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Abnormal vitamin D metabolism in cirrhosis

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SUMMARY Vitamin D metabolism was investigated in 10 patients with cirrhosis. Mean plasma 25 hydroxycholecalciferol (25 OHD) concentration in alcoholic cirrhosis was lower than in controls but the difference was not significant. In three patients restudied after the summer, plasma 25 OHD had risen. In contrast to the finding in normal subjects, the half-life of intravenously administered ^{3}H cholecalciferol was short in cirrhotics and showed no correlation with plasma 25 OHD. Furthermore, the appearance of ^{3}H 25 OHD from ^{3}H cholecalciferol was reduced compared to the control group four hours after injection. Increased rate of metabolism of cholecalciferol and deficient production of 25 OHD contribute to vitamin D deficiency in liver disease.

The association of osteomalacia and cirrhosis has been described (Atkinson *et al.*, 1956). Vitamin D is hydroxylated in the liver to 25 OHD, the main circulating metabolite. Superimposed liver disease in experimental animals with rickets has been shown to increase the amount of vitamin D required to heal the rickets (Heymann, 1938). Recently, low concencentrations of plasma 25 OHD have been reported in many types of cirrhosis, including cirrhosis due to alcoholism (Hepner *et al.*, 1976; Long *et al.*, 1976). The reason for low plasma 25 OHD in cirrhosis is not completely understood. We have therefore studied vitamin D metabolism in detail in 10 patients with cirrhosis.

Methods

Ten cirrhotics (five male, five female), proven by biopsy, were investigated between December and March. Eight had alcoholic cirrhosis and two cryptogenic cirrhosis. Their ages ranged from 50 to 71 years. Twelve control subjects (of both sexes) aged 30 to 70 years were investigated. Informed consent was obtained from all persons.

Patients were admitted to hospital for 10 days. Serum calcium, phosphate, alkaline phosphatase, bilirubin, albumin, and 24 hour urinary calcium and phosphate estimations were performed by Autoanalyser. A dietary history was taken with special emphasis on intake of calcium and vitamin D. Bone biopsy was taken from the iliac crest using a trephine needle. iPTH was estimated by antiserum 211/32 and vitamin D-binding protein in plasma by immunoelectrophoresis (Dann, 1978). Plasma 25 OHCC was measured by a competitive protein-binding assay (Edelstein *et al.*, 1974). Calcium absorption was measured by a forearm counting technique (Chalmers *et al.*, 1973) after seven days on a 400 mg (10 mmol) calcium diet.

Vitamin D_3 half-life, and 25 OHD production were measured after the injection of 2·5 μC 1α2α (n) ^{3}H cholecalciferol (2-10 C/mmol, Radiochemical Centre, Amersham) in absolute alcohol. Blood was sampled at four, eight, 12, 24, and 48 hours and plasma extracted by the method of Bligh and Dyer (1959). The chloroform extract was evaporated on a rotary evaporator and the cholecalciferol and 25 OHD separated by thin layer chromatography. The radioactivity from each fraction was counted in a Packard 2425 liquid scintillation counter. ^{3}H Cholecalciferol half-life was calculated from a semilog plot of $^{3}HD_3$ dpm/ml of plasma against time of the values from four to 48 hours.

Statistical analysis was performed using standard parametric tests.

Results

The plasma 25 OHD in the eight alcoholic cirrhotics was 8·7 $\pm$ 3·99 ng/ml (mean $\pm$ SD). In the two cryptogenic cirrhotics 25 OHD levels were 12·6 and 15·6 ng/ml. Plasma 25 OHD from a normal population studied at the same time of year (winter) was

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10·4 ± 3·8 ng/ml. The 25 OHD level in the alcoholic cirrhotics was thus lower than in the normal population but failed to achieve a significant degree of difference. Response to ultra-violet light was assessed by reinvestigating four alcoholic cirrhotics in the late summer. There was a considerable rise of plasma 25 OHD in three patients (Table).

