

Plasma alkaline phosphatase isoenzymes in hepatobiliary disease

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SYNOPSIS By cellulose acetate or acrylamide gel electrophoresis it is possible to separate these alkaline phosphatase isoenzymes from serum: [anode] fast liver, slow liver, placenta/Regan, bone, intestine, bile [cathode]. Heat or chemical inhibition can confirm the differentiation.

Normal adult serum always contains slow-liver isoenzyme, and sometimes bone isoenzyme: the latter is always present in serum of children. In hepatobiliary disease slow-liver isoenzyme was always increased: intestinal isoenzyme appeared in many cases of cirrhosis (of blood groups B and O) but fast-liver and bile isoenzymes were occasionally seen in miscellaneous cases. The findings in other diseases included Regan isoenzyme in six out of 45 cases of malignant disease.

Four major isoenzymes of alkaline phosphatase (orthophosphoric-monoester phosphohydrolase: EC 3.1.3.1) have been known for some time and can be easily separated in normal and abnormal serum: they are produced respectively by liver parenchyma, placenta, intestinal mucosa, and osteoblasts (Arfors, Beckman, and Lundin, 1963; Green, Cantor, Inglis, and Fishman, 1972; Fishman, Bardawil, Habib, Anstiss, and Green, 1972). The predominant liver isoenzyme is 'slow' or ' α_2 '. More recently, a second liver isoenzyme ('fast' or ' α_1 ') has been demonstrated in the serum of some patients with hepatobiliary disease but its origin is uncertain (Rhone and Mizuno, 1973; Fritsche and Adams-Park, 1972). The separate isoenzyme found in bile arising from duct cells may occasionally be detected in serum (Kaplan and Rogers, 1969). The isoenzyme present in chyle has properties similar to those of intestinal isoenzyme (Inglis, Fishman, Stolbach, Warsaw, and Fishman, 1972). Regan isoenzyme represents the related isoenzymes with properties similar to placental isoenzyme that may be produced by malignant tissues (Fishman, Inglis, Stolbach, and Krant, 1968).

There is uncertainty about the diagnostic value of determining serum alkaline phosphatase isoenzymes. We have therefore investigated the iso-

enzyme patterns in a variety of hepatobiliary disorders and in some other conditions for comparison. The opportunity has been taken to compare the acrylamide gel (Smith, Lightstone, and Perry, 1968; Canapa-Anson and Rowe, 1970) and the cellulose acetate strip (Fritsche and Adams-Park, 1972) methods of separating isoenzymes.

Materials and Methods

Normal sera were obtained from fasting laboratory staff and from specimens of child inpatients with unrelated complaints such as tonsillitis. Abnormal sera were obtained from fasting inpatients, and occasionally from outpatients, of the Royal Free Hospital. Diagnoses were based on clinical assessment and on full laboratory and radiological studies, supplemented when appropriate by liver biopsy.

Serum was separated as soon as possible. The sera were first analysed for total alkaline phosphatase activity by the kinetic method of Moss (1966), adapted by Stevens and Thomas (1972), in order to determine the amount of serum needed for electrophoresis. On cellulose acetate 6 μ l of serum containing less than 20 KA units/dl was used, while 3 μ l was sufficient for serum containing higher activities. On acrylamide gel 25 μ l of serum containing less than 30 KA units/dl was used: if 30-60 KA units/dl, 20 μ l; 60-90 KA units/dl, 15 μ l; 90-120 KA units/dl, 10 μ l; 120-150 KA units/dl, 5 μ l. The serum was diluted with aqueous sucrose solution (20g/dl) if the activity exceeded 150 KA units/dl.

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Portions of the sera were frozen at -20°C until analysed for isoenzymes: they were all analysed within one week of obtaining the specimens. This delay did not alter the isoenzyme distribution.

