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Electrophoretic separation of tissue-specific serum alkaline phosphatases

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SYNOPSIS Previous electrophoretic methods for the separation of tissue-specific serum alkaline phosphatases have either been unable to separate the liver and bone enzymes or have been too involved for routine clinical use. A relatively simple electrophoretic method is described which separates placental, liver, bone, and intestinal alkaline phosphatases in serum. The clinical applications of such a method appear to be mainly in the differential diagnosis of liver and bone disease, especially in complicated hypercalcaemic states where tumour metastases can affect both bone and liver, in children, and possibly in cirrhosis of the liver.

No differences in electrophoretic mobility could be seen between zymograms of different diseases affecting the same organ. Patients presenting with hepatic cirrhosis all showed a marked serum intestinal alkaline phosphatase zone as well as a liver zone on electrophoresis. An intestinal zone was not present with other types of hepatobiliary disease.

The heterogeneity of total serum alkaline phosphatase activity in normal subjects is demonstrated, alkaline phosphatases of liver and bone, and sometimes of intestine being present in normal serum.

Results obtained in women in the last trimester of pregnancy and in old people are also discussed.

Alkaline phosphatase activity in human serum is derived from liver, bone, intestine, or placenta. In hepatobilary and osteoblastic bone disease the increase in total serum alkaline phosphatase activity is due to the release of tissue-specific liver and bone alkaline phosphatase into the serum. In a clinical context the significance of a raised serum enzyme level is usually obvious, but the potential value of identifying the tissue or tissues responsible for the rise, and of establishing their respective contribution to the total increase, has long been recognized. A method for doing this and our experience with the method in routine clinical practice is described in the present paper.

Material and Methods

Sera from 186 subjects were analysed. They were divided on clinical grounds into eight groups as shown in Table I.

Total serum alkaline phosphatase activity was estimated by a routine adaptation of the method of Babson (1965) using phenolphthalein monophosphate as the substrate. Alkaline phosphatase activities are expressed in King-Armstrong units per 100 ml (K-A u%).

The amount of serum used for electrophoresis varied from 2 to 40 μl, depending on the activity of the sample. Alteration of the amount of serum used within this range did not affect the mobilities of the various zones of alkaline phosphatase.

Primary reference standards were prepared from tissue homogenates as described by Smith, Lightstone, and Perry (1968). Sera from patients with established uncomplicated liver and bone disease, from normal adults, and from normal children were used as secondary reference standards. Our results on patients for diagnosis are compared with the results from the groups of

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established liver and bone disease. The groups of pregnant women and old people are compared with the normal adult group. The results from the group of sick children are compared with those obtained from normal children.

Using the method described below zones of alkaline phosphatase activity extracted from liver, bone, placenta, or gut had similar mobilities to the corresponding zones of serum activity.

Group	*Numbers in Each Group*
Patients with oesteoblastic bone disease (Table III)	16
Patient with hepatobiliary disease with hyperbilirubinaemia (Table IV)	16
Normal adults aged 18 to 56 years (Table V)	30
Pregnant women in the last trimester (Table VI)	14
Normal children aged 2 to 14 years (Table VII)	8
Sick children aged 1 day to 12 years (Table VIII)	14
Old people aged over 75 years with evidence of osteoporosis (Table IX)	25
Patients for diagnosis (Table X)	62
Cases 1 to 21, suspected of liver disease, diagnosis supported by electrophoresis	
Cases 22 to 24, liver disease suspected plus bone disease	
Cases 25 to 28, liver disease suspected, also showing an intestinal zone on electrophoresis	
Cases 29 to 36, bone disease suspected, diagnosis supported by electrophoresis	
Cases 37 to 39, bone disease suspected, diagnosis supported by electrophoresis which also showed a liver zone	
Cases 40 to 49, bone disease suspected, diagnosis not supported by electrophoresis	
Cases 50 to 59, presenting with raised serum AP, liver zone shown on electrophoresis	
Case 60, presenting with a raised serum AP, bone zone shown on electrophoresis	
Cases 61 and 62, presenting with raised serum AP, liver and bone zones shown on electrophoresis	
Total	185

