Short report

Neurological involvement in hereditary transcobalamin II deficiency

PK THOMAS,* AV HOFFBRAND,† IS SMITH*‡

From the Departments of Neurological Science* and Haematology,† Royal Free Hospital School of Medicine, London

SUMMARY   A case of hereditary transcobalamin II deficiency with neurological involvement is described. The patient presented in early infancy with megaloblastic anaemia and was treated with folinic acid from 6 weeks of age. The diagnosis of transcobalamin II deficiency was not made until he was 2 years old when he showed severely retarded intellectual development, ataxia and a pyramidal deficit in the limbs. Following treatment with hydroxocobalamin, his condition has slowly improved but he has remained with a severe neurological deficit. The consequences of vitamin B_{12} deficiency on neurological development in infancy are discussed.

Inherited lack of transcobalamin II (TC II), one of the two main vitamin B_{12} binding proteins in serum, is known to give rise to megaloblastic anaemia in the neonatal period.[1-3] Neurological abnormalities were present in the case described by Burman et al,[3] but this child had received vitamin B_{12} irregularly since infancy. Recently a patient with an abnormal rather than absent TC II has been reported.[4] The present report describes a further case of this type which displayed both haematological and neurological involvement. It demonstrates the effect of severe vitamin B_{12} deficiency during the first two years of life on the development of the nervous system.

Case report
The patient (Royal Free Hospital No 019548) is a male child aged 7 yr who was born in Australia to healthy unrelated Maltese parents in April, 1974. A brother had died aged 6 months and had shown peripheral pancytopenia and a megaloblastic marrow. He has two healthy older sisters. He was admitted to hospital at the age of 26 days because of feeding difficulties. His haemoglobin level was normal (11·7 g/dl), but fell to 7·4 g/dl within 2 weeks. His bone marrow was megaloblastic. A diag-

‡Present address: Department of Clinical Neurophysiology, National Hospital, Queen Square, London.

Address for reprint requests: Prof PK Thomas, Department of Neurological Science, Royal Free Hospital, Pond Street, London NW3 2QG, UK.

nosis of dihydrofolate reductase deficiency was made and he was started on daily parenteral treatment with folinic (5-formyltetrahydrofolic acid) (see Tauro et al,[5] Case 2). At 6 months of age he was stated to have had normal mental and physical development.

He was first seen in London in 1976. He had continued to receive injections of folinic acid (6 mg daily). His peripheral blood count had remained normal. He became able to sit up unsupported at 9 months and at one year was able to pull himself up and walk if his hands were held. He had never said any clear words. After 12 months of age both his mental and motor development regressed. He had suffered from repeated chest infections related to inhalation of liquids. When examined at the age of 2 years, his head circumference was 46 cm (below third centile for age). He was able to support his head. His fundi, pupils and external ocular movements were normal, but his face was hypotonic with salivary drooling. His limbs were hypotonic. There was a jerky ataxia of the upper limbs during movement. He was unable to sit unsupported. His tendon reflexes were all depressed except for normal knee jerks and both plantar responses were extensor. He responded to painful stimuli bilaterally. Frequent spontaneous attacks of short duration were observed in which his head became extended and the limbs rigid. During these he was unresponsive. His peripheral blood count showed a haemoglobin level of 11·8 g/dl. PCV 34·6%, RBC 4·63 × 10^{12}/l, MCV 74 fl, MCH 25 pg; WBC 8·4 × 10^9/l, 28% neutrophils, 60% lymphocytes, 11% monocytes and 1% eosinophils; platelets 180 × 10^9/l. The serum vitamin B_{12} level was 490 ng/l (normal range 160-925 ng/l) and serum folate 32 µg/l (normal range 3-20 µg/l). The detailed haemato-

logical and biochemical studies will be presented elsewhere (AV Hoffbrand, M Frater-Schroder, E Tripp, BFA Jackson and WE Luck, to be published). A liver biopsy showed normal dihydrofolate reductase activity. Methylmalonic acid was not detected in the urine. Measurement of the total unsaturated serum vitamin B_{12} binding capacity and of the binding capacities of the individual transcobalamins I, II and III were performed on the patient, his parents and his two sisters. The patient showed absence of TC II binding and his parents and sibs approximately half normal values. Immunological testing, however, has revealed the presence of some TC II which is functionally inactive (AV Hoffbrand *et al*, to be published). Lumbar puncture revealed normal cell and protein contents in the cerebrospinal fluid (CSF). The CSF vitamin B_{12} level was 10 ng/l and the folate level 79 μg/l. His electroencephalogram showed mixed theta and delta activity symmetrically over both cerebral hemispheres. During the recording a tonic seizure lasting 11 s occurred at which time sharp components were seen bilaterally, most obvious in the left temporal region. A CT brain scan demonstrated moderate enlargement of the lateral and third ventricles, and of the cortical sulci, Sylvian and interhemispheric fissures. There were diffuse zones of low density in both cerebral hemispheres in the region of the central white matter.