INHIBITION

Since the heat-stable placental and Regan isoenzymes have a mobility similar to but distinguishable from the bone isoenzyme, their presence was confirmed by repeating the electrophoretic separation after destroying the bone isoenzyme by heat at 65°C for 30 minutes (Aoba, Hariu, and Yamaguchi, 1967). Differentiation of intestinal from liver isoenzyme was confirmed by its sensitivity to 5 mmol/l concentration of L-phenylalanine, while bone and liver isoenzymes were characterized by their sensitivity to 25 mmol/l concentration of L-homoarginine (Green *et al*, 1972).

ELECTROPHORESIS

For electrophoresis on acrylamide gel (borate buffer pH 8.8; preparation buffer tris-citrate pH 8.8, Smith *et al*, 1968) and on cellulose acetate (tris-barbitone buffer pH 8.8, Fritsche and Adams-Park, 1972) the substrate was β -naphthyl phosphate and the stain Fast Blue RR. Acrylamide electrophoresis was performed in the cold room at 4°C for six hours at 90 V, cellulose acetate electrophoresis at room temperature for 18 min (0.3 h) at 200 V.

Results

COMPARISON OF SEPARATION PROCEDURES

The figure shows the distribution of isoenzymes in relation to serum proteins. The analyses are of sera from typical clinical cases. The order of separation is (anode): fast liver, slow liver, placental Regan, bone, intestinal, bile (origin): (cathode).

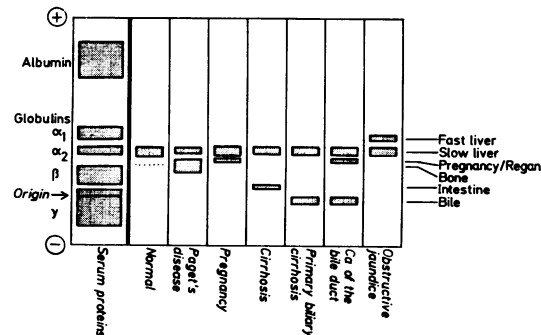


Fig Electrophoretic separation of alkaline phosphatase isoenzymes of human serum in health and disease compared with the pattern of normal serum proteins.

The acrylamide gel method separates the bone (osteoblastic) and 'slow' liver (α_2) isoenzymes more clearly but the bile isoenzyme cannot be identified with certainty. Results are otherwise identical with either method. The ease of setting up and the short time required for adequate electrophoretic separation make the cellulose acetate strip a suitable method for the routine analysis of alkaline phosphatase isoenzymes in serum and there has been no difficulty in detecting individual increases in liver and bone isoenzymes.

PATTERN IN HEALTH AND DISEASE (TABLE 1)

Liver isoenzyme (slow α_2) was universally detected. Normal adult serum usually showed only the liver band, but in 13% of samples a slight bone band was also detected. In growing children the bone band was always seen, the total serum alkaline phosphatase values being between 15 and 28 KA units/dl. In normal pregnancy the placental band was first seen at 15 weeks, and in the last trimester was detected in 28% of samples: placental isoenzyme was always found when the total alkaline phosphatase exceeded 20 KA units/dl. One each of five cases of normal twins, of three cases of toxæmia, of six cases of hypertension, and of three cases of post-mature pregnancy showed a placental band, the total alkaline phosphatase activity being in the range of 20-27 KA units/dl.

All patients with hepatobiliary disease who were studied had raised serum total alkaline phosphatase. In cases of cirrhosis 22% showed the intestinal band. Fast (α_1) liver isoenzyme was seen in a few cases of infective hepatitis, obstructive jaundice, or primary biliary cirrhosis but not in cirrhosis. The bile isoenzyme occurred only occasionally, with or without the fast liver isoenzyme, in primary biliary cirrhosis and in carcinoma of the bile duct.

Of the 38 cases of intestinal disease examined, six had a raised total alkaline phosphatase (17-21 KA units/dl). An intestinal band was seen in one case of malabsorption syndrome due to coeliac disease, with a total alkaline phosphatase of 18 KA units/dl.

