of aldehyde dehydrogenase. Our finding of a biphasic acetaldehyde metabolism accords with earlier studies on purified sheep liver and human liver cytosolic enzyme.[12 14] Biphasic kinetics may have several causes: (*a*) enzymes may exhibit co-operative behaviour,[16] (*b*) different isoenzymes may be present, and (*c*) one enzyme may consist of non-equivalent subunits which have a different affinity for the substrate. The difference in metabolic rates between flushers and non-flushers was unlikely to be due to a difference in enzyme concentration, since the elimination in phase 1 was almost identical for the two groups. Our data support the hypothesis of a difference in aldehyde dehydrogenase activity between flushers and non-flushers, which is probably due to two types of high affinity binding sites for acetaldehyde. Hypothetically this difference in enzyme activity between those at risk of developing late complications and those who are not could be used after further refinement to identify patients at risk early by using a simple blood test. Determining the biochemical basis of protection against late complications through the change in enzyme activity will be an even more important step.

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High serum vitamin B_{12} binding capacity as a marker of the fibrolamellar variant of hepatocellular carcinoma

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Abstract

Ten (9·3%) of 107 patients with hepatocellular carcinoma had considerably increased serum unsaturated vitamin B_{12} binding capacity. All 10 were young (mean 21 years), had no serum alpha-fetoprotein, and no underlying cirrhosis; all had a longer survival compared with patients without increased serum unsaturated vitamin B_{12} binding capacity in the study. Seven of the 10 patients had fibrolamellar hepatocellular carcinoma, a recently recognised histological variant, which was found in only one young patient without increased serum unsaturated vitamin B_{12} binding capacity and no alpha-fetoprotein among the remaining 97. This high degree of correlation between increased serum unsaturated vitamin B_{12} binding capacity and fibrolamellar hepatocellular carcinoma has not been reported before. Increased serum unsaturated vitamin B_{12} binding capacity may be of considerable help in diagnosis, prognosis, and monitoring treatment of this well-defined group of patients with hepatocellular carcinoma but no alpha-fetoprotein.

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Introduction

A fibrosing variant of hepatocellular carcinoma found in young people was first noted by Edmondson in 1958,[1] but the clinical features of this variant, including its development in the non-cirrhotic liver and its relatively good prognosis, were not reported until 1976.[2] Because of the distinct eosinophilic cytoplasm of the tumour cells and the parallel arrangement of the collagen in the conspicuous fibrous septa the tumour was called eosinophilic hepatoma with lamellar fibrosis, which was later shortened to fibrolamellar carcinoma.[3] There have also been reports[4-6] of

hepatocellular carcinoma in adolescents and young adults (adolescent hepatoma) with no underlying cirrhosis and appreciably increased serum vitamin B_{12} binding capacity due either to production or to modification of the binding protein transcobalamin I by the tumour,[7 8] but there are no detailed histological studies of hepatocellular carcinoma associated with increased serum unsaturated vitamin B_{12} binding capacity.

In a study of the clinical and histological features of 107 patients with hepatocellular carcinoma we measured their serum unsaturated vitamin B_{12} binding capacity and alpha-fetoprotein concentrations. A raised concentration of serum vitamin B_{12} binding capacity occurred mainly in patients with fibrolamellar carcinoma and can be used as a marker of this type of tumour.

Patients and methods

The 107 patients (aged 15-75 years) with histologically confirmed hepatocellular carcinoma comprised 87 men and 20 women, nine of whom had taken oral contraceptive preparations at some time. Sixty-eight had underlying cirrhosis and 78 had raised serum concentrations of alpha-fetoprotein (ranging from 35 to 1·25 ng/ml by radioimmunoassay). The material examined came from needle biopsies, surgical biopsies, or tumour resection specimens. Three biopsy specimens from patients with increased serum unsaturated vitamin B_{12} binding capacity and fibrolamellar carcinoma were also examined by electronmicroscopy. Serum unsaturated vitamin B_{12} binding capacity was measured by radioassay[9] in serum samples collected before treatment and stored at −20°C (normal values 0·7-1·6 μg/l).