The laboratory findings established that the patient was suffering from transcobalamin II deficiency and not dihydrofolate reductase deficiency as had earlier been thought. Folinic acid therapy was discontinued and he was started on treatment with 1000 μg hydroxocobalamin intramuscularly thrice weekly and this has been continued ever since. His mental and neurological state have slowly improved. When examined in January, 1980, he was 101·5 cm in height with a head circumference of 47 cm (both below third centile for age). His intellectual development was severely subnormal. He was able to comprehend speech reasonably well but had a very limited vocabulary for speech utterance with about 12 recognisable single words. His vision appeared normal, as was hearing. There was no optic atrophy or nystagmus. The other cranial nerves were normal. No facial weakness was evident, his jaw jerk was not increased, there was no pout reflex and swallowing was normal. In the upper limbs there was no wasting and tone and power seemed normal. He had no obvious ataxia but finger movements were clumsy. His spine was normal but his trunk was unsteady when sitting unsupported. In the lower limbs, he showed no muscle wasting or skeletal deformity. Tone was slightly increased. Power was difficult to assess. He was unsteady on his legs and walked with assistance with both feet in an equinus position. His tendon reflexes were normal in the upper limbs, as were the knee jerks; both ankle jerks were absent. The abdominal reflexes were symmetrical but both plantar responses were extensor. He responded normally to light touch and pin prick over the trunk and limbs; vibration and joint position sense could not be assessed.

Electromyography (concentric needle electrode) of the right quadriceps and tibialis anterior muscles revealed no denervation potentials and motor unit potentials were normal. Motor nerve conduction velocity in the right

peroneal nerve on recording with surface electrodes over extensor digitorum brevis was normal at 42 m/s, as was the distal motor latency (3·8 ms). The amplitude of the evoked muscle action potential (negative component) was normal with knee and ankle stimulation (3·6 mV at both sites). The right sural nerve action potential obtained with subcutaneous needle electrode recording at the ankle and percutaneous stimulation at midcalf level was of normal amplitude (20 μV) and inflection velocity (41 m/s). A repeat CT head scan revealed similar but less marked changes than those observed in 1976. At lumbar puncture, the CSF showed no pleocytosis and the protein content was 0·1 g/l. Protein electrophoresis demonstrated sharp haptoglobin oligomers in the gamma region consistent with a high molecular weight transudate. The vitamin B_{12} concentration was 100 ng/l and the folate level 19 μg/l.

Discussion

Transcobalamin II (TC II) is the principal plasma transport protein for vitamin B_{12}. Its deficiency leads to reduced delivery of this vitamin to the bone marrow and other tissues. There is also impaired absorption of vitamin B_{12}. Hereditary TC II deficiency is of presumed autosomal recessive inheritance. The disease has been reported in two female siblings born to unaffected parents.[1] In the family reported by Hitzig *et al*,[2] the parents were first cousins; one male child was shown to have TC II deficiency and two other male siblings, who died in early infancy, may also have been affected. Heterozygotes have reduced TC II binding capacity.[1][2] In the present case, TC II binding capacity was absent, but abnormal TC II was detectable on immunological testing. The inheritance in this family is presumably autosomal recessive, the patient and one brother who died in infancy being probable homozygotes and the parents and both sisters probable heterozygotes. The patient reported by Seligman *et al*[4] had a clinical history compatible with hereditary absence of TC II but possessed an abnormal TC II protein that failed to bind cobalamin. The results obtained suggested to the authors that she was a compound heterozygote, possessing the gene for absence of TC II inherited from her mother and a gene for abnormal TC II inherited from her father.

It is of interest to compare the consequences of vitamin B_{12} deficiency during the early developmental period with those seen in adult life. The child reported by Burman *et al*[3] had presented at the age of 6 weeks with diarrhoea and vomiting. He was found to be anaemic with a megaloblastic marrow. After treatment with cyanocobalamin he rapidly recovered. A haematological relapse occurred at the end of the first year of life which again responded to treatment with vitamin B_{12}. He was then maintained

on folic acid (but not vitamin B_{12}). When assessed 16 months later, developmental progress had not been normal over the intervening period. He had begun to walk at the age of 2 years and at the age of $2\frac{1}{2}$ he had a wide unsteady gait. Over the succeeding $2\frac{1}{2}$ months he had deteriorated with frequent generalised convulsions and became unable to walk, sit or speak. He responded dramatically to injections of vitamin B_{12} and within 4 weeks was again walking. His seizures later ceased.