All patients with bone disease had an increased bone band when there was an increased total serum alkaline phosphatase and increased osteoblastic activity, as did some patients with chronic renal failure: the one fracture case without a detectable bone band was a 70-year-old woman with a fracture that had failed to unite and a total alkaline phosphatase of 9 KA units/dl. In multiple myeloma with no osteoblastic activity and a normal alkaline phosphatase, no band was seen except for one case in which the bone band was no more than was found in the occasional normal adult patient mentioned

	Type of Disease	No. of Cases	Alkaline Phosphatase Isoenzymes					
			Slow Liver	Fast Liver	Bile	Intestine	Bone	Placenta
Normals	Adults	46	46				6	
	Growing children 3-12 years	21	21				21	
	Pregnancy—1st trimester	41	41					
	2nd trimester	29	29					3
	3rd trimester	47	47					13
Diseases Liver	Infective hepatitis	38	38	4				
	Obstructive jaundice (gallstones)	26	26	4				
	Primary biliary cirrhosis	42	42	2		3		
Intestinal	Cirrhosis of the liver	54	54			12		
	Ulcerative colitis	2	2					
	Malabsorption syndrome	9	9					
	Coeliac disease	4	4			1		
Bone	Duodenal ulcer	22	22					
	Fractures	10	10				9	
	Rickets	6	6				6	
Renal	Paget's disease	3	3				3	
	Multiple myeloma	4	4				1	
	Chronic renal failure	12	12			3	5	
	Glomerulonephritis	7	7					
Primary malignant disease	Liver	3	3					1
	Lung	9	9					1
	Bile duct	15	15	2		1		3
	Pancreas	4	4					
	Breast	6	6					
	Prostrate	3	3					
	Colon	2	2					
	Vulva	1	1					
	Stomach	1	1					
	Tongue	1	1					

Table I Serum alkaline phosphatase isoenzymes in normal subjects and in disease

found in the occasional normal adult patient mentioned earlier.

Patients with acute glomerulonephritis and pyelonephritis without uraemia had no abnormal band, whereas some patients with advanced chronic renal failure showed the intestinal band.

The sera from patients with malignant disease were particularly examined for Regan isoenzyme which was detected in six out of 45 miscellaneous cases. Of interest is its occurrence in three out of 15 cases of carcinoma of the bile duct, one of which also had fast-liver and bile isoenzymes.

We have not investigated chyle.

INTESTINAL ISOENZYME AND BLOOD GROUP

Table II shows that the intestinal isoenzyme often

Blood Groups	No. of Patients (cirrhosis)	Isoenzymes	
		Liver	Intestine
O	26	26	10
A	20	20	—
B	6	6	2
AB	2	2	—
Total	54	54	12

Table II Alkaline phosphatase isoenzymes and blood groups

demonstrable in cirrhosis is not present in patients with blood group A or AB. There were sufficient group A patients for this to be significant.

DIFFERENTIAL EFFECT OF INHIBITORS

Table III confirms that various inhibitors can be used, mostly in conjunction with electrophoresis, for the identification of abnormal alkaline phosphatase isoenzymes.

Isoenzyme	Heat (65°C 5 min)	L-phenylalanine (5 mmol/l)	L-homoarginine (25 mmol/l)
	Degree of Inhibition		
Bone	++++	+	++++
Liver	++	+	++++
Intestine	+	++++	—
Placenta	—	++++	—
Regan	—	++++	—

Table III Effect of inhibitors on alkaline phosphatase isoenzymes

Discussion

In normal subjects, previous authors (Fritsche and Adams-Park, 1972) have found the slow (α_2) liver band with bone or intestinal band or both, while

Rhone and Mizuno (1973) have found only the slow (α_2) liver band in 19 out of 24 subjects. We have found only the slow (α_2) liver band in 40 out of 46 normal sera, the other six sera showing the slow (α_2) liver band and the bone band.

