172-96

**ROYAL FREE HAMPSTEAD NHS TRUST**

**ETHICAL PRACTICES SUB-COMMITTEE PROTOCOL AND PRO FORMA**

*ALL SECTIONS TO BE COMPLETED*

1. Responsible Consultant(s):

   Name: Professor John Walker-Smith

   Department: Department of Paediatric Gastroenterology

   Name: Dr Simon Murch

   Department: Department of Paediatric Gastroenterology

   Name: Dr A J Wakefield

   Department: Academic Department of Medicine

Signature denoting approval of Head of Department:

.....

Date 6/8/96

**Name(s), qualification(s) and status of research worker(s) directly involved:**

Name: Dr Javed Subhani Status: Clinical Research Fellow

Qualifications: MB BA BChir MRCP

Name: Dr David Casson Status: Lecturer in Paediatric Gastroenterology

Qualifications: BA MB BS MRCP

**Are other Departments involved?** YES

If YES, which: Department of Neurology
Dept of Child Health (Child Psychiatry)

**Signature(s) of Head(s) of Collaborating Department(s) denoting approval:**

Dr P Harvey.....Department Neurology

Dr M Berelowitz.....Department Child Health (Child Psychiatry)

2. **TITLE OF PROJECT** (Capital letters)

A NEW PAEDIATRIC SYNDROME: ENTERITIS AND DISINTEGRATIVE DISORDER FOLLOWING MEASLES/RUBELLA VACCINATION

3. **OBJECTIVE** (eg hypothesis which it is intended to test)

We will test the hypothesis that in genetically susceptible children, measles vaccination is associated with persistent enteric (and possibly CNS) infection, enteritis and malabsorption of vitamin B12. In the rapidly myelinating brain, which is particularly susceptible to vitamin B12 deficiency, the latter predisposes to encephalopathy.

This encepahlopathy may, in the future, be amenable to replacement of B12, and control of the putative underlying enteritis.

4. **DESIGN OF THE STUDY** (describe briefly, including proposed methods for the analysis of results):

Children, referred either by their GP, or via the vitamin B12 unit at the Chelsea and Westminster Hospital, who manifest disintegrative disorder and symptoms and signs of intestinal disease will be admitted to Malcolm ward for a period of one week, under the care of Professor J Walker-Smith. With fully informed parental consent, children will undergo the following investigations in an attempt to characterise the intestinal and cerebral pathologies that potentially underly this condition.

- General and neurological histories and examinations plus a structured assessment for disintegrative disorder.
- Routine ileocolonoscopy and upper gastrointestinal endoscopy with large and small bowel biopsy under either standard sedation (pethidine/Diazamuls) or general anaesthesia, as indicated.
- A barium follow-through will be performed, if indicated by the presence of endoscopic abnormalities.
- MRI, EEG and visual, somato-sensory and brain stem auditory evoked potentials.
- Lumbar puncture for measurement of CSF antibody and cytokine profiles.
- Serology for measles and rubella IgM and IgG, routine haematology, biochemistry and immunology profiles, and isolation of DNA from peripheral blood mononuclear cells for complement genotyping.
- In addition, studies of B12 status and metabolism will be performed on a serum sample and a 24 hour urine collection. A full protocol is attached.

5. **SCIENTIFIC BACKGROUND** (Please give a brief review of the relevant literature. (If this investigation has been done previously with human subjects why repeat it? If it has not been done previously with human subjects, has the problem been worked out as fully as possible in animals, in order to refine analytical techniques and to assess possible toxic effects?)

**Introduction**

There are indications of the emergence of a new syndrome comprising disintegrative disorder and a possible enteritis associated with vitamin B12 deficiency. The syndrome has been linked - anecdotally but consistently - with either measles or measles/rubella vaccination. It appears to occur in previously well and developmentally normal children following exposure to these vaccines.

