

Altered Calcium Metabolism in Epileptic Children on Anticonvulsants

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Summary

A survey of 105 epileptic children aged 10-16 years at a residential school who were taking anticonvulsant drugs showed reduced serum calcium levels in 30% and a raised serum alkaline phosphatase in 24%. Urinary D-glucuronic acid excretion, a quantitative index of hepatic enzyme induction, was raised in 94% of the children, and statistical analysis showed a significant inverse correlation with the level of serum calcium. These findings give further support for the view that an important factor in the development of the hypocalcaemia and occasional clinical osteomalacia in epileptics on anticonvulsant drugs is an alteration of vitamin-D metabolism in the liver as a result of microsomal enzyme induction. As a consequence there is an increased requirement for vitamin D which may not be met by average intakes in Britain.

Introduction

Anticonvulsant drugs such as phenobarbitone, phenytoin, and primidone are known to cause induction of hepatic microsomal enzymes (Conney, 1967) and an increased breakdown of vitamin D in the liver has recently been implicated in the aetiology of the osteomalacia which has been reported in epileptic patients on these drugs (Dent *et al.*, 1970; Richens and Rowe 1970). In a study of adult epileptics these workers showed a significant relationship between serum calcium levels and the total dosage of anticonvulsant drugs taken. They also described in the rat that the hypercalcaemia and renal calcification produced by vitamin D intoxication could be reduced by the simultaneous administration of phenobarbitone. Though direct assay of microsomal enzymes in liver biopsy specimens is possible in man, it is rarely feasible. However, we have recently shown that it is possible to assess microsomal enzyme activity quantitatively by measurement of the urinary excretion of D-glucuronic acid, a product of the glucuronic acid pathway of the liver (Hunter *et al.*, 1971a). We have used this method in the present study to determine the relationship of hepatic enzyme induction to the level of serum calcium and other biochemical criteria of osteomalacia in a series of 105 children with epilepsy at a residential school.

Methods

Serum calcium, phosphate, and alkaline phosphatase values were determined by means of the SMA 12/60 AutoAnalyzer in

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non-fasting venous blood samples taken between 9 and 11 a.m. If the serum specific gravity differed from 1027 the value for the serum calcium was corrected accordingly (Dent, 1962). The isoenzymes of alkaline phosphatase were separated by electrophoresis on polyacrylamide gel (Canapa-Anson and Rowe, 1970), the relative proportions of bone and liver alkaline phosphatase being determined visually.

The concentration of D-glucuronic acid in a sample of urine obtained at the same time was determined by the inhibitory effect of glucarolactone, to which D-glucuronic acid is converted by heating at pH 2, on β -glucuronidase as described by Marsh (1963). The result was related to the concentration of creatinine determined in the same urine sample—a ratio which we have previously found both in epileptic and in normal subjects to correlate closely with the total daily D-glucuronic acid excretion when measured in a complete 24-hour urine collection ($r=0.97$). The results were compared with values found in a control series of urine samples obtained from 15 children aged 10-15 years who were resident in a home for the mentally retarded, and on no drug therapy.

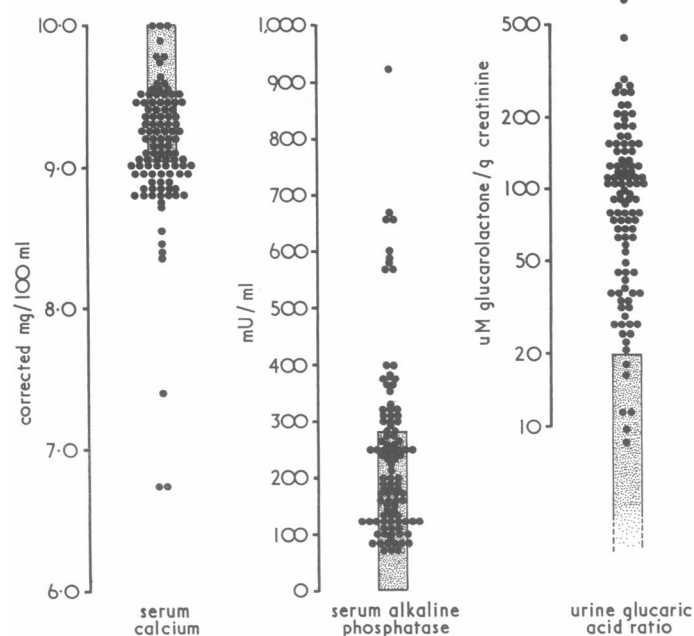
Results

The 105 children investigated, aged 10-16 years, included 60 boys and 45 girls. Many were of subnormal intelligence, and it was not possible to obtain individual dietary histories, but according to their supervisors none had noticeable food fads, and calculation of the vitamin D content of their diet over two separate weeks indicated an intake of 140-290 IU daily.

