⁴⁷Calcium absorption in parenchymatous and biliary liver disease

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SUMMARY As measured by whole body retention of isotopic calcium given in milk, absorption of calcium was impaired in 10 patients with chronic parenchymal non-biliary liver disease who were icteric. Mean absorption was normal in 15 patients with parenchymal liver disease who were anicteric although some individual patients absorbed less than any of the controls. Depressed absorption of calcium was seen in 10 patients with primary biliary cirrhosis and seven patients with intra- or extra-hepatic biliary obstructive disease. The most likely cause for this malabsorption is reduced bile salt secretion into the intestinal lumen which impairs vitamin D and fat absorption. The finding that parenteral vitamin D increased calcium absorption to normal levels in five patients with primary biliary cirrhosis suggests that deficiency of this vitamin is a major and correctable factor leading to calcium malabsorption in such patients. Precipitation of calcium salts by excess intraluminal fat appears to be a further possible factor reducing calcium absorption in these patients. These findings emphasize the importance of parenteral vitamin D therapy in patients with chronic obstructive biliary diseases. They also suggest that certain patients with chronic parenchymatous liver disease, particularly those who are icteric, may also occasionally require therapy with vitamin D.

Patients with any form of chronic liver disease may develop bone thinning. This is characteristically seen following prolonged cholestatic jaundice (Atkinson, Nordin, and Sherlock, 1956) but may also occur in association with anicteric liver disease (Lichtwitz, Cachin, Hioco, Tutin, and de Seze, 1959; Huguenin, Sudaka, Monier, and Monier, 1960; Teng, Daeschner, Singleton, Rosenberg, Cole, Hill, and Brennan, 1961; Summerskill and Kelly, 1963; Paterson and Losowsky, 1967). Osteoporosis is the commonest abnormality noted but osteomalacia also occurs and indeed the two may coexist (Atkinson *et al*, 1956; Badenoch, 1960; Deller, Edwards, and Addison, 1963). A possible cause for osteomalacia is malabsorption of calcium and vitamin D due to reduced intraluminal bile salt concentrations. In patients with primary biliary cirrhosis calcium absorption as measured by whole body retention of ⁴⁷Ca has been shown to be inversely correlated with the depth of jaundice, presumably reflecting the

degree of biliary obstruction, and depressed absorption is restored to normal by intramuscular vitamin D (Kehayoglou, Holdsworth, Agnew, Whelton, and Sherlock, 1969). Likewise, it has been suggested that malabsorption of calcium might be a factor in the development of bone disease in patients with chronic non-biliary liver disease (Paterson and Losowsky, 1967) and the present study was undertaken to assess calcium absorption in a group of such patients. These were compared with a group of patients suffering from various forms of biliary disease and with a group of normal subjects.

Patients and Methods

Twenty-five patients with non-biliary liver disease (15 with cryptogenic cirrhosis and 10 with active chronic hepatitis) were studied. These patients were subdivided into icteric (serum bilirubin greater than 2 mg/100 ml) and non-icteric (serum bilirubin less than 2 mg/100 ml) groups. Five patients with cryptogenic cirrhosis and five with active chronic hepatitis were icteric. Seventeen patients with cholestatic liver disease (10 with primary biliary cirrhosis, five with

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surgical extrahepatic biliary obstruction, and two with intrahepatic cholestasis) were studied. Eleven patients in this group had serum bilirubin levels greater than 2 mg/100 ml at the time of study. The diagnosis was made according to clinical and biochemical criteria, liver histology, and, where applicable, serological tests (Sherlock, 1968). Clinical details, serum bilirubin, alkaline phosphatase, and calcium levels and 24-hour urinary calcium excretion results are given in Table I. Five of the patients with primary biliary cirrhosis were studied again after at least two months' intramuscular therapy with vitamin D in a dose of 100,000 i.u. weekly for one month and then monthly. Control studies were carried out in 16 subjects without jaundice or liver disease. These comprised mainly staff volunteers and patients with chronic bronchitis. Individual diagnoses of other control patients studied were angina pectoris, gallstones, abdominal pain of undetermined origin, cellulitis, epilepsy, and recovered meningitis.

