Hence CPD might be a better anticoagulant for blood for transfusion than ACD. It is yet to be determined however whether a relatively low DPG-level in blood for transfusion is of clinical importance. However, an increase in the shelf life of stored blood may have advantages, particularly in hospital blood banks in which there is a relatively slow turnover of blood.

Some purine nucleosides when added to anticoagulant solutions increase ATP concentration and consequently the shelf-life of stored blood and the post-transfusion survival of stored cells. An ACD-adenine solution, in which blood for transfusion is stored for up to 35 days, is used in Uppsala.[17]

Storage of blood in the frozen state has been extensively studied. Krijnen *et al.*,[18] suspend packed cells from ACD blood in 20% (w/v) glycerol and then freeze the cells in liquid nitrogen at −196°C. Blood may be stored in this way for years. When required for transfusion the cells are thawed at 40°C, washed in 16% sorbitol and 0·9% sodium chloride. The 24-hour post-transfusion survival of the cells is over 90%.

Frozen blood has several advantages. It is leucocyte-free and devoid of hepatitis virus. It is particularly valuable for storing patients' own cells for subsequent transfusion in connexion with transplant surgery, in which it is important to avoid cytotoxic incompatibility of lymphocytes, or when the patient has a very rare blood group and has developed antibodies against the red cells of almost everyone else.

When blood of a rare group has been kept in reserve at 2-6°C and has not been used, it can be rejuvenated by the addition of inosine[19] and then stored in the frozen state.

Conclusion

Large volumes have been written on the red cell, and indeed on haemolysis alone. The foregoing account is but a brief essay on a vast and fascinating subject. Further details may be obtained from various monographs[20 21 11] from contributions to recent symposia,[22 23] and from the July and October 1970 numbers of *Seminars in Heamatology*.

This article is based on a lecture given in the Birmingham course under the title "The Scientific Basis of Clinical Practice" (see B.M.J., 27 November 1971, p. 510).

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For Debate . . .

Folate Deficiency after Anticonvulsant Drugs: An Effect of Hepatic Enzyme Induction?

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Summary

Serum and red cell folate levels were reduced in 59% and 58% respectively of 75 children with epilepsy attending a residential school. The degree of folate deficiency was significantly related to increased hepatic microsomal enzyme activity, assessed from increased urinary excretion of D-glucaric acid and also correlated with the daily dose of anticonvulsant taken. Anticonvulsant drugs are known to have inducing properties, and since folate is required as a cofactor in drug hydroxylations it is suggested that folate depletion results from increased demand for the cofactor after induction of drug-metabolizing enzymes. As folate deficiency may ultimately limit drug metabolism this hypothesis would explain why blood phenytoin levels decrease and fit control may worsen after correction of folate deficiency in epileptic patients.

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Introduction

Anaemia occurring after treatment with anticonvulsant drugs was first reported in 1952,[1] and it is now recognized that the

cause is folate deficiency resulting in a high incidence of macrocytosis (11-53%) and megaloblastic changes in the marrow and that this complication may follow treatment with any of the commonly used anticonvulsants.[2 3]

The proposed mechanisms include impairment of folate (monoglutamate) absorption,[4] which in the case of phenytoin has been attributed to alkalinization of the upper small intestine.[5] This has not been confirmed in recent studies[6 7] and neither has the suggestion that phenytoin inhibits intestinal conjugates[6 8] which are responsible for deconjugating polyglutamates (that account for about 75% of folate in a normal diet) to the monoglutamate form, which is absorbed.[9] Displacement of folate from its carrier plasma protein by anticonvulsant drugs[10] though it would account for lowering the serum folate would not explain the reduction in tissue folate also observed.[11] Nor is there any evidence that anticonvulsants interfere with the uptake of folate by cells in the marrow[12] or that they behave as folate antagonists[13] despite the structural similarities.[14]

Anticonvulsant drugs are powerful enzyme-inducing agents.[15] As folate is a cofactor for certain hydroxylations performed by microsomal enzymes[16 17] it seemed that folate deficiency occurring after anticonvulsant therapy might result from increased metabolic requirements for folate by the liver as a result of increased activity of drug-metabolizing enzymes. A quantitative assessment of microsomal enzyme activity in man can be obtained, as we have recently shown, by measurement of the urinary excretion of D-glucaric acid, an end product of glucuronic acid metabolism in the liver.[18 19] Using this technique we have assessed the relation of folate deficiency to microsomal enzyme activity in an unselected group of 75 children with epilepsy attending a residential school.