Results

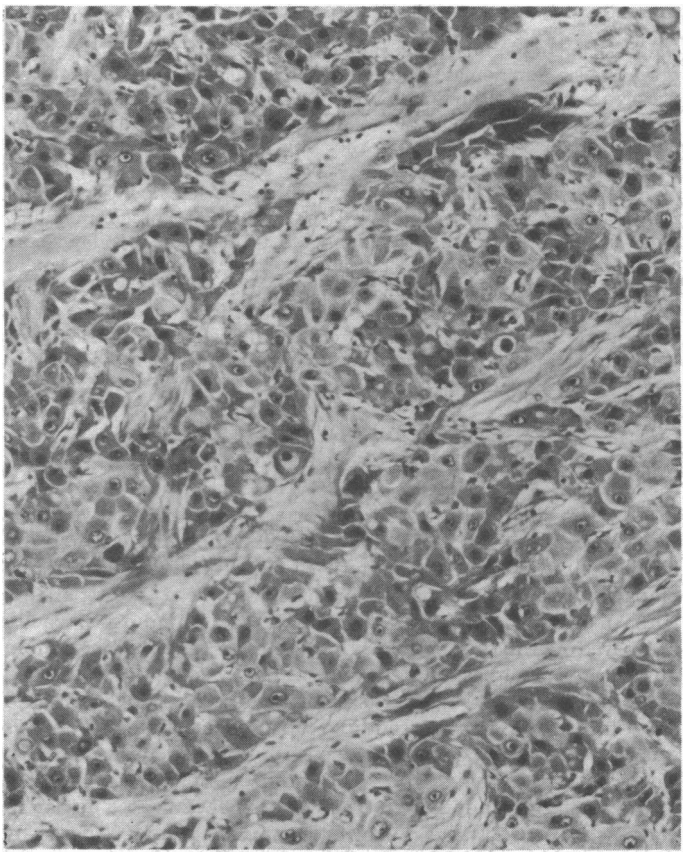
CLINICAL FEATURES

Eight of 107 patients had characteristic features of fibrolamellar carcinoma (figure); of these, seven as well as three others without diagnostic fibrolamellar features had considerably increased serum unsaturated vitamin B_{12} binding capacity (table I), the correlation between increased serum unsaturated vitamin B_{12} binding capacity and fibrolamellar carcinoma being highly significant (table II). Abnormally high serum vitamin B_{12} concentrations were present in the six patients in whom these were measured in the same pretreatment samples (table I).

Serial values were available in seven of the patients with increased serum unsaturated vitamin B_{12} binding capacity. In four, who showed no clinical evidence of response to chemotherapy, values gradually rose, with death occurring at six, 12, 14, and 14 months after diagnosis. The maximum increase in serum unsaturated vitamin B_{12} binding capacity between diagnosis and death was 75%. In a fifth patient the serum unsaturated vitamin B_{12} binding capacity fell by 75% during a remission induced by adriamycin and subsequently rose again to exceed the presentation value, with relapse, disease progression, and death at 20 months. In the two patients who underwent hemihepatectomy serum unsaturated vitamin B_{12} binding capacity returned to normal after operation.

The one-year projected survival by the life-tables method shows that 79% of patients with hepatocellular carcinoma associated with increased serum unsaturated vitamin B_{12} binding capacity were alive, which compares favourably with 28% (22 of 77 patients not withdrawn from the study) of live patients with other types of hepatocellular carcinoma ($\chi^2=7·37$; $p<0·01$).

None of the patients with fibrolamellar carcinoma or the three others with increased serum unsaturated vitamin B_{12} binding capacity had a raised serum alpha-fetoprotein concentration. In contrast, 78 of 97 (81%) with normal serum unsaturated vitamin B_{12} binding capacity had a raised serum alpha-fetoprotein concentration.