Table *Response to ultraviolet light—four patients*

Patient	*25 OHD ng/ml*	*^3H Vitamin D_3 (Half-life in hours*)*
1		
Winter	1·7	6·7
Summer	12·7	9·6
2		
Winter	9·0	10·8
Summer	13·1	8·7
3		
Winter	6·7	7·5*
Summer	10·9	6·7
4		
Winter	7·4	5·5*
Summer	7·7	5·7

*Half-lives to 24 hours compared as no ^3H cholecalciferol was detected at 48 hours.

Dietary intake was assessed in eight patients. Calcium intake was 911 ± 301 mg/24 h (22·8 ± 7·5 mmol/24 h) and the vitamin D intake was 4·4 ± 4·9 μg/24 h (11 ± 12·3 nmol/24 h). Dietary intake of vitamin D did not correlate with plasma 25 OHD.

Calcium absorption was within normal limits (35-72%). Three patients had a raised serum bilirubin (39·3, 47·9, 88·9 μmol/l—that is, 2·3, 2·8, 5·2 mg/dl respectively) and all 10 patients had normal serum calcium and phosphate. Twenty-four hour urinary calcium was low in all patients (3·25 ± 1·12 mmol/24 h—that is, 130·1 ± 44·8 mg/24 h, normal range 5-10 mmol/24 h) while on a 1000 mg (25 mmol) calcium diet.

iPTH was raised in only one case. Bone biopsy revealed no evidence of osteomalacia or hyperparathyroidism.

Vitamin D-binding protein level in the cirrhotics was 298 ± 72 μg/ml (normal range 300-550 μg/ml). Vitamin D-binding protein correlated with serum albumin (r = 0·735, P < 0·02).

Vitamin D half-life was short in the cirrhotic patients (Fig. 1). In three patients no ^3H cholecalciferol could be detected in plasma at 48 hours. Vitamin D half-life may be influenced by the state of vitamin D nutrition (Mawer *et al.*, 1971). We, therefore, compared the vitamin D half-life with the prevailing plasma 25 OHD level (Fig. 1). Controls showed a significant correlation (r = 0·78, P < 0·01). Although the cirrhotic patients appeared to cluster around the projected control regression line; they did not, as a population, show a significant correlation (r = 0·23, P = NS). Vitamin D half-life remained short in the four cirrhotics who were investigated again in late summer (Table), despite the rise in 25 OHD values in three patients.

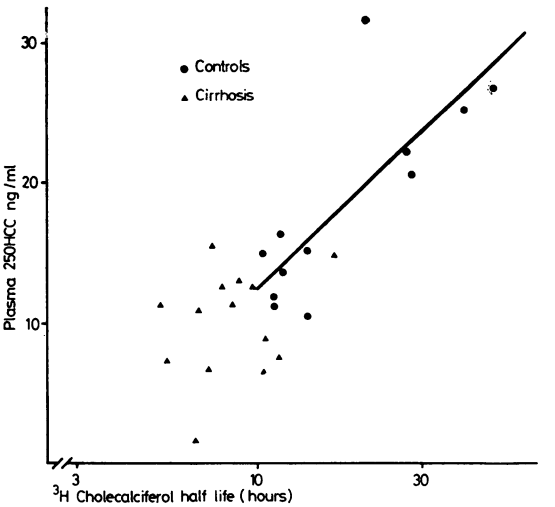

Fig. 1 *Relationship of plasma 25 OHD to ^3H cholecalciferol half-life. The line depicts the correlation in the control group (r = 0·78, P < 0·02). No correlation exists for the cirrhotics (see text). The cirrhotic group includes four patients reinvestigated in summer.*

We investigated the appearance of ^3H 25 OHD in plasma after an injection of ^3H cholecalciferol. ^3H 25 OHD was expressed as a percentage of the total ^3H dpm appearing in plasma at four hours after the injection of ^3H cholecalciferol. In both controls and cirrhotics the percentage of dpm as ^3H 25 OHD at four hours correlated with ^3H cholecalciferol half-life (Fig. 2. Controls r = 0·7, P < 0·02, cirrhotics r = 0·75, P < 0·01). However the slopes of the two regression lines differed significantly (P < 0·02), the cirrhotic groups showing a reduction in the appearance of ^3H 25 OHD in plasma when compared with controls. No significant difference was found at eight, 12, or 24 hours after injection of ^3H cholecalciferol and the majority of counts at these times were as 25 OHD in both groups.