Subjects and Methods

Seventy-five children (39 boys and 36 girls) aged 12 to 16 years were examined with parental consent. Many were retarded and it was not possible to obtain a reliable dietary history but all were receiving the standard school diet and had been resident for at least three months at the time of study. None were on folic acid or other vitamin supplements. Some of these children were investigated at the same time as they were being screened for osteomalacia, the results of which have been recently reported.[20]

Serum and red cell folate concentrations were measured in non-fasting blood taken between 9 and 11 a.m., by using microbiological assay with *Lactobacillus casei* as the test organism.[21 22] The concentration of D-glucaric acid in a urine sample collected at the same time was determined from the inhibitory effect of glucarolactone, to which it is converted by boiling at pH 2, on β-glucuronidase.[23] This was related to the concentration of creatinine in the same sample and expressed as μmol of glucarolactone/g of creatinine. We have previously found in epileptic and normal subjects that this ratio bears a close relation to the total 24-hour excretion of D-glucaric acid.[20]

The results of D-glucaric acid excretion were compared with values obtained in urine samples collected from a control group of 15 children of the same age in an institution for the mentally retarded, none of whom were on drug treatment. The values for serum and red cell folate were compared with the established normal range for these tests in our laboratory.

Results

Serum folate was reduced below 3·5 ng/ml, the lower limit of normal, in 44 (59%) of the 75 children investigated. The lower limit of normal for red cell folate is 100 ng/ml, and values lower than this were present in 42 (58%) of the 72 children in whom measurements were made (Fig. 1). There was a significant correlation between serum and red cell folate ($r = 0·39$, $P < 0·002$). Both sexes had a similar frequency of reduced red cell and serum folate levels.

Increased concentrations of glucaric acid were found in 69 (90%) of the epileptic children (Fig. 2). Four of the six children with normal D-glucaric acid values were not on anticonvulsants as they had not had fits for some time, and the other two were

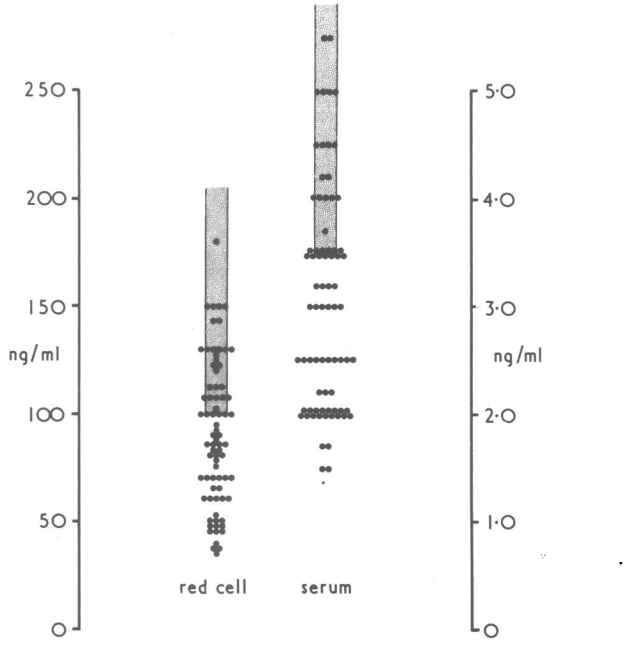

FIG. 1—Serum and red cell folate levels in 75 epileptic children. Hatched areas are normal ranges for our laboratory.

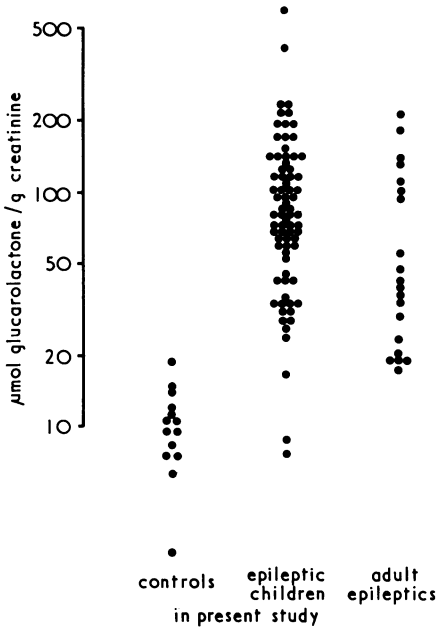

FIG. 2—Glucaric acid excretion (expressed as μmol of glucarolactone/g of creatinine) in 15 control and 75 epileptic children and in 20 epileptic adults.

on very small doses. The range of D-glucaric acid values in the epileptic children (9-631 μmol/g) was comparable to that found by us in a study of adult epileptics (Fig. 2).