The results of the biochemical screening showed that the serum phosphate value was within the normal range in every instance, the mean value for the group being 3.8 mg/100 ml (range 2.5-4.8). Serum alkaline phosphatase levels were raised in 25 children to above 295 mU/ml, the upper limit of normal for this age group (Bauer and De Vito, 1969). The highest level was 910 mU/ml, and nine children had levels above 500 mU/ml (see Fig.). In every patient with values above 295 mU/ml electrophoresis showed that the percentage of bone isoenzymes was very high, comprising 85-100% of the total amount of alkaline phosphatase present. In only nine patients was the liver isoenzyme the dominant one, and in all these the total serum level fell within the normal range.

In 31 children (14 boys and 17 girls) the serum calcium was below 9 mg/100 ml, which is regarded as the lower limit of the normal range for the SMA 12/60 AutoAnalyzer (G. Walker, personal communication, 1971). Six children had a serum calcium of less than 8.5 mg/100 ml, and in four of these the calcium phosphate product ($\text{Ca} \times \text{P}$) was below 30 mg/100 ml, the lower limit of normal (Fraser and MacIntyre, 1970). In three of these children, as well as in the two with a normal $\text{Ca} \times \text{P}$ product, the serum alkaline phosphatase was markedly raised (298-640 mU/ml). These six children were considered to have biochemical osteomalacia. All had been on anticonvulsant therapy for at least eight years, and though most of the pupils in the school are of British descent, this group included two West Indians and one Italian. Two of the others had been resident at the school for less than 12 months.

A detailed record of the present anticonvulsant regimen and of the duration of therapy was taken in each case. The length of drug treatment varied widely from 2 to 15 years, as did the current dosage of drugs. In many of the children control of



Values for serum calcium, alkaline phosphatase, and urinary glucuronic acid excretion in 105 children with epilepsy shown in relation to the normal range (hatched area).

fits had finally been achieved only by the use of several drugs in combination. With the simple scoring scheme of Richens and Rowe (1970) an index of the total daily dose of drugs was calculated for each child, but no correlation could be detected between this and the serum calcium level ($r = -0.14$, $P > 0.1$).

Relationship to Urinary D-glucuronic Acid

Increased concentrations indicating a raised total urinary excretion of D-glucuronic acid were found in 99 of the 105 children. Four of the six children with values within the normal range had stopped anticonvulsant drugs several months previously because they had been free of fits for some years, and two were currently on small doses only. The increases in D-glucuronic acid excretion were often considerable, the highest being $631 \mu\text{M/g}$ creatinine as compared with a mean in control subjects of $12 \mu\text{M/g}$ (see Fig.). The six children with biochemical evidence of osteomalacia all had a raised urinary D-glucuronic acid, though the range of levels was wide ($66-279 \mu\text{M/g}$).

Statistical analysis showed a significant correlation between the concentration of D-glucuronic acid and the serum calcium level ($r = -0.275$, $P < 0.01$). This correlation was slightly higher when those on anticonvulsant drugs for more than eight years were considered separately ($n = 69$, $r = -0.312$, $P < 0.01$), and an analysis of those on treatment for a shorter period than this failed to show a significant correlation ($n = 33$, $r = -0.087$, $P > 0.1$). No correlation could be detected between urinary D-glucuronic acid and serum alkaline phosphatase levels ($r = -0.040$, $P > 0.1$).

Discussion

The frequency of biochemical abnormalities in this series of epileptic children—namely, 30% with hypocalcaemia and 24% with a raised alkaline phosphatase—is very similar to that reported by Richens and Rowe (1970). In their series of 160 adults with epilepsy 22.5% had serum calcium levels

below $9 \text{ mg}/100 \text{ ml}$, and 29% had raised alkaline phosphatase levels. Unlike these workers, however, we were unable to find any correlation between reduction in serum calcium and the total daily dose of anticonvulsant drugs that the patient was receiving. A possible explanation for this is that many of our patients were on combinations of drugs, including some of the less frequently used ones, such as carbamazepine and sulthiame, whose inducing properties are unknown.

The high levels of D-glucuronic acid found in virtually all the epileptic children in this survey are indicative of pronounced induction of the hepatic microsomal enzymes. Though the exact step at which the glucuronic acid pathway in the liver is stimulated is uncertain we have been able to show a close relation between D-glucuronic acid excretion and the dose of inducing drugs taken. There is also a significant inverse relationship to the lowering of plasma bilirubin in epileptics which may be another consequence of microsomal enzyme induction (Hunter *et al.*, 1971a). Furthermore, in guinea-pigs, which like man are unable to synthesize ascorbic acid (which in other mammals is the end product of the glucuronic acid pathway), we have found a highly significant relationship between the urinary excretion of D-glucuronic acid and the total liver content of the microsomal enzyme cytochrome P450 (Hunter *et al.*, 1971b). In the present study, therefore, the significant correlation found between urinary D-glucuronic acid and serum calcium level is strong supporting evidence, though indirect, for the suggestion that enzyme induction is the underlying cause of the hypocalcaemia associated with anti-convulsant therapy.