As the aim of the study was to assess calcium absorption under, as far as possible, normal day-to-day conditions, no attempt was made to control diet. Likewise, no patients received calcium supplements during the period of the study. Calcium intake before the study was calculated from a dietary history. Mean estimated daily intake of patients with non-icteric, non-biliary liver disease was 932 mg, for patients with icteric non-biliary disease 1,100 mg, and in those with primary biliary cirrhosis 1,154 mg.

Absorption of ⁴⁷Ca was measured by a modification of the method of Agnew, Kehayoglou, and Holdsworth (1969). Ten μ Ci ⁴⁷Ca was given orally to fasting subjects in 200 ml of milk (calcium content approximately 240 mg). Subjects remained fasting for three hours after taking the isotope. Retention was assessed by measuring whole body radioactivity three hours after the dose and again after seven days. After subtraction of background and correction for

radioactive decay, the seven-day ⁴⁷Ca retention was calculated as follows:

$$\text{retention} = \frac{\text{whole body count rate at seven days}}{\text{whole body count rate at three hours}} \times 100.$$

Duplicate blood samples were taken at three hours for determination of ⁴⁷Ca activity. A four-day urine collection was made to check renal excretion of ⁴⁷Ca. In no patient was urinary excretion sufficiently large to invalidate the whole body counter method of measuring absorption (Agnew *et al*, 1969). Faecal fat was measured in three-day specimens by the method of van de Kamer, ten Bokkel Huinink, and Weyers (1949) on the inpatients who were studied. Twenty-four-hour urinary calcium on a free diet was measured by the method of Baron and Bell (1959). Standard radiographs of the hands and lumbar spine were taken on all patients and none had radiological evidence of osteomalacia.

Results

The results of the whole body ⁴⁷Ca retention measurements are shown in Figure 1. The mean seven-day retention in the control group was 18.8% $\pm$ 6.4% (1 SD) while in the 25 patients with chronic non-biliary liver disease it was 15.6 $\pm$ 8.3%. The difference between these two groups as a whole is not statistically significant but in the 10 jaundiced patients taken as a separate group the mean retention (10.7 $\pm$ 5.4%) was significantly depressed ($p < 0.01$ Student's *t* test). The presence of portacaval shunts (five patients) or the administration of corticosteroids (five patients) did not significantly alter mean retention in these cases. Retention in the patients with primary biliary cirrhosis (11.1% $\pm$ 5.6%) was significantly lower than in the control group ($p < 0.01$). Retention improved in four of the five patients with primary biliary cirrhosis studied again

Group	No. and Sex		Age (years)		Serum Levels							
					Bilirubin (mg/100 ml)		Calcium (mg/100 ml)		Alkaline Phos- phatase (KA Units)		Albumin (g/100 ml)	
	M	F	Mean	Range	Mean	Range	Mean	Range	Mean	Mean	Mean	Range
Control	10	6	55	30-88	<1.0	0.5-0.8	9.6	8.5-10.4	<13	>3.5	110	68-178
Non-biliary liver disease (anicteric)	7	8	48	24-59	0.8	0.5-1.6	9.3	8.4-9.9	18	3.6	196	61-620
Non-biliary liver disease (icteric)	1	9	46	29-56	3.6	2.5-24	8.7	7.6-9.4	55	3.0	183	55-335
Primary biliary cirrhosis	1	9	51	38-65	3.8	0.3-15.0	9.3	8.2-10.0	55	3.4	150	87-270
Intra- and extra- hepatic biliary tract disease	2	5	53	34-62	6.0	0.6-11.5	9.0	8.2-9.6	70	3.8	132	65-180

Table I Clinical and biochemical data of patients and control subjects

Fig. 1 ^{47}Ca retention in non-biliary and biliary liver disease. Significance figures relate mean of each group to mean of controls (Student's *t* test).

after vitamin D therapy and remained unaltered in one; the mean improvement (5.3%) just failed to reach statistical significance.

