ and total vitamin $B_{12}$. Further studies of the pertinent transport system(s) and enzymes will be required to distinguish the exact lesion or lesions. Thus it is quite possible that within the small group of patients already studied there are represented different underlying metabolic abnormalities. The extent to which this possibility accounts for chemical and/or clinical diversity within this group remains to be determined.

The case of M.M. illustrates the possibility that a patient with aberrant vitamin $B_{12}$ metabolism may first attract clinical attention because of megaloblastic anaemia. It seems quite possible that other such patients will first be discovered by demonstration of methylmalonic aciduria, or a low plasma Me-$B_{12}$. In two previous patients, E.M. and J.R., homocystinuria was the first biochemical abnormality to come to light. The demonstration of any one of these findings should therefore stimulate a thorough search for additional components of this syndrome. In this context, it may be well to emphasize that the block in homocysteine metabolism, which may be crucial in helping to pinpoint the underlying defect, has been reflected in previous patients by the excretion of a relatively small amount of homocysteine, which, although definitely elevated above normal, is not as great as the amount excreted in the more widely known cystathionine synthase deficiency and may require sensitive methods to permit detection. The type of homocystinuria in question may be accompanied by homocystinaemia, cystathioninaemia, cystathioninuria and hypomethioninaemia (Levy et al., 1970), or these abnormalities may not be detectable (Goodman et al., 1970).

Theoretical considerations lead to the expectation that patients with aberrant vitamin $B_{12}$ metabolism may benefit from treatment with large doses of vitamin $B_{12}$. These expectations are supported by the demonstrations that the metabolic abnormalities in fibroblasts of E.M. were partially overcome by growth in the presence of high concentrations of OH-$B_{12}$ (Mudd et al., 1970b) and that the methylmalonate excretion of J.R. decreased more than 90% after parenteral administration of a total of 3.7 $\mu$mol (5000 $\mu$g) of OH-$B_{12}$ over a 5-day period. A large dose was required, since a single dose of 0.37 $\mu$mol (500 $\mu$g) of this compound produced little, if any, effect. CN-$B_{12}$ appeared to be somewhat less effective (Goodman, Keyser, Mudd, Schulman, Turse & Lewy, 1972), and Ado-$B_{12}$ was not more effective than OH-$B_{12}$ (S. I. Goodman, personal communication). In the present studies we show that the methylmalonate excretion of M.M. was not significantly decreased in the 10 days after an intramuscular dose of 0.63 $\mu$mol (1000 $\mu$g) of Ado-$B_{12}$. In view of the data on J.R., this finding does not preclude the possibility that similar patients may respond to higher doses of vitamin $B_{12}$, perhaps given as OH-$B_{12}$, and a trial of such therapy is certainly merited in future cases.

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