Table I *Groups of subjects studied and the numbers in each group*

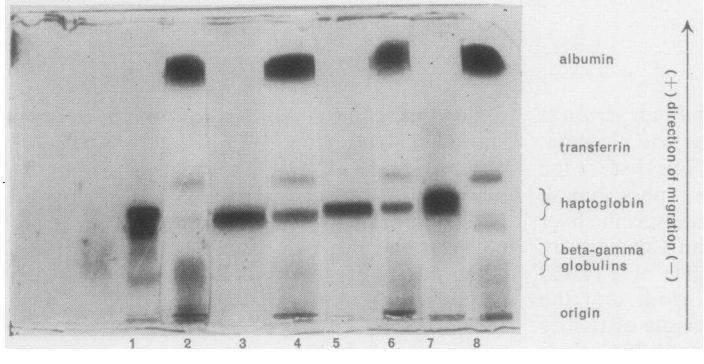

Fig. 1 *Mobility of zones of alkaline phosphatase activity in relation to other serum protein fractions.*

Samples 1, 3, 5, and 7 were stained for alkaline phosphatase (AP) and samples 2, 4, 6, and 8 for total protein. (1: liver and intestinal AP; 3: bone AP; 5: liver AP; and 7: placental AP.)

This electrophoretic run was prematurely terminated in order to demonstrate the serum albumin zones. Consequently, differences in electrophoretic mobility between the tissue-specific alkaline phosphatases shown in this figure are less than those usually obtained.

Electrophoresis was carried out on vertical polyacrylamide gel slabs, essentially as described by Akroyd (1967). The dimensions of the cell were 16 × 10 × 0·3 cm.

Forty ml of 8% Cyanogum 41 (acrylamide monomer plus N N′-methylene-bis-acrylamide, British Drug Houses Ltd) in Tris-citrate buffer, plus 0·2 ml β-dimethylaminopropionitrile, and 0·3 ml 7% ammonium persulphate was pipetted into the cell, overlaid with distilled water, and allowed to set. In mixing these substances, as far as possible contact with the air was avoided.

The composition of the Tris-citrate 'gel' buffer (*p*H 8·8) was 1·52 ml 2 M Tris-(hydroxymethyl)-aminomethane plus 0·2 ml 1 M citric acid made up to 40 ml with distilled water. The electrode compartment buffer (*p*H 8·8) was 60 ml 0·5 M boric acid plus 4·5 ml 2·5 M sodium hydroxide made up to 1 litre with distilled water.

Some improvement in the resolution between the various alkaline phosphatase zones, especially when assaying sera with normal alkaline phosphatase levels, could be achieved by adding a 'spacer' gel to the system. This was done by gelling 35 ml 8% Cyanogum 41 as described above, removing the excess liquid, and adding 5 ml 4% gel solution. This was similarly overlaid with water and allowed to set.

Temporary sample compartments were made along the upper surface of the gel by inserting small pieces of Teflon tubing, approximately 5 mm in length, until they sat firmly on the upper surface of the gel, which was washed several times with water and left filled with water. Samples, prestained with bromphenol blue, were pipetted into the compartments. A 15-mA constant current was passed until the samples had just migrated into the gel. The current was turned off while the sample compartments were removed. When this was completed the wick was replaced, and electrophoresis at 30 mA constant current was continued until one and a half hours after the albumin bands had migrated out of the bottom of the gel. The gel was then removed from the cell and zones of alkaline phosphatase activity were located by incubating the gel in a solution of sodium 2-naphthyl phosphate and fast blue BB (F. T. Gurr Ltd) in the dark, at room temperature, at *p*H 9·7 (Smith *et al*, 1968) until staining was optimal. This time was usually up to two hours. Low activity samples can be incubated in two changes of 'location' reagent to increase the intensity of staining.