In the present case, the child is microcephalic and the neurological picture is dominated by retarded intellectual function. Epilepsy occurred at an earlier stage. There is no clinical evidence of visual impairment or optic atrophy, although visual evoked potentials have not been examined. Bilateral pyramidal signs are evident in the limbs. Although he displays ataxia of gait and truncal ataxia, it is not possible to ascertain whether lack of proprioceptive function contributes to this. There is no evidence of peripheral neuropathy from the nerve conduction studies, but the absence of ankle jerks despite the normal sural sensory nerve action potentials calls for comment. It is possible that this is the result of a process that has given rise to a selective degeneration of the centrally directed fibres derived from the appropriate dorsal root ganglion cells, with preservation of the peripherally directed fibres. This may lead to interruption of the monosynaptic tendon reflex arc.[6] Fine and Hallett,[7] from studies on spinal somatosensory evoked potentials in subacute combined degeneration from vitamin B_{12} deficiency in adults, have demonstrated that the changes may selectively affect the central responses with relative preservation of sensory conduction in the peripheral nerves.

Both of these cases suggest that cerebral function is particularly susceptible to vitamin B_{12} deficiency during infancy. The clinical picture in our case was dominated by severe retardation and regression of intellectual development and this was also true of the patient reported by Burman *et al*.[3] An encephalopathy may be caused by vitamin B_{12} deficiency in adults. Vitamin B_{12} deficiency in childhood may also arise because of congenital intrinsic factor deficiency. In this condition, deficiency does not occur before the age of 1-2 years, but some neurological involvement then develops with ataxia and mental changes.[8] Occasional cases of pernicious anaemia in adults exhibit impaired memory or frank dementia,[9] [10] although the incidence of encephalopathy is very difficult to assess.[11] Pathologically, multiple perivascular foci of demeylination have been found in the cerebral cortex.[12] Electroencephalographic changes are frequent in adults with vitamin B_{12} deficiency, but correlate poorly with the severity of mental

disturbance.[13] Epileptic seizures were a prominent feature in the present case and in the boy described by Burman *et al*,[3] which is not true for vitamin B_{12} deficiency in adults. Both displayed ataxia, although the nature of this is uncertain. Corticospinal tract dysfunction was evident in the second. Peripheral neuropathy has not been obvious; the depressed ankle jerks in the present case may be related to intramedullary pathology in view of the normal nerve conduction studies.

Patients with vitamin B_{12} deficiency excrete increased quantities of methylmalonic acid (MMA) in the urine.[14] Desoxyadenosylcobalamin participates in the breakdown of methyl malonyl-CoA to succinyl-CoA in the conversion of propionic acid to succinic acid. If vitamin B_{12} is lacking, MMA accumulates. It has been reported that individuals with neurological involvement secondary to vitamin B_{12} deficiency excrete larger quantities of MMA than those with haematological manifestations alone.[15] On the other hand, patients with neurological involvement tend to have more severe deficiency, as assessed by serum vitamin B_{12} assays. It was also found that ^{14}C-labelled propionate accumulated in the sural nerve of a patient with pernicious anaemia and neurological involvement, but not in a patient with pernicious anaemia alone.[15] This raised the possibility of a particular relationship between abnormalities of propionate metabolism and neurological damage. This seems unlikely as in the case of hereditary TC II deficiency reported by Burman *et al*[3] with neurological involvement, urinary MMA excretion was only modestly elevated, and it was not detected in the urine in the present case at a time when neurological involvement was marked.

Vitamin B_{12} is implicated in a further metabolic pathway. Methylcobalamin is necessary for the demethylation of methyltetrahydrofolate to tetrahydrofolate (THF). This involves the methylation of homocysteine to methionine. THF (or formyl THF) is required for the production of the active folate coenzymes (THF polyglutamates) which are necessary for DNA synthesis and aminoacid interconversions. Methyl THF is not a direct substrate for folate polyglutamate formation. Nitrous oxide (N_2O) is known to inactivate methylcobalamin but not adenosylcobalamin and N_2O exposure gives rise to megaloblastic change in bone marrow.[16] Prolonged exposure of monkeys to N_2O causes subacute combined degeneration[17] with degeneration in the lateral and posterior columns in the spinal cord. A myeloneuropathy has also been observed in man after prolonged exposure to N_2O.[18] Dinn *et al*[19] found that if monkeys are exposed to an atmosphere of N_2O and given oral methionine supplements, the development of subacute combined degeneration is

prevented. These workers have therefore suggested that the neurological deficit in vitamin B_{12} deficiency is due to a deficiency of methyl groups resulting from inadequate methionine and S-adenosyl methionine (SAM) synthesis. Earlier work in mice has shown that cycloleucine, which inhibits SAM synthesis from methionine, causes a neurological disorder resembling subacute combined degeneration.[20] Thus it seems possible that deficiency of SAM rather than of methionine is the cause of subacute combined degeneration. If so, it remains unclear why folate deficiency, which would impair methionine and SAM synthesis, does not cause this disease. It is also unclear why folate therapy corrects the anaemia but possibly aggravates the neurological complications of vitamin B_{12} deficiency (see Addendum).