Vitamin D is excreted largely as an inactive glucuronide, and this process may be expected to increase with microsomal enzyme induction. However, it is also known that cholecalciferol (D₃), the main form of the vitamin in man, is converted in the liver to a metabolite with greater antirachitic activity—namely, 25-hydroxycholecalciferol (Ponchon *et al.*, 1969). Induction could lead to an increased production of this metabolite but the situation is complex for it has also been shown recently that 25-hydroxycholecalciferol is further metabolized in the liver and other organs to more polar compounds (Cousins *et al.*, 1970) such as 1,25-hydroxycholecalciferol (Lawson *et al.*, 1971) and 21,25- and 25,26-dihydroxycholecalciferol (Suda *et al.*, 1970a, 1970b). The first of these metabolites has greater antirachitic activity than 25-hydroxycholecalciferol but the two other compounds have much less. Thus induction could result in a change in the normal breakdown of vitamin D with the production of less active compounds. Similar changes in the metabolism of cortisol in man have also been described after the administration of barbiturates and phenytoin, with increased hydroxylation to 6B-hydroxycortisol—a more polar and probably less active compound (Werk *et al.*, 1964; Kuntzman *et al.*, 1968).

Vitamin D deficiency, though generally rare in Britain, has been described in immigrants (Dunnigan *et al.*, 1962; Benson *et al.*, 1963), and it may be relevant that three of the six epileptic children with biochemical evidence of osteomalacia were immigrants. The daily amount of vitamin D available to these children, estimated from the content of the school diet, was 140-290 IU, but as already mentioned it was difficult to assess how closely the actual intake was related to this. Lumb *et al.* (1971) estimated that many people in north-west England have a dietary intake of only 30-60 IU, though Dent and Smith (1969) regarded 75 IU to be the minimum adult requirement. In Britain, with such levels of dietary intake and with relatively little exposure to sunlight to compensate, it is not perhaps surprising that deficiency arises when demands for the vitamin are increased following the hepatic enzyme induction which results from anticonvulsant therapy. This may explain why there have so far been no reports of this complication from the U.S.A. or Canada, where the fortification of foodstuffs with vitamin D provides a large daily intake—often as much as 2,000-3,000 IU (American Academy of Pediatrics, 1963).

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Hyposensitization with *Dermatophagoides pteronyssinus* Antigen: Trial in Asthma Induced by House Dust

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Summary

A double-blind clinical trial of hyposensitization with aqueous extracts of *Dermatophagoides pteronyssinus* (the house-dust mite) and human skin scales showed a substantial improvement in symptoms in 11 asthmatics allergic to house dust treated with the *D. pteronyssinus* extract and a reduction in their need for other therapy. Five patients were well for a year but six relapsed. These results contrasted with the generally unfavourable course of the patients treated with the extract of human skin scales. Asthma due to house-dust allergy may be substantially improved by hyposensitization with *D. pteronyssinus* extract.

Introduction

The role of the house-dust mite (*Dermatophagoides pteronyssinus*) as the major allergen in house dust has been well established (Voorhorst *et al.*, 1967; Maunsell *et al.*, 1968; Morrow Brown and Filer, 1968; Pepys *et al.*, 1968). Patients known to be allergic to house dust also show marked skin, nasal, and bronchial allergy to the house-dust mite. The treatment of house-dust allergy by hyposensitizing injections has been disappointing (McAllen, 1961; British Tuberculosis Association, 1968), presumably because of the low concentration of mite antigen in the extracts used. We therefore carried out a double-blind trial to determine the hyposensitizing effects of injections of aqueous extract of *D. pteronyssinus* in a small group of patients with asthma induced by house dust.

Patients and Methods

Twenty-two patients were selected who had perennial asthma, with or without rhinitis; attacks of asthma on contact with house dust (as in sweeping or making beds) and frequent nocturnal and early morning attacks of asthma; an immediately positive result to a prick test with 1:10,000 extract of *D. pteronyssinus*; no history of other allergies; and negative skin tests to *Aspergillus fumigatus*, other moulds, pollens, and animal fur. Further information about them is presented in Table I. All had suffered from asthma for many years.

TABLE 1—Distribution of the Patient's Characteristics between the Two Treatment Groups

	Mite-treated Group	Control-treated Group
Total No.	11	11
Age range (years)	11-47	13-48
Males/females	5/6	9/2
Smokers	4	1
Atopic family history . . .	10	9
Allergic rhinitis	10	8
Severity of asthma: (Grade)		
Mild	0	0
Moderate	5	3
Severe	4	7
Very severe	2	1
No. on steroid	3	4
No. on disodium cromoglycate	1	1

The grading of asthma before entering the trial was based on the number of days off work in the previous six months, the severity of breathlessness, the frequency of nocturnal attacks, the frequency with which an aerosol bronchodilator was used during the month before treatment, and the type of therapy previously received by the patient.

One group of 11 patients received house-dust mite extract and the other 11 patients an extract of human skin scales. The mite extracts were prepared by Dr. Kate Maunsell in the way she has described (McAllen *et al.*, 1970), and the control extracts were prepared in the same fashion. There was no loss of skin-reacting property in the mite extract after heating, and the strength was unimpaired after storage at room temperature for 12 months.