**Disintegrative disorder (Heller's disease)**

Disintegrative disorder (or Heller's disease) occurs when normally developing children suddenly, or over a period of several months, show marked behaviour changes and developmental regression after age 2, often in association with some loss of co-ordination and bowel or bladder function (Reviewed by Rutter et al (1). Behavioural changes include social withdrawal, reduced response to sounds, complete loss of communication, unusual sensory behaviours and development of simple rituals and hand and finger stereotypes, much like those of autistic children. However, disintegrative disorder differs from autism in the loss of motor and self-help skills and usually, too, in the lack of more complex stereotype behavioral patterns (although simple motor stereotypes may occur). This rare disorder can sometimes be linked to measles encephalitis, cerebral lipoidoses, leukodystrophies or other neurological conditions but in most cases no clear cause is ever identified. Even in cases where progressive neurological disorder is eventually identified, initial medical tests are often negative and sometimes diagnoses of hysterical reactions are considered. Thus, it is important to repeat medical investigations if a child's conditions does not improve.

Two different courses are typical of children with regressions occurring after the first few years. Most common are regressions that extend over several months and then plateau, resulting in a developmental and behavioral pattern that looks much like autism with severe mental handicap. In some cases, deterioration continues, with increased motor dysfunction, development of seizures and localized neurological signs.

Another disorder that overlaps in symptomatology but that does not have quite so poor a prognosis is Landau-Kleffner syndrome of acquired aphasia with epilepsy. Children with this disorder lose receptive and expressive language, usually over a period of months, typically in conjunction with the development of seizures or transient EEG abnormalities. Some social withdrawal and unusual behaviours may occur, but usually relatively normal social relationships are maintained with parents and others known to the child. Non-verbal cognitive functioning remains intact. In most cases, the outlook for these children is better than for children with disintegrative psychoses or autism, and sometimes language is eventually regained.

Evidence is accumulating for the participation of immune mechanisms in the pathogenesis of these conditions, including the presence of circulating antibodies to myelin basic protein (MBP) (2,3) and the putative brain serotonin receptors (4). A recent study identified an increase in circulating DR+ (activated) T cells in affected subjects without an increase in IL-2 receptor+ cells (5), suggesting incomplete activation of the immune system, a finding that has been observed in several established autoimmune disorders (6-9), including chronic autoimmune hepatitis, a disease in which a role for measles virus has been implicated.

*Complement, measles and distintegrative disorder:* The association between measles vaccine and disintegrative disorder has been noted independently by Warren R et al in Utah, where they have linked susceptibility to this condition (which they have termed "secondary autism") to a congenital deficiency of the C4B complement protein (10, 11). They have identified a deficient form (null allele) of the C4B gene located in the class III region of the MHC complex, which occurred more than twice as often in affected individuals as in a matched, normal population. The C4B gene products are crucial to activation of the classical

complement pathway, a vital mechanism for protection against invading microorganisms. The actual relationship of the C4B null allele to the induction of disintegrative disorder is not known. Hypothetically, individuals inheriting one or two C4B null alleles may not be able to clear certain viruses completely, including live "attenuated" vaccine strains. Persistent infection may induce enteritis, encephalitis and/or the development of chronically activated T cell clones with reactivity to epitopes in the central nervous system such as MBP. Immune mediated damage to, for example, myelin in the developing brain may result in clinical disease. The study will seek to identify and characterise inherited abnormalities of complement (C4B) genotype, based upon the hypothesis that abnormal complement regulation is central to the susceptibility and subsequent expression of disease.

*Enteritis*: mothers of those children reported to us, describe a variety of abdominal signs and symptoms suggesting intestinal pathology. These include pain, bloating, alternating constipation and diarrhoea, steatorrhoea and failure to thrive. Behavioral symptoms parallel intestinal disturbances. Some children have documented macrocytic or iron deficiency anaemias. As yet, the pathogenesis of these features have not been established. This study aims to identify and characterise any morphological and immunohistochemical changes in intestinal tissues of individuals affected by the syndrome and to seek, using established techniques, the presence of measles and rubella virus proteins and RNAs, using appropriate positive and negative controls.