Children with high D-glucaric acid values were found to have reduced folate levels, and a linear relation was found to exist between D-glucaric acid concentration when plotted logarith-

mically and the serum folate and red cell folate values. Statistical analysis disclosed a significant inverse correlation between log glucaric acid and serum folate ($r = -0.35$, $P < 0.002$) and red cell folate ($r = -0.34$, $P < 0.002$). There was also a significant correlation between the levels of serum and red cell folate in the individual children and the total daily dose of anticonvulsant drugs when expressed in units[24] ($r = -0.41$, $P < 0.001$, and $r = -0.55$, $P < 0.001$, respectively).

Discussion

We have found the urinary excretion of D-glucaric acid to be a sensitive indicator of hepatic microsomal enzyme activity. In man the excretion of this metabolite is related to the amount of inducing drug administered,[18] and in guinea-pigs treated with varying doses of phenobarbitone we have found a significant correlation between urinary D-glucaric acid excretion and the total liver content of cytochrome P-450, an enzyme of central importance in microsomal hydroxylations.[19] Further studies in which phenobarbitone was administered to guinea-pigs together with an inhibitor of protein synthesis showed that this relation still obtained.

The finding of a significant association between the extent of microsomal enzyme induction and the levels of both serum and red cell folate is strong evidence, though not direct proof, for our hypothesis that folate deficiency results from accelerated metabolism of folate after the induction of hepatic drug-metabolizing enzymes caused by anticonvulsant drug therapy. This would also explain the occurrence of folate depletion in women on oral contraceptives[25 26] and the effect of chronic ethanol ingestion in accelerating the development of serum and tissue folate depletion in subjects on folate-deficient diets,[27] for despite considerable differences in chemical structure ethanol and oestrogens, as in the case of the anticonvulsant drugs, have the property of inducing the activity of microsomal enzymes.[15 28 29]

In most reported cases patients with folate deficiency occurring after anticonvulsant therapy have been on combinations of drugs, and the effect appears to be dose-related.[2] There is no evidence that any single anticonvulsant drug is particularly apt to produce these changes. In the cases reported by Hawkins and Meynell[30] macrocytosis occurred in 27% of patients on phenytoin alone and 34% of those on phenobarbitone alone, while macrocytosis occurred in 45% of those receiving both drugs.

This hypothesis also provides a logical explanation for the finding that administration of folic acid to epileptic patients on phenytoin results in a lowering of the blood phenytoin levels[31] and an increase in the urinary excretion of its principal metabolite[32] and for the clinical observation that deterioration in fit control may ensue.[2] This is based on evidence suggesting that folic acid may be required as a necessary cofactor in the hydroxylation of drugs as well as certain natural substrates. Tietz *et al.*[17] showed that liver microsomes lost their ability to oxidise batyl alcohol and other glyceryl ethers after repeated washing but that activity was fully restored and even augmented by the addition of folate or certain pteridines, and these compounds have been shown to be required in the hydroxylation of phenylalanine.[33 34]

Experiments in vitro have shown that folate is required as a cofactor in the microsomal hydroxylation of progesterone,[16] and the clinical studies of Kutt *et al.*[32] and of Baylis *et al.*[31] suggested that folate depletion may limit the rate of metabolism of diphenylhydantoin, which like all inducing agents is metabolized by microsomal enzymes. Thus the prolonged administration of any drug capable of inducing the activity of microsomal drug-metabolizing enzymes is likely to result in an increased demand for folate. As body stores are limited[35] and dietary intake is usually sufficient only for normal requirements folate levels will fall until an equilibrium is reached in which any further increase in enzyme induction is not accompanied by increase in drug-metabolizing capacity of the liver as a result of lack of the cofactor. If this limitation is removed by administration of folic acid supplements an increase in the rate of drug metabolism may be expected to occur with more rapid breakdown and thus reduction in blood levels of the drug (as has been shown for phenytoin) and possible deterioration in fit control.

Thus folate deficiency may have to be added to the list of possible adverse effects of prolonged induction of hepatic microsomal enzymes. The problems of anticoagulant control in patients on barbiturates are now well known.[15] Recently the disorders of calcium metabolism and frank osteomalacia seen in some epileptic patients have been attributed to enzyme induction by anticonvulsant drugs with accelerated metabolism of vitamin D.[20 36] Regular screening of patients at risk is essential and the place of dietary supplements before deficiency has occurred requires further study.

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