The regular finding in growing children between 3 and 12 years of age of a bone band in addition to the slow (α_2) liver band confirms the osteoblastic origin of the bone isoenzyme (Nagant de Deuxchaisnes and Krane, 1964). In normal pregnancy, the placental isoenzyme reaches its peak in maternal serum between 37 and 40 weeks at which time the growth of the microvilli declines rapidly (Fox, 1968; Fishman *et al.*, 1972). We have found the placental isoenzyme most frequently in the third trimester of pregnancy, in nearly one-third of subjects.

There have been many detailed studies of the changes in serum alkaline phosphatases, both total and differential in abnormal pregnancy (Fishman and Ghosh, 1967; Fox, 1968; Fishman *et al.*, 1972).

The first report of the intestinal band in cirrhosis was by Fishman, Inglis, and Krant (1965). Their patients were of blood groups B and O: other series (Kaplan and Rogers, 1969) have confirmed their finding. The intestinal band has been reported in normal subjects of blood groups B and O (Arfors *et al.*, 1963; Bamford, Harris, Luffman, Robson, and Clegghorn, 1965): Beckmann (1964) related this to ABH secretor status. The presence of intestinal isoenzyme is also related to diet, the ingestion of fat elevating the intestinal isoenzyme level in the sera of blood group O secretor individuals (Langman, Leuthold, Robson, Harris, Luffman, and Harris, 1966; Walker, Eze, Tweedie, and Evans, 1971). This finding is in conformity with ours in 12 out of 54 cirrhotics showing the intestinal band, all 12 being of blood group B or O. In two of our patients who were studied serially, the intestinal isoenzyme disappeared from serum as the aspartate transaminase and alkaline phosphatase returned towards normal. The finding of intestinal alkaline phosphatase in a suspected cirrhotic patient is confirmatory evidence of the diagnosis and may be useful in monitoring the course of the disease; absence of the isoenzyme has no diagnostic importance.

It is of interest that the intestinal isoenzyme was found in some cases of chronic renal failure, the total alkaline phosphatase being raised in all of these cases. The presence of the intestinal isoenzyme is unrelated to the plasma urea concentration but the patients are again of blood group B or O. Further investigation of this finding is necessary. The presence of the bone isoenzyme found in chronic renal failure may be due to increased osteoblastic activity.

The fast (α_1) liver isoenzyme has been reported

in a wide variety of hepatobiliary diseases whether primarily hepatocellular or primarily cholestatic (Korner, 1962; Taswell and Jeffers, 1963; Rhone and Mizuno, 1973) and this has been our experience. It is completely unrelated to total alkaline phosphatase and its presence, although it indicates hepatobiliary disease, is of no diagnostic or prognostic importance.

Bile isoenzyme has previously been reported in the serum of cases of primary biliary cirrhosis (Kaplan and Rogers, 1969); it was found here in cases of primary biliary cirrhosis and of carcinoma of the bile duct, one of which also had the slow and fast liver bands. It was never detected in obstructive jaundice from other causes and its significance is unknown.

Detection of a definite bone band is an established method of assessing increased osteoblastic activity (Nagant de Deuxchaisnes and Krane, 1964). It is practicable in the presence of an increased liver band, and may be useful as an index of active bone repair, for example, in assessing the union of fractures. Electrophoresis is as simple as, and more specific than, separation of the isoenzyme by heat (Posen, Neale, and Clubb, 1965). Many like to measure a separate enzyme whose serum activity increases in cholestasis but not in bone disease, eg, 5' nucleotidase or γ -glutamyltranspeptidase, the latter being more sensitive but less specific for disease of the liver (Whitfield, Pounder, Neale, and Moss, 1972).

The nature of the Regan isoenzyme has been widely discussed in recent years (Fishman, 1973). The incidence of three in 15 cases of carcinoma of the bile duct is much higher than reported in tumours generally (Fishman *et al.*, 1968) and this isoenzyme, though found in tumour tissue of carcinoma of bile duct (Jacoby and Bagshawe, 1971) has not been reported before in the serum in this condition.

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