*$B_{12}$ (Cobalamin: Cbl) metabolism:* The bulk of the vitamin B12 (Cbl) in the body is present as its two co-enzymes, methyl cobalamin (MeCbl) and adenosylcobalamin (AdoCbl). Both are required for normal cellular metabolism (12). Cbl is known to be involved in only 3 reactions in human tissues: as Ado-Cbl in the isomerisation of methylmalonyl CoA to succinyl CoA and of α-leucine to ß-leucine, and as Me-Cbl in the methylation of homocysteine to methionine, a reaction which also requires methyltetrahydrofolate. A tissue deficiency of MeCbl leads to increased plasma homocysteine levels and urinary excretion of homocystine (12). AdoCbl is the co-enzyme to methylmalonyl CoA mutase and is required in the final step or propionate to succinate catabolism. Depletion of cellular AdoCbl leads to raised levels of methylmalonic acid (MMA) in plasma and increased urinary MMA excretion. Absorption of Cbl is not a simple process, as it involves release of the vitamin from food, binding first to a salivary glycoprotein (R-binder) in the stomach, then to intrinsic factor (IF) in the jejunum and finally to receptors in the terminal ileum. There, by a process of receptor-mediated endocytosis it is absorbed into the bloodstream and bound to the plasma-Cbl protein transcobalamin II. The routine Schilling test can identify Cbl malabsorption due to lack of IF, but malabsorption due to a failure to release Cbl from food will not be detected. The study seeks to identify the cause of any Cbl malabsorption by the use of a modified Schilling test in which the administered radio-labelled Cbl is first bound to scrambled egg.

*The role of cobalamins and pathophysiology of cobalamin deficiency in the central nervous system.*

The evidence that Cbl plays a role in the central nervous system comes from several sources. These include case reports describing neurological complications in patients with Cbl deficiency and in patients with inborn errors affecting Cbl metabolic pathways. In addition, animal models have provided experimental evidence that Cbl is necessary for normal function of the nervous system. It is notable that neurological symptoms of Cbl deficiency may be the presenting features, and neurological changes frequently occur without anaemia or macrocytosis (reviewed by Green and Jacobsen: ref. 13).

*Clinical features of Cbl-deficient myeloneuropathy.*
The principal role of Cbl appears to be in myelin-producing cells (oligodendroglia), and therefore the pathological effects of deficiency are largely as a consequency of myelopathy. The central nervous system appears to be more susceptible to Cbl-deficiency in infants and children than adults, and involvement is diffuse rather than localised. This may be due to rapid and more widespread myelinogenesis during this period of active growth and development of the nervous system: accordingly, in Cbl-deficient infants and children, a somewhat different spectrum of neurological complications is seen compared with adults. In paediatric patients Cbl-deficiency is usually associated with inborn errors of Cbl metabolism, Cbl-dependent enzymes, or binding proteins which mediate transport of Cbl (14). The more common neurological manifestations encountered in such patients include seizures, mental retardation and abnormal cerebellar and spinal cord function. In addition to metabolic errors, a syndrome of Cbl-deficiency has been described in breast-fed infants of strictly vegetarian mothers consisting of apathy, developmental regression and involuntary movements of the head, trunk and limbs.

Electro-physiological abnormalities associated with Cbl myelopathy include slowed nerve conduction, abnormal electroencephalogram tracings, delayed visual evoked responses, and abnormal brain stem auditory evoked responses and sensory evoked responses.

*Pathogenesis of Cbl-deficient myeloneuropathy.*
The mechanism whereby Cbl-deficiency results in myelopathy is not known. Several hypotheses have been advanced, although none fully or satisfactorily explains the pathogenesis of neurological damage. Overall, however, aberrant myelin synthesis appears to be central to the pathological changes that are observed.

*Cbl-deficiency and disintegrative disorder.*
A recent, as yet, unreported pilot study of cobalamin metabolism in children with disintegrative disorder and/or attention deficit/hyperactive disorder (AD/HD) has shown that many had abnormally increased MMA excretion and other signs of impaired cobalamin function (Linnell J, personal communication). Some then received high dosage cobalamin treatment which reduced MMA excretion to normal and appeared to confer other benefits, although these were difficult to quantify. Most of these children had a Cbl profile indicative of intestinal malabsorptio. The proposed investigation will include an assessment of intestinal status and function in addition to a range of metabolic tests to assess cobalamin function and metabolism.

Functional cobalamin status is better indicated by direct measurement of the cobalamin co-enzymes or the relevant metabolites than by assaying the 'serum B12' (15). This is because most of the circulating cobalamin is firmly attached to an inactive protein binder of long half-life which does not promote tissue uptake of the vitamin (16).