To localize zones of alkaline phosphatase activity to specific serum protein fractions duplicate samples were run side by side on the same gel (Fig. 1). After electrophoresis the gel was sliced, one duplicate being stained with Lissamine Light Green and cleared in dilute acetic acid for total protein, and the other for alkaline phosphatase activity as described above. Both strips were then left overnight in dilute acetic acid to attain the same degree of shrinkage

Sample No.	*Alkaline Phosphatase (K-A u%)*	*Calculated Percentage Activities of Liver and Bone AP*		*Visual Assessment of Proportionate Activities of Percentage Alkaline Phosphatase*							
		Liver	*Bone*	*Liver*	*Bone*	*Liver*	*Bone*	*Liver*	*Bone*	*Liver*	*Bone*
1	100	73	27	75	25	75	25	70	30	80	20
2	110	57	43	60	40	60	40	60	40	65	35
3	130	40	60	40	60	40	60	45	55	40	60
4	150	25	75	30	70	35	65	40	60	20	80
5	150	25	75	30	70	35	65	35	65	20	80
6	55	25	75	30	70	30	70	40	60	30	70
7	30	25	75	30	70	30	70	40	60	30	70
8	15	25	75	30	70	35	65	40	60	40	60

Table II *Visual assessments of the proportions of liver and bone AP activity in eight samples*

Case No.	*Age*	*Sex*	*Diagnosis*	*Serum Calcium (mg %)*	*Serum Inorganic Phosphate (mg %)*	*Total Serum Alkaline Phosphatase (K-A u%)*	*Relative Percentage Proportions of Alkaline Phosphatase*		*Absolute Amounts of Alkaline Phosphatase (K-A u%)*	
							Liver	*Bone*	*Liver*	*Bone*
1	64	F	Osteomalacia, secondary adult coeliac disease	6·9	2·8	32	10	90	3	29
2	84	F	Osteomalacia, vegetarian, pernicious anaemia	8·1	4·4	30	10	90	3	27
3	69	F	Osteomalacia, malabsorption	8·8	3·6	40	0	100	None[1]	40
4	60	M	Osteomalacia	6·9	2·2	31	0	100	None	31
5	88	M	Osteomalacia	8·8	3·7	34	15	85	5	29
6	83	F	Osteomalacia	9·4	2·7	17	20	80	3	14
7	27	M	Hereditary vitamin D-deficient rickets	10·1	1·7	21	20	80	4	17
8	7	F	Hereditary vitamin D-deficient rickets	9·3	1·8	40	0	100	None	40
9			Hereditary vitamin D-deficient rickets			16	20	80	3	13
10			Hereditary vitamin D-deficient rickets			20	0	100	None	20
11	14	M	Nutritional rickets	9·2	4·2	55	0	100	None	55
12	71	M	Paget's disease			85	5	95	4	81
13	79	M	Paget's disease	7·6	3·6	108	0	100	None	108
14	85	F	Paget's disease	8·1	2·7	45	15	85	7	38
15	55	M	Paget's disease	9·1	4·2	31	0	100	None	31
16	80	F	Paget's disease			108	0	100	None	108

Table III *Reference table of patients with osteoblastic bone disease*

[1]In this, and all subsequent tables, where 'none' is entered in the right hand column, this is taken to mean that any activity in the serum from the tissue in question was insufficient to show up as a zone on staining.

Case No.	*Age*	*Sex*	*Diagnosis*	*Serum Bilirubin (mg %)*	*SGOT (u/ml)*	*SGPT (u/ml)*	*Thymol Turbidity (u %)*	*Serum Alkaline Phosphatase (K-A u%)*	*Percentages of Alkaline Phosphatase*		*Actual Amounts of Alkaline Phosphatase (K-A u%)*	
									Liver	*Bone*	*Liver*	*Bone*
1	62	M	Choledocholithiasis	6·1	700	860	1	21	100	0	21	None
2	69	F	Choledocholithiasis	2·2	400	248		37	100	0	37	None
3	35	M	Empyema of gall bladder	6·1	145		2	52	100	0	52	None
4	70	M	Choledocholithiasis	6·7	77			65	100	0	65	None
5	78	M	Choledocholithiasis	2·0		49	2	30	100	0	30	None
6	87	F	Choledocholithiasis	10·0	139	112	1	43	90	10	39	4
7	36	F	Glandular fever	2·6	18		9	52	100	0	52	None
8	22	F	Infectious hepatitis	12·8		416	10	22	100	0	22	None
9	64	F	Infectious hepatitis	17·0	196	172	1	110	100	0	110	None
10	66	M	Infectious hepatitis	2·4	286	127	1	19	100	0	19	None
11	75	F	Chlorpromazine jaundice	22·6			3	114	100	0	114	None
12	88	M	Obstructive jaundice	1·7	76	61		40	100	0	40	None
13	42	M	Stab wounds in liver	9·2			1	41	100	0	41	None
14	70	M	Obstructive jaundice	6·4	70	35	2	23	100	0	23	None
15	85	M	Obstructive jaundice	4·7	72	53	2	39	100	0	39	None
16	70	F	Obstructive jaundice	11·8		151	1	84	100	0	84	None

Table IV *Reference table listing cases with hepatobilary disease and hyperbilirubinaemia*

Results were photographed to provide a permanent record.