Discussion

Low levels of plasma 25 OHD have been reported in larger series of alcoholic cirrhotics (Hepner *et al.*, 1976; Long *et al.*, 1976). The alcoholic cirrhotics in our series had lower values than the controls, but this did not reach a significant level. All our controls and cirrhotics were studied in winter when a difference may not be so apparent.

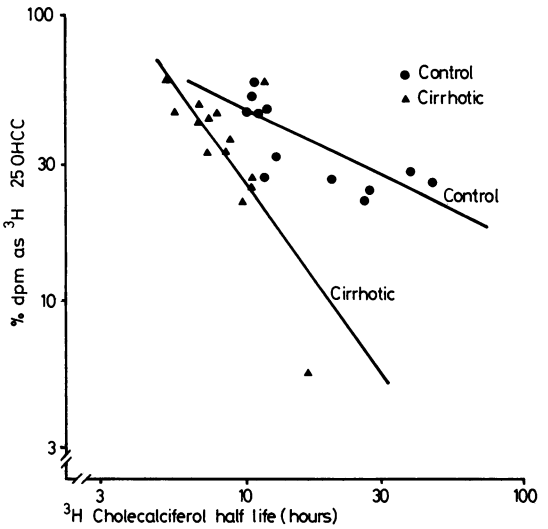

Fig. 2 *The relationship of percentage dpm in plasma as ^{3}H 25 OHD at four hours after an intravenous injection of ^{3}H cholecalciferol and ^{3}H cholecalciferol half-life in controls and cirrhotics.*

The dietary intake of vitamin D in our Caucasian cirrhotics was adequate and showed no correlation with plasma 25 OHD level, in contrast to the situation found in an immigrant population (Hunt *et al.*, 1976). In view of the rise of plasma 25 OHD at the end of summer, ultra-violet light may be more important in maintaining plasma 25 OHD in cirrhotics without biliary obstruction. It is noteworthy that patient 1 (Table) showed a considerable rise in plasma 25 OHD by the late summer in spite of a serum bilirubin of 47·9 μmol/l (2·8 mg/dl).

Vitamin D and 25 OHD are transported in plasma on a binding protein (DBP) (Haddad and Walgate, 1976). Levels in cirrhotics were low and correlated with serum albumin; this may reflect impaired liver function. However, it seems unlikely that the low levels of DBP are the cause of low 25 OHD, as the remaining DBP has a considerable reserve of binding capacity; in normal persons DBP is 2-3% saturated (Haddad and Walgate, 1976).

We found an abnormally rapid disappearance of ^{3}H cholecalciferol in cirrhosis in contrast with the findings of Avioli *et al.* (1967). No correlation existed between ^{3}H cholecalciferol half-life and prevailing level of plasma 25 OHD. Moreover, ^{3}H cholecalciferol half-life remained short in spite of a rise of plasma 25 OHD in late summer. We found a reduced appearance of ^{3}H 25 OHD in plasma at four hours in cirrhosis. This accords with the finding of an impaired rise in plasma 25 OHD 24 hours after the parenteral administration of 120 μg vitamin D (Hepner *et al.*, 1976). In hepatectomised rats in which the appearance of 25 OHD in plasma six hours after the intravenous administration of ^{3}H cholecalciferol was considerably reduced, the removal of ^{3}H cholecalciferol from plasma was slow (Olson *et al.*, 1976). On the other hand, in the cirrhotic patients a reduced appearance of ^{3}H 25 OHD in plasma was associated with a short cholecalciferol half-life.

It is not clear whether the vitamin D was disappearing into liver or into other tissues. Extrahepatic tissues appear to remove vitamin D only slowly from plasma in hepatectomised rats (Olson *et al.*, 1976), suggesting that the rapid disappearance of ^{3}H cholecalciferol in cirrhosis may be due to increased uptake by liver. If this is the major route of removal it seems likely to involve increased metabolism *via* pathways other than 25 hydroxylation.

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