The proposed studies are designed to determine whether B12 deficiency in affected children occurs as a consequence of failure of terminal ileal reabsorption due to enteropathy, or alternatively, whether there is a innate error of B12 metabolism in such individuals.

**Working hypotheses**
The possible link between an environmental insult (measles/rubella vaccine) in a previously healthy child, who may be genetically susceptible to responding inappropriately to the viruses via abnormal complement regulation, and the subsequent development of enteritis, Cbl-deficiency and disintegrative disorder, permits several working hypotheses to be proposed.

1) **Enteritis hypothesis:** the simplest model may be as follows: In the genetically susceptible individual, the virus (measles and/or rubella) does not induce an appropriate cellular immune response leading to persistent infection and inflammation.

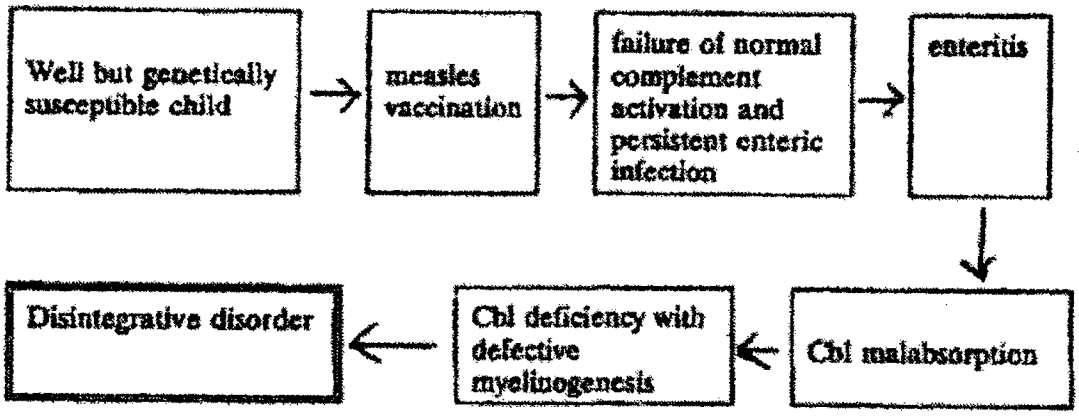


Figure 1. The "enteritis" model for disintegrative disorder

This scenario is supported, indirectly and anecdotally, by the clinical improvement in some affected children treated with Cbl replacement (Linnell J, personal communication). It also suggests that therapeutic intervention directed at controlling the enteritis and replacing Cbl, may be of benefit.

2. **Encephalitis/enteritis hypothesis**

This invokes that in the genetically susceptible infant, vaccination leads to persistent virus infection of both the gut and the brain. It is possible that ensuing inflammation may occur either as a direct result of infection, or via the immune response to persistent infection. Any Cbl deficiency that occurs as a consequence of the enteritis, may then exacerbate neurological damage, but is not its prime cause. The observation that disintegrative disorder may follow acute measles encephalitis, supports this hypothetical pathway. This scenario would be less likely to be amenable to specific therapy since persistent measles virus infection cannot, as yet, be eradicated, although Cbl replacement may still be of some benefit.

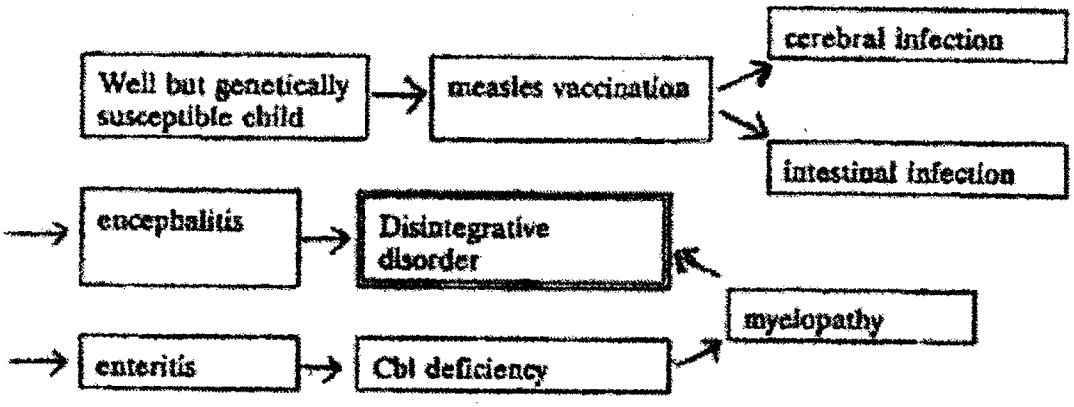


Figure 2. The "encephalitis/enteritis" model for disintegrative disorder

Evidence for the role of these hypothetical pathways can be generated by the proposed study.