The relative intensity of electrophoretic zones of alkaline phosphatase activity was assessed visually. When more than one zone was present in any sample the activity of each was expressed as a percentage of the total activity. The validity of this method was tested by showing four laboratory technicians, unfamiliar with the technique, a gel on which had been run eight samples with total alkaline phosphatase activities varying between 15 and 150 K-A u%. The samples contained different proportions of liver and bone alkaline phosphatase from 25 to 75% of the total activity. The subjects were asked to assess the proportions of the two zones in each sample.

Case No.	Age (yr)	Serum Alkaline Phosphatase (K-A u%)	Percentages of Alkaline Phosphatase in			Actual Amounts of Alkaline Phosphatase (K-A u%) in		
			Liver	Bone	Intestine	Liver	Bone	Intestine
Males								
1	19	7	40	60	0	3	4	None
2	22	4	15	85	0	0·5	3·5	None
3	22	7	75	25	0	5	2	None
4	24	6	70	30	0	4	2	None
5	24	8	30	60	10	2·5	4·5	1
6	24	7	40	50	10	2·5	3·5	1
7	25	6	55	45	0	3·5	2·5	None
8	27	6	45	45	10	3	3	0·5
9	28	5	80	20	0	4	1	None
10	28	6	30	60	10	2	3·5	0·5
11	39	6	50	50	0	3	3	None
12	42	6	90	10	0	5·5	0·5	None
Females								
13	18	5	40	60	0	2	3	None
14	18	5	20	80	0	1	4	None
15	19	7	40	50	10	3	3·5	0·5
16	19	4	60	40	0	2·5	1·5	None
17	19	6	65	35	0	4	2	None
18	19	4	40	60	0	1·5	2·5	None
19	19	5	50	50	0	2·5	2·5	None
20	21	7	50	50	0	3·5	3·5	None
21	21	7	55	35	10	4	2	1
22	22	4	65	35	0	3	1	None
23	22	5	80	20	0	4	1	None
24	23	6	80	20	0	5	1	None
25	23	4	30	50	20	1·5	2	0·5
26	23	5	20	80	0	1	4	None
27	28	5	70	30	0	3·5	1·5	None
29	32	8	40	20	40	3	2	3
30	52	6	70	30	0	4	2	None
31	56	7	30	60	10	2	4	1

Table V *Reference table of normal adults aged 18 to 56 years*

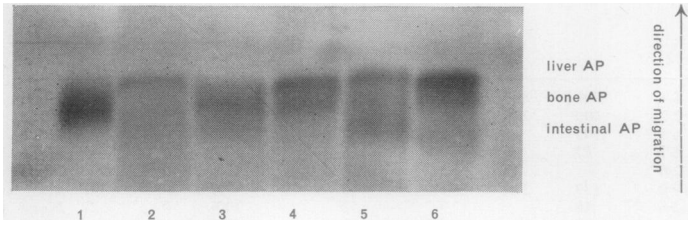

Fig. 2 *Heterogeneity of serum alkaline phosphatase activity in normals.*

1 Child aged 11 years (AP = 14 K-A u%).
2 Female aged 23 years (AP = 5 K-A u%).
3 Male aged 24 years (AP = 8 K-A u%).
4 Male aged 28 years (AP = 5 K-A u%).
5 Female aged 32 years (AP = 8 K-A u%).
6 Male aged 42 years (AP = 6 K-A u%).