Fibrolamellar carcinoma composed of eosinophilic cells forming cords separated by parallel collagen lamellae. (Haematoxylin and eosin × 120)

TABLE II—*Correlation between histology and increased serum unsaturated vitamin B_{12} binding capacity (UBBC)*

Histology	Increased UBBC (No of patients): With	Without	Total
Fibrolamellar hepatocellular carcinoma	7	1	8
Other hepatocellular carcinoma	3	96	99
Total	10	97	107

$\chi^2=52·8$, $p<0·001$.

TABLE I—*Clinicopathological details of 10 patients with hepatocellular carcinoma associated with increased unsaturated vitamin B_{12} binding capacity*

Patient	Age	Sex	UBBC (μg/l) (normal 0·7-1·6)	Vitamin B_{12} (ng/l) (normal 160-750 ng/l)	Specimen	Histology	Cirrhosis	Follow up (months)	Treatment
1	26	M	170·0		Needle	Fibrolamellar	No	Died (14)	Embolisation + adriamycin
2	20	M	39·5		Surgical	Fibrolamellar	No	Alive (15)	Embolisation + adriamycin
3	18	M	82·0		Surgical	Trabecular/acinar	No	Died (14)	Embolisation + adriamycin
4	20	F	379·0	995	Needle	Trabecular/acinar	No	Alive (22)	Adriamycin
5	16	F	183·0		Surgical	Fibrolamellar	No	Died (12)	Embolisation + adriamycin
6	28	F	29·6	1 345	Needle	Trabecular/acinar	No	Alive (4)	Adriamycin
7	16	M	144·0	3 500	Surgical	Fibrolamellar	No	Died (20)	Adriamycin
8	15	F	414·0	15 500	Needle	Fibrolamellar	No	Died (6)	Adriamycin
9	30	F	79·0	13 000	Resection	Fibrolamellar	No	Alive (50)	Resection + adriamycin
10	21	F	39·7	>900	Resection	Fibrolamellar	No	Alive (10)	Resection + adriamycin

UBBC = Unsaturated vitamin B_{12} binding capacity.

HISTOLOGICAL AND ELECTRONMICROSCOPIC FEATURES

Intracellular globular inclusions were seen in seven of the eight fibrolamellar carcinomas. They were periodic acid Schiff-positive and diastase-resistant, although they stained less strongly than comparable inclusions known to consist of alpha-fetoprotein or alpha 1 antitrypsin. On electronmicroscopy they appeared to correspond to electron-dense membrane-bound inclusions. Pale inclusions and similar material in cells and pseudoacini (probably the "pale bodies" of Craig *et al*[3]) stained more consistently with alcian blue and orcein methods, indicative of strongly sulphated acid glycoprotein content. They corresponded electron microscopically to granular and basement membrane-like material both free and within dilated endoplasmic reticulum lakes. In the fibrotic lamellar areas the main stromal cell was the fibroblast, although some macrophages were also seen. In the non-fibrotic areas the main stromal cell was endothelial. No lipocytes were seen.

Discussion

Our data show a hitherto unreported association between increased serum unsaturated vitamin B_{12} binding capacity and the fibrolamellar histological pattern of hepatocellular carcinoma. Hepatocellular carcinoma was present in seven of the 10 patients with increased unsaturated vitamin B_{12} binding capacity compared with only one of the other 97 patients in whom unsaturated vitamin B_{12} binding capacity was not increased ($p < 0.001$). The three patients (cases 3, 4, and 6) with increased unsaturated vitamin B_{12} binding capacity in whom no fibrosis was seen could very possibly also have had fibrolamellar carcinoma, since fibrosis in this type of tumour is not always present throughout the tumour, which may be trabecular or acinar in places.[3] The association is, however, not complete, as shown by the one patient with fibrolamellar hepatocellular carcinoma and normal serum unsaturated vitamin B_{12} binding capacity.