**References**


1. Rutter M, Taylor E, Hersor L. In Child and Adolescent Psychiatry. Blackwells Scientific Publications. 3rd Edition London pp 581-582.

2. Weizman A, Weizman R, Szekeley GA, Wijsenbeek H, Livini E. Abnormal immune response to brain tissue antigen in the syndrome of autism. Am J Psychiatry. 1982;139:1462-1465

3. Singh VK, Warren RP, Odell JD, Warren WL, Cole P. Antibodies to myelin basic protein in children with autistic behaviour. Brain, Behaviour and Immunity 1993;7:97-103.

4. Todd R, Ciaranello R. Demonstration of inter and intra species differences in serotonin binding sites by antibodies from an autistic child. Proc Natl Acad Sci USA, 1985;82:612-616.

5. Warren RP, Yonk J, Burger RW, Odell D, Warren WL. DR-positive T cells in autism: association with decreased plasma levels of the complement C4B protein. Neuropsycobiology 1995;31:53-57.

6. Burmester GR, Jahn B, Gramatzki M, Zacher J, Kalden JR. Activated T cells in vivo and in vitro: divergence in expression of Tac and Ia antigens in the non-blastoid small T cells of inflammation and normal T cells activated in vitro. J Immunol. 1984;133:1230-1231.

7. Berfroth V, Konthinen YT, Pelkonen P, Haapala M, Haapasaari J, Nordstrom D, Kunnamo I, Friman C. Synovial fluid lymphocytes in different subtypes of juvenile rheumatoid arthritis. Rheumatology 1989;31:780-783.

8. Hafler DA, Hemler ME, Christenson L, Williams JM, Shapiro HM, Strom TB, Strominger JL, Weiner HL. Investigation in vivo T cells in multiple sclerosis and inflammatory central nervous system diseases. Clin Immunol Immunopathol. 1985;37:163-171.

9. Vergani D, Larcher VF, Davies ET, Wells L, Nasaruddin BA, Mieli-Vergani G, Mowat AP, Genetically determined low C4: a predisposing factor to autoimmune chronic hepatitis. Lancet 1985;ii:294-298.

10. Warren RP, Burger RA, Odell D, Anthony TR, Warren L. Decreased plasma concentrations of the C4B complement protein in autism. Arch Pediatr Adolesc Med 1994;148:180-183.

11. Warren RP, Singh VK, Cole P, Odell JD, Pingree CB, Warren WL, White E. Increased frequency of the null allele at the complement C4B locus in autism. Clin Exp Immunol. 1991;83:438-440.

12. Linnell JC and Bhatt HR (1995). Inborn errors of B12 metabolism and their management. Baillière's Clinical Haematology 8, 567-601.

13. Green R and Jacobsen DW (1990). The role of cobalamins in the nervous system. In: Biomedicine and Physiology of vitamin B12; Eds. JC Linnell & HR Bhatt. Children's Medical Charity, London; pp 107-119. ISBN 0-9514084-0-2.

14. Dillon MJ, England JM, Gompertz D et al. (1974). Mental retardation, megaloblastic anaemic, homocystine metabolism due to an error in B12 metabolism. Clin.Sci.Mol.Med. 47;43-61.

15. England JM and Linnell JC (1980). Problems with the serum vitamin B12 assay. Lancet 2;1072-1074.

16. Linnell JC (1981). The value of radioisotopic essays for 'serum B12' in the diagnosis of cobalamin-deficiency disorders. Clin. & Lab. Haematol.3;99-107.

*Who has verified the scientific validity of merit of this study?* - Review Committee, Senior expert or colleague, NOT directly involved in or responsible for (eg; as Head of Department) the study.