The results of the three-hour plasma levels of radioactivity are shown in Table II. They show a

	Urinary Excretion of ^{47}Ca (% of administered dose)		Three-hr Plasma Level of ^{47}Ca (% of administered dose)	
	Mean	SD	Mean	SD
Control	2.6	1.4	1.3	0.3
Non-biliary liver disease (anicteric)	6.2	4.5	1.3	0.5
Non-biliary liver disease (icteric)	2.6	1.9	0.7	0.4
Primary biliary cirrhosis	2.1	2.3	0.8	0.5
Biliary tract disease	1.4	0.9	0.8	0.4

Table II Four-day urinary excretion and three-hour plasma levels of ^{47}Ca after administration of oral dose

similar distribution to the results for whole body retention, the normal group having the highest mean value ($1.3\% \pm 0.3$). Anicteric patients with non-biliary liver disease likewise had similar values ($1.3\% \pm 0.4$). Icteric patients had low levels ($0.7\% \pm 0.4$) as had those with primary biliary cirrhosis ($0.8\% \pm 0.4$) and extra- and intra-hepatic cholestasis ($0.8\% \pm 0.4$).

The patients with primary biliary cirrhosis given

vitamin D showed a mean increase in three-hour plasma radioactivity from 0.64% to 1.12% after treatment and there was a good correlation between this improvement and the improvement in whole body retention (Fig. 2).

In the 17 patients (eight primary biliary cirrhosis, five intra- and extra-hepatic cholestasis, and four non-biliary disease) in whom faecal fat was measured there was a significant negative correlation ($r = -0.68$; $p < 0.01$) between faecal fat and whole body retention (Fig. 3).

Fig. 2 Correlation between plasma radioactivity three hours after dose and ^{47}Ca retention in five patients with primary biliary cirrhosis before and after vitamin D treatment.

Fig. 3 Correlation between degree of steatorrhoea and whole body ⁴⁷Ca retention in 17 patients with hepato-biliary disease.

Discussion

Although metabolic balance studies are probably the most accurate method of measuring calcium absorption they are tedious, difficult, and necessarily in-patient investigations. Isotopic methods, while imperfect (Deller, Worthley, and Martin, 1965), are rapid and straightforward and can be applied to the ambulant outpatient. While the figure for retention of isotope cannot indicate actual rates of absorption they can probably be safely used to compare the facility for absorption between groups of patients (Agnew *et al*, 1969). When the isotope is given in aqueous solution with an ionized calcium salt as the carrier, the measurement of isotopic retention should be regarded as predominantly a test of small intestinal mucosal function rather than a test of ability to absorb dietary calcium (Kehayoglou *et al*, 1968). In the present investigations, isotopic calcium was given with milk, that is, with a much greater amount of cold calcium (approximately 240 mg) in a form common in food. In this way it was hoped that the test might be influenced not only by defects in intestinal mucosal function but also those due to intraluminal disturbances. This modified test was utilized since the aim of these investigations was to assess absorption in a group of ambulant patients in whom it was suspected that any malabsorption might be slight. Deller (1966) has shown that using milk instead of an ionized calcium salt as the carrier increases the proportion of subnormal absorption results in patients with post-gastrectomy malabsorption or pancreatic steatorrhoea and that the use of

milk as the carrier may therefore give a better guide to the patient's true absorption of dietary calcium. Retention of a ⁴⁷Ca dose given with milk cannot, however, give an accurate measure of dietary calcium absorption as there are many other substances in a normal diet which may influence calcium absorption.

Total absorption of the isotope as indicated by whole body retention measured on the seventh day after ingestion was considerably less in normal subjects when the isotope was given in milk in this study ($18.8\% \pm 6.4\%$, mean $\pm$ SD) than when given with the smaller dose of calcium in water in the previous tests ($48.6\% \pm 6.6\%$). This decrease in normal absorption is due partly to the increase in carrier calcium level (Deller *et al*, 1965) and partly to the use of milk rather than ionized calcium as the carrier (Deller *et al*, 1965; Arman, Nilsson, and Reizenstein, 1970).

In the earlier study using 10 mg of cold calcium as the carrier, the mean retention of ⁴⁷Ca was not significantly less in jaundiced patients with primary biliary cirrhosis than in a group of normal subjects (Kehayoglou *et al*, 1968). In the present study, however, where the isotope was given in milk, a significant depression of mean retention was seen in jaundiced patients with both biliary and non-biliary liver disease.