Results

The mobilities of the major zones of alkaline phosphatase activity occurring in serum in relation to other serum protein fractions are shown in Figure 1. In 8% polyacrylamide all major zones of activity run in a region between transferrin and the beta-globulin-gamma-globulin complex. Placental alkaline phosphatase travels farthest, followed by that of liver, bone, and intestine in that order. In 5% polyacrylamide the mobility of all the serum alkaline phosphatase zones is increased relative to transferrin, and they migrate in a region in front of, and behind, transferrin (unpublished results).

The validity of visual assessment as a measure of the relative intensities of zones of alkaline phosphatase is shown in Table II. No assessment varied by more than 15% of the calculated percentage, and most assessments were accurate to within ±5%.

Results obtained from a reference series of 16 patients with osteoblastic bone disease are summarized in Table III. These patients were suffering from osteomalacia, vitamin D-resistant rickets, nutritional rickets, or Paget's disease. Their total serum alkaline phosphatase activities were between 16 and 108 K-A u%, of which the serum bone zone seen after electrophoresis was not less than 85% of the total activity. The remainder of the activity was of liver type.

A summary of the results obtained on a reference series of 16 patients with hepatobiliary disease and hyperbilirubinaemia is given in Table IV. All these subjects had a raised serum alkaline phosphatase activity which, in 15 of the cases, was of liver type only. One serum was also found to contain a zone of bone alkaline phosphatase. The level of activity in this zone was within the limits for bone activity seen in adult normals.

The results in a reference series of 31 normal adults are shown in Table V. The total serum alkaline phosphatase activity was between 4 and 8 K-A u%. Both liver and bone zones were seen in all these subjects. Variations in the proportions of alkaline phosphatase in individual sera appeared to be random with regard to age and sex, and varied between 15 and 90% for liver and between 10 and 85% for bone (Fig. 2). Intestinal alkaline phosphatase activity was seen in six female and four male subjects, and in one case (no. 29) accounted for 40% of the total activity.

Further evidence that intestine was the tissue of origin of this zone was given by the addition of L-phenylalanine (0·005 M) to the incubation solution after electrophoresis. This resulted in specific inhibition of the serum intestinal alkaline phosphatase activity (Fishman, Green, and Inglis, 1963).

The results from 14 women in the last trimester of pregnancy are summarized in Table VI. Total serum alkaline phosphatase activity was significantly greater than the levels of activity seen in our adult normals. Placental activity was seen in 11 out of the 14 cases; no intestinal activity could be demonstrated in any of the sera. We were able to detect an increase in serum bone alkaline phosphatase activity over the levels seen in the adult normals in only two cases (nos. 11 and 14). Presumably the mother's dietary intake of calcium and phosphorus is sufficient to supply the foetal requirements for these chemicals.

Case No.	*Serum Alkaline Phosphatase (K-A u%)*	*Percentages of Alkaline Phosphatase in*			*Actual Amounts of Alkaline Phosphatase (K-A u%) in*		
		Placenta	*Liver*	*Bone*	*Placenta*	*Liver*	*Bone*
1	12	0	95	5	None	11	1
2	8	0	75	25	None	6	2
3	10	45	35	20	5	3	2
4	15	60	20	20	9	3	3
5	18	45	25	30	8	5	5
6	16	20	80	0	3	13	None
7	10	0	80	20	None	8	2
8	12	30	70	0	4	8	None
9	12	33	33	33	4	4	4
10	13	25	50	25	3	6	3
11	13	30	10	60	4	1	8
12	15	60	5	35	9	1	5
13	12	50	25	25	6	3	3
14	25	65	10	25	16	3	6

Table VI *Pregnant women in the last trimester*

Case No.	*Age (yr)*	*Alkaline Serum Phosphatase (K-A u%)*	*Percentages of Alkaline Phosphatase in*		*Actual Amounts of Alkaline Phosphatase (K-A u%) in*	
			Liver	*Bone*	*Liver*	*Bone*
1	2	17	10	90	2	15
2	11	22	10	90	2	20
3	11	14	10	90	1·5	12·5
4	12	17	10	90	2	15
5	13	24	5	95	1	23
6	13	19	10	90	2	17
7	14	15	10	90	2	13
8	14	17	10	90	2	15

Table VII *Reference table of normal children aged 2 to 14 years*

Results on a reference series of eight children, all apparently normal, are summarized in Table VII. In all cases the total serum alkaline phosphatase levels were higher than those seen in normal adults, the increase being due to the high circulating levels of bone alkaline phosphatase normally seen in growing children. None of these eight cases showed less than 90% bone alkaline phosphatase activity on electrophoresis. The remainder of the activity was invariably of hepatic type and was present in amounts similar to those seen in our normal adults.