Addendum

Since this article was submitted, these questions have been further considered (Scott JM, Dinn JJ, Wilson P, Weir DG. *Lancet* 1981;2:334-7 and Scott JM, Weir DG. *Lancet* 1981;2:337-40).

References

1 Hakami N, Nieman PE, Canellos GP, Lazerson J. Neonatal megaloblastic anaemia due to inherited transcobalamin II deficiency in two siblings. *New Engl J Med* 1971;**285**:1163-70.

2 Hitzig WH, Dohmann U, Pluss HJ, Vischer D. Hereditary transcobalamin deficiency: clinical findings in a new family. *J Pediat* 1974;**85**:622-8.

3 Burman JF, Mollin DL, Sourial NA, Sladden RA. Inherited lack of transcobalamin II in serum and megaloblastic anaemia: a further patient. *Br J Haematol* 1979;**43**:27-38.

4 Seligman PA, Steiner LL, Allen RH. Studies of a patient with megaloblastic anaemia and an abnormal transcobalamin II. *New Engl J Med* 1980;**303**:1209-12.

5 Tauro GP, Danks DM, Rowe PB, Van der Weyden MB, Schwarz MA, Collins VL, Neal BW. Dihydrofolate reductase deficiency causing megaloblastic anemia in two families. *New Engl J Med* 1976;

294:466-70.

6 Thomas PK, Jefferys JGR, Smith IS, Loulakakis D. Spinal somatosensory evoked potentials in hereditary spastic paraplegia. *J Neurol Neurosurg Psychiatry* 1981;**44**:243-6.

7 Fine EJ, Hallett M. Neurophysiological study of subacute combined degeneration. *J Neurol Sci* 1980;**45**:331-6.

8 Chanarin I. *The megaloblastic anaemias*, 2nd ed. Oxford: Blackwell, 1979.

9 Holmes J MacD. Cerebral manifestations of vitamin B_{12} deficiency. *Br Med J* 1956;**2**:1394-8.

10 Shulman R. Psychiatric aspects of pernicious anaemia: a prospective controlled investigation. *Br Med J* 1967;**3**:266-70.

11 Pallis CA, Lewis PD. *The neurology of gastrointestinal disease*. Philadelphia: WB Saunders, 1974:49.

12 Adams RD, Kubik CS. Subacute degeneration of the brain in pernicious anemia. *New Engl J Med* 1944;**231**:1-9.

13 Walton JN, Kiloh LG, Osselton JW, Farrall J. The electroencephalogram in pernicious anaemia and subacute combined degeneration of the cord. *EEG Clin Neurophysiol* 1954;**6**:45-64.

14 Flavin M, Ortiz PJ, Ochoa S. Metabolism of propionic acid in animal tissues. *Nature* 1955;**176**:823-6.

15 Vivaqua RJ, Myerson RM, Prescott DJ, Rabinowitz JL. Abnormal propionic-methyl malonic-succinic acid metabolism in vitamin B_{12} deficiency and its possible relationship to the neurologic syndrome of pernicious anaemia. *Am J Med Sci* 1966;**251**:507-15.

16 Amess JAL, Burman JF, Rees GM, Nancekieville DG, Mollin DL. Megaloblastic haemopoiesis in patients receiving nitrous oxide. *Lancet* 1978;**2**:339-41.

17 Dinn JJ, McCann S, Wilson P, Reed B, Weir D, Scott J. Animal model for subacute combined degeneration. *Lancet* 1978;**2**:1154.

18 Layzer R. Myeloneuropathy after prolonged exposure to nitrous oxide. *Lancet* 1978;**2**:1227-30.

19 Dinn JJ, Weir DG, McCann S, Reed B, Wilson P, Scott JM. Methyl group deficiency in nerve tissue: a hypothesis to explain the lesion of subacute combined degeneration. *Irish J Med Sci* 1980;**149**:1-4.

20 Jacobsen W, Gandy G, Sidman RL. Experimental subacute combined degeneration of the cord in mice. *J Path* 1973;**109**:xiii p.