The age ranges were similar in each of the groups studied so that differences in absorption due to variation with age can be discounted here (Avioli, McDonald, and Lee, 1965; Bullamore, Gallagher, Wilkinson, Nordin, and Marshall, 1970). Although absorption of isotopic calcium is markedly influenced by the patient's dietary intake preceding the test (Spencer, Lewin, Fowler, and Samachson, 1969) this also is unlikely to be an important cause of variation in absorption in these investigations since dietary intake appeared to be similar in each of the groups studied.

The impaired retention of calcium demonstrated here in the icteric group of patients with parenchymatous liver disease and in the group with biliary disease could have been due to a mucosal defect in calcium absorption secondary to vitamin D deficiency or to a defect in the intraluminal phase of calcium absorption. Both of these possible mechanisms may be acting together in individual patients. Calcium absorption may be impaired directly as a result of the formation of insoluble calcium soaps in the presence of excess fat in the lumen and there is supportive evidence to suggest that excess intraluminal fat interferes directly with calcium absorption (Southgate, Widdowson, Smits, Cooke, Walker, and Mathers, 1969; Agnew and Holdsworth, 1971). Vitamin D deficiency may arise because of failure to absorb the vitamin adequately. Alternatively, as the liver

appears to be the major site of transformation of vitamin D to its active metabolite (Ponchon and DeLuca, 1969), there may be a failure to metabolize the vitamin adequately and there is some evidence that this may occur in hepatic cirrhosis. The finding that parenteral vitamin D therapy corrected the absorptive defect for calcium in patients with primary biliary cirrhosis (admittedly in dosage greater than required to correct nutritional vitamin D deficiency) suggests that deficiency of the vitamin is an important factor in the calcium malabsorption seen in association with this disorder. This conclusion is strongly supported by the observation that calcium absorption from milk in this study, as well as from water (Kehayoglou *et al*, 1968), was improved by vitamin D therapy. Furthermore, on this evidence, failure to metabolize vitamin D to its active form would not appear to be a factor of major significance, at least in the patients with biliary disease.

Significant depression in retention of isotopic calcium was not demonstrated in patients with anicteric parenchymatous liver disease. It is possible that these patients had less severe liver disease than the icteric group and indeed the mean serum albumin of the group (3.6 g/100 ml) was appreciably higher than that of the jaundiced patients (3.0 g/100 ml). Thus even though there was no collateral clinical evidence that liver disease was significantly more severe in the jaundiced patients, a defect in vitamin D metabolism due to parenchymal damage cannot be excluded as an additional cause for the malabsorption seen in the icteric subgroup.

Since bile is necessary for the adequate absorption of vitamin D (Schachter, Finkelstein, and Kowarski, 1964), it is reasonable to suggest that vitamin D malabsorption may be due to defective bile salt liberation into the duodenum. This is particularly likely in the patients with biliary obstructive diseases and may also be true of those patients with parenchymatous liver disease who were icteric. It is interesting that even in the anicteric subgroup, where the mean retention of calcium was similar to normal, some individual patients retained less isotope than any of the normal subjects. A relative deficiency of intraluminal bile salts, in the absence of a block to biliary flow, has been demonstrated in cirrhosis (Badley, Murphy, and Bouchier, 1969) and it is possible that this is a factor in the vitamin D and hence the calcium malabsorption of these anicteric individuals. It is of interest in this respect that isotope retention was inversely correlated with faecal fat levels in those patients in whom this was measured. This is a not unexpected finding since the malabsorption of vitamin D and fat both probably result from bile salt deficiency. The presence of unabsorbed fats in the intestinal lumen may also be an additional factor

in depressing calcium absorption in patients with chronic liver disease, although this seems less significant than vitamin D deficiency.

All the patients who absorbed calcium poorly are presumably liable to develop metabolic bone disease if they survive long enough and if they are not treated (Atkinson *et al*, 1956). Whatever the cause of this malabsorption in jaundiced patients the therapeutic implications are clear. Parenteral vitamin D restores calcium absorption to normal, although this does not always halt bone thinning in patients with chronic liver disease. The importance of replacement therapy with vitamin D and calcium in patients with primary biliary cirrhosis is established. The present study emphasizes that similar replacement therapy may also be necessary in some patients with non-biliary liver disease. This particularly applies to patients who are jaundiced for any length of time.

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