In a series of 14 sick children aged between 1 day and 12 years (Table VIII), the levels of total serum alkaline phosphatase activity varied between wider limits than those seen in normal children. This was due to much greater variation in the serum levels of bone and liver alkaline phosphatase. In the normal children bone activity lay between 12·5 and 23 K-A u% and there was never more than 10% liver activity present. In the sick children levels of serum bone alkaline phosphatase varied between 4 and 60 K-A u% (10 to 100% of the total serum activity).

An intestinal alkaline phosphatase zone was seen in case 2, amounting to 25% of the total serum activity. A similar zone of activity was not demonstrated in any of the other children, either sick or well. The relevance of this zone is not known.

The results in a series of 25 old people, aged over 75 years, are summarized in Table IX. Total

Case No.	*Age*	*Diagnosis*	*Serum Alkaline Phosphatase (K-A u%)*	*Percentages of Alkaline Phosphatase in*			*Actual Amounts of Alkaline Phosphatase in*		
				Liver	*Bone*	*Intestine*	*Liver*	*Bone*	*Intestine*
1	1 day	Metabolic acidosis	60	0	100	0	None	60	None
2	1 day	Congenital rubella infection	35	0	75	25	None	27	8
3	5 weeks	Jaundice two weeks postpartum	49	0	100	0	None	49	None
4	2 years	Jaundice, favism	27	0	100	0	None	27	None
5	1 day	Neonatal hypocalcaemia	33	0	100	0	None	33	None
6	1 day	Neonatal hypocalcaemia	16	0	100	0	None	16	None
7	7 years	Acute glomerulonephritis	16	35	65	0	6	10	None
8	9 years	Polyarthritis	31	80	20	0	25	6	None
9	9 years	Thalassaemia	12	50	50	0	6	6	None
10	10 years	Epilepsy	29	25	75	0	7	22	None
11	12 years	Recurrent rheumatic fever, hepatomegaly	12	50	50	0	6	6	None
12	12 years	Aspirin overdose	16	0	100	0	None	16	None
13	7 years	Hepatitis	29	65	35	0	19	10	None
14	8 years	Fever, rash, hepatomegaly	34	90	10	0	30	4	None

Table VIII *Sick children aged 1 day to 12 years*

Case No.	Age	Sex	Additional Diagnoses	Serum Calcium (mg %)	Serum Inorganic Phosphate (mg %)	Serum Alkaline Phosphatase (K-A u%)	Percentages of Alkaline Phosphatase in		Actual Amounts of Alkaline Phosphatase (K-A u%) in	
							Liver	Bone	Liver	Bone
1	80	F	Kyphosis, pneumonia	9·4	2·9	4	80	20	3	1
2	75	F	Cancer of stomach	8·2	2·0	4	85	15	3·5	0·5
3	80	F	Kyphosis, dementia	8·8	2·7	12	65	35	8	4
4	93	F	Rheumatoid arthritis, dementia	9·4	4·1	11	70	30	8	3
5	83	F	Kyphosis, hemiplegia	9·1	3·5	8	65	35	5	3
6	80	F	Fractured femur	9·5	3·4	10	65	35	7	3
7	88	F	Osteoarthritis	9·1	3·2	6	85	15	5	1
8	86	F	Pneumonia	8·6	3·8	8	90	10	7	1
9	84	F	Hemiplegia, breast cancer	8·8	3·2	7	80	20	5·5	1·5
10	78	F		9·1	3·2	7	80	20	5·5	1·5
11	84	F	Pneumonia	9·3	2·5	10	50	50	5	5
12	83	F	Kyphosis, dementia	8·8	3·2	6	100	0	6	None
13	84	F	Gallstones, dementia	8·1	3·4	5	80	20	4	1
14	82	F	Kyphosis, duodenal ulcer	8·8	2·4	12	60	40	7	5
15	77	F	Osteoarthritis	9·4	3·9	8	80	20	6	2
16	81	F	Gross osteoarthritis	9·4	3·9	9	60	40	5	4
17	80	F	Kyphosis, peripheral vascular disease	9·4	5·1	7	75	25	5	2
18	92	F	Collapsed thoracic and lumbar vertebrae	7·8	2·5	5	80	20	4	1
19	86	F	Myelomatosis, liver secondaries			6	100	0	6	None
20	78	M	Congestive cardiac failure, uraemia			9	90	10	8	1
21	85	M	Kyphosis, dementia	8·8	3·2	6	100	0	6	None
22	75	M	Kyphosis, Parkinson's disease	8·9	2·9	7	100	0	7	None
23	78	M	Kyphosis, dietary anaemia	8·8	2·7	12	65	35	8	4
24	86	M	Congestive cardiac failure, bronchitis	9·4	4·2	9	90	10	8	1
25	86	M	Kyphosis, dementia	8·4	2·5	10	65	35	6·5	3·5