The increased serum unsaturated vitamin B_{12} binding capacity in some previously reported cases of hepatocellular carcinoma was due to an increase in a vitamin B_{12} binding protein which differs from normal transcobalamin I only in that it contains more sialic acid residues.[7 8] Whether it is produced by the tumour or stored and modified within it by addition of sialic acid has not been established. Transcobalamin I was not measured in this series, but it is of interest that acid glycoproteins were detected histochemically and basement membrane-like material electron microscopically. Whether these features are morphological manifestations of the presence of transcobalamin I must await the development of specific antibodies. It is not known if the presence of transcobalamin I could stimulate fibrogenesis, which is otherwise uncommon in hepatocellular carcinoma. In six patients with hepatocellular carcinoma associated with unsaturated vitamin B_{12} binding capacity in whom vitamin B_{12} was measured in the same pretreatment sera as unsaturated vitamin B_{12} binding capacity, the concentration of vitamin B_{12} was found to be raised (table I). A combination of serum unsaturated vitamin B_{12} binding capacity and vitamin B_{12} measurements may be more informative than estimations of unsaturated vitamin B_{12} binding capacity alone, since marginal rises of transcobalamin can be masked by a high concentration of serum vitamin B_{12}.[6]

The description of a characteristic histological picture in addition to the previously reported clinical features makes tumours associated with unsaturated vitamin B_{12} binding capacity a distinct subgroup of hepatocellular carcinoma. Determination of unsaturated vitamin B_{12} binding capacity does not, however, replace histological diagnosis, since increased binding capacity in serum has been described in patients with disseminated neoplasms from other primary sites, particularly when hepatic metastases are present[10]; in these patients serum unsaturated vitamin B_{12} binding capacity is seldom as high as in hepatocellular carcinoma associated with increased serum unsaturated vitamin B_{12} binding capacity and patients have been older and in the terminal stages of their disease.

Our observations confirm earlier findings[6] that changes in serum unsaturated vitamin B_{12} binding capacity, even in the absence of vitamin B_{12} and transcobalamin I determinations, reflect disease progression. Serum unsaturated vitamin B_{12} binding capacity thus seems to be a useful serum marker in this group, particularly since the main serum marker of hepatocellular carcinoma (namely alpha-fetoprotein) is consistently absent. Whereas serum alpha-fetoprotein behaves as if it reflects tumour cell mass, rising exponentially with time in the absence of effective treatment, unsaturated vitamin B_{12} binding capacity changes much less appreciably. Serum alpha-fetoprotein concentrations may increase more than 10-fold between presentation, diagnosis, and death, whereas unsaturated vitamin B_{12} binding capacity increased by a maximum of 75% in this series. It seems unlikely therefore that the actual increase in unsaturated vitamin B_{12} binding capacity bears any direct relation to tumour size.

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ONE HUNDRED YEARS AGO Mr Anthony Trollope's illness, which terminated fatally on Wednesday night, presents many points of medical interest. In January last he consulted Dr Murrell for shortness of breath on exertion, and other symptoms which had been attributed to angina pectoris. On inquiry, it was found that there had been no true anginal attack, but an examination disclosed the existence of an aortic diastolic murmur. It was decided that Mr Trollope should take a rest, and he accordingly went to Ireland for a holiday. In October he returned to town, and resumed his literary labours. On November 3rd he was suddenly seized with aphasia and paralysis of the right arm and leg, evidently due to embolism. At first there was great mental excitement, which was with difficulty subdued by the free administration of bromide of sodium. For some days he was able to utter only a few simple words, which served to express his wants, but, little by little, the power of language returned and the paralysed limbs regained power. Last week he had so far recovered as to take outdoor exercise, and there was every prospect of a speedy restoration to health. On Saturday last there was some return of the mental irritability, and it was apparent that further changes were taking place in the brain-substance. He gradually became comatose, and for the last three days of his life took nothing by mouth, with the exception of a little iced water. Predigested foods were used in the form of enemata, and suppositories of peptones were found useful. The immediate cause of death was congestion of the lungs. (*British Medical Journal*, 1882.)