Dr O Epstein

6. **RADIOACTIVE SUBSTANCES (If radio-isotopes are to be used you are required to register the project with the Radiation Protection Adviser and to obtain approval of the Health Ministers through the Department of Health.)**

*Are radio-isotopes to be used?* YES

**If yes, provide evidence of approval from the Radiation Protection Adviser and/or a copy of authority certificate and any additional comments received from the Department of Health.**

Either routine or a modified Schilling test will be carried out to assess intestinal cobalamin absorption from food (Linnell JC. Clin Lab Haematol. 1981;3:99-107). Approximately 1mCi Co-cyanocobalamin (CNCbl) in cooked egg is ingested, and a flushing dose of 1mg CNCbl is administered intramuscularly. Radiolabelled CNCbl excretion is measured in a 24 hour urine sample.

7. **RADIOLOGICAL INVESTIGATIONS (If included, please indicate number and frequency of exposures, and total calculated dosage.)**

Barium follow through. Dose = 948 CGYs 1?4*min screening time.

**Subjects**

(a) Selection criteria

- presence of disintegrative disorder
- symptoms and signs of intestinal dysfunction
- parental request for investigation to be undertaken

(b) Exclusion citeria

- Egg allergy - children will be excluded from modified Schilling test

8. SUBJECTS: *How many are needed?* 25

**(b) Are subjects to be used - under the age of 16, pregnant women, psychiatric or elderly patients?**

**If yes, describe safeguards.**

All subjects will be under the age of 16, and all will manifest disintegrative symptoms and signs to differing extents. They will be accompanied at all times by a parent, and will have a designated nurse(s) in attendance throughout the week. Invasive procedures and MRI will be carried out under either standard sedation or general anaesthetia (ileocolonoscopy and biopsy and the lumbar puncture will be performed on the same morning), as indicated. Parents will be aware that they can withdraw their child from the study at any stage.

**(c) Are normal volunteers involved?**

NO

**(d) How much will each volunteer be paid for participating in the study?**

9. **CONTROLS:** NIL

10. **SUBSTANCES TO BE GIVEN TO SUBJECTS** (Special diets, drugs, isotopic tracers, etc).

1mCi cyanocobalamin as Schilling test.

State dose, mode of administration, frequency, trial or exemption certificate number, product licence, any precautions ie: storage.

**How are the substances for this study being provided, and how is the study being funded?**

Clinical research at the Royal Free Hospital (E.C.R.).

11. **PROCEDURES OR SAMPLES TO BE TAKEN FROM SUBJECTS**

**(Venepunctures, arterial blood, urine, tissue biopsy, etc.) State type, frequency and amount.**

1) Two venepunctures of 10-20 ml each
2) Ileocolonic tissue biopsies 10 per patient
3) One 24-hour urine collection
4) Cerebrospinal fluid 3 ml

**Would the procedure(s) or sample(s) be taken, especially for this investigation, or as part of normal patient care?**

Yes: in view of the symptoms and signs manifested by these patients, all of the procedures and the majority of samples are clinically indicated. Additional intestinal biopsies (5 per patient) will be taken for viral analysis. DNA for genotyping will use blood cells isolated from the rotine blood sample, and will not require an additional sample.

12. **DISCOMFORT** **What discomfort or interference with their usual activities may be suffered in all or any of the subjects? (List expected side effects of drugs, procedures, etc.) Any statement made here must also appear in the patient information sheet.**

Ileocolonoscopy, upper endoscopy and lumbar puncture are invasive procedures that are performed routinely at the Royal Free Hospital by members of the Department of Paediatric Gastroenterology. In order to avoid or reduce discomfort, ileocolonoscopy is performed under sedation given as an injection, or under a general anaesthetic. Lumbar puncture is performed under a local anaesthetic injected into the skin of the lower back. The injection itself produces a mild and brief stinging sensation. Thereafter, the procedure is painless.

13. **INSURANCE** ***What arrangements have been made to cover the possibility of liability claims arising from this project?***

CLINICAL RESEARCH AT THE ROYAL FREE HOSPITAL AND SCHOOL OF MEDICINE.

14. **COPY OF HANDOUT EXPLAINING PROJECT IN LAY TERMS TO BE GIVEN TO PATIENT OR OTHER PARTICIPANT**

**Code Number** _____

**TITLE** _____

Please attach copy of handout and consent form.

(Copy of handout and consent form to be retained in patient's hospital notes).