Table IX *Old people with clinical or radiological evidence of osteoporosis aged over 75 years*

serum alkaline phosphatase levels were all within the usually quoted normal range of 3 to 13 K-A u%. The upper limit of serum alkaline phosphatase activity in these people (12 K-A u%) was greater than that seen in our normal adults. This was always due to an increase in the serum liver alkaline phosphatase fraction. A bone alkaline phosphatase zone was also seen in the majority of these sera. These patients were selected because all had clinical or radiological evidence of osteoporosis. No significant difference in serum bone alkaline phosphatase activity (either quantitative or qualitative) could be demonstrated between these sera and those from normal adults.

Sixty-three patients for diagnosis are summarized in Table X. The results obtained on these patients are arranged as listed in Table I, on the bases of initial diagnosis and electrophoretic results. Total serum alkaline phosphatase activities lay between 9 and 385 K-A u%, the majority being above normal limits. Intestinal zones were seen only in cases 25 to 28.

Discussion

Our experience shows that polyacrylamide gel electrophoresis offers a simple and reliable means for separating serum alkaline phosphatase activity into a number of well defined fractions. The fractions correspond to tissues of origin: in particular a fraction in liver, bone, intestine, and a placenta can be identified.

Our designation of serum alkaline phosphatase zones as 'liver', 'bone', etc, fractions was based on two sets of preliminary investigations. First, the bands corresponded in mobility to the main zones of activity of tissue extracts. These extracts were run both as 'pure' tissue preparations and as admixtures to normal and abnormal sera. Second, the bands corresponded to the patterns observed in series of 'reference' sera, which were obtained from patients with firmly established diagnoses of advanced but uncomplicated liver and bone disease with high total serum alkaline phosphatase activities, and from normal children and adults. Although all the results from the two sets of investigations were in close agreement, they cannot be taken to provide absolute proof of the source of serum enzyme fractions. This is a theoretical limitation applying to all serum enzyme studies.

Confirmatory evidence for the tissues of origin of specific serum alkaline phosphatase zones was provided by two other findings. First, as mentioned before, the addition of L-phenylalanine to the alkaline phosphatase location reagent inhibited intestinal alkaline phosphatase zones to a greater extent than other zones. Second, sera and tissue extracts containing high proportions of bone alkaline phosphatase demonstrated a lower resistance to heat denaturation than did samples containing high proportions of liver,

intestinal, or placental alkaline phosphatase (Posen, Neale, and Clubb, 1965).

The fractions seen after electrophoresis can be estimated semiquantitatively by visual assessment. This method would be unacceptable for estimating total serum alkaline phosphatase activity with any accuracy but this can, of course, be measured accurately by conventional methods. Visual assessment merely compares relative intensities of electrophoretic zones. It has proved accurate and reproducible even among untrained observers.

Although we are satisfied that the technique described here clearly separates serum alkaline phosphatase activities derived from different tissues, we have been unable to establish, so far, patterns characteristic of different types of disease. For example, the intense liver alkaline phosphatase zone associated with hepatic carcinomatous deposits (cases 18 to 21, Table X) did not differ from the pattern associated with gallstone disease (cases 1, 2, 4 to 6, Table IV), and the increased bone alkaline phosphatase activity in Paget's disease was electrophoretically similar

Case No.	*Age*	*Sex*	*Provisional Diagnosis*	*Serum Calcium (mg %)*	*Serum Phosphate (mg %)*	*Serum Bilirubin (mg %)*	*SGOT (u/ml)*	*SGPT (u/ml)*	*T.T. (u%)*	*Serum Alkaline Phosphatase (K-A u%)*	*Percentages of Alkaline Phosphatase in*			*Actual Amounts of Alkaline Phosphatase (K-A u%) in*		
											Liver	*Bone*	*Gut*	*Liver*	*Bone*	*Gut*
1	42	M	Cholecystitis			0·6	68	198		53	100	0	0	53	None	None
2	29	M	Infectious hepatitis			8·7	787	640	9	12	80	20	0	9·5	2·5	None
3	79	M	Postchlorpromazine jaundice			0·5	3	17	1	23	100	0	0	23	None	None
4	89	F	Jaundice of unknown aetiology			11·4	95		1	32	100	0	0	32	None	None
5	58	M	Jaundice of unknown aetiology			4·5	294		2	38	100	0	0	38	None	None
6	76	M	Jaundice, cancer of the pancreas			20·8	98	118	2	256	100	0	0	256	None	None
7	64	M	Jaundice, cancer of the pancreas			3·2	103		3	65	100	0	0	65	None	None
8	39	M	Cancer of oesophagus, palpable liver			3·2	144	82	1	58	95	5	0	55	3	None
9	63	M	Jaundice, cancer of the pancreas			2·2	120	103		15	100	0	0	15	None	None
10	67	F	Jaundice, pancreatitis, palpable liver			22·0	40	71	1	14	100	0	0	14	None	None
11	69	M	Jaundice, congestive cardiac failure			1·3	292	480		17	100	0	0	17	None	None
12	66	F	Breast cancer, malignant nodes in porta hepatis			6·2	159	225	1	88	100	0	0	88	None	None
13	80	M	Jaundice, cancer of the bladder			5·6	78	105	1	42	90	10	0	38	4	None
14	72	F	Hemiplegia, hepatomegaly			0·6	21	9		33	100	0	0	33	None	None
15	68	F	Acromegaly, fatty degeneration of liver			0·3			2	16	100	0	0	16	None	None
16	66	M	Congestive cardiac failure, hepatomegaly			0·8	25	44	2	16	100	0	0	16	None	None
17	70	F	Pneumonia, liver disease, pyelonephritis			0·5				24	100	0	0	24	None	None
18	80	F	Breast cancer, hepatic secondaries			0·5				22	100	0	0	22	None	None
19		M	Stomach cancer, hepatomegaly			1·0	177		1	44	100	0	0	44	None	None
20	60	M	Paraplegia, reticulosarcoma, hepatic secondaries			1·2	115	160	1	33	100	0	0	33	None	None
21	80	F	Rectal carcinoma, knobbly liver			1·0			3	80	100	0	0	80	None	None
22	60	M	Paget's disease, infectious hepatitis							36	50	50	0	18	18	None
23	67	F	Paget's disease, infectious hepatitis			0·6	114	170	3	76	50	50	0	38	38	None
24	75	F	Breast cancer, on testosterone, jaundice opacities compatible with gallstones			19·0	178	149	2	32	55	45	0	18	14	None
25	60	F	Jaundice, serum hepatitis			8·2	70	122	7	33	80	0	20	26	None	7
26	54	M	Large, hard liver, diabetes; acquired porphyria, cirrhosis			0·6	156	157	5	24	80	0	20	19	None	5
27	37	M	Alcoholic cirrhosis			0·5				24	50	0	50	12	None	12
28		M	Cirrhosis			0·4	266	135		18	45	10	45	8	2	8
29	86	M	Cachexia, cancer of pancreas, sclerotic bone deposits	8·6	6.3					50	0	100	0	None	50	None
30	3	F	Hereditary vitamin D-deficient rickets	9·3	2·7					31	0	100	0	None	31	None

Table X *Patients for diagnosis*

Case No.	Age	Sex	Provisional Diagnosis	Serum Calcium (mg %)	Serum Phosphate (mg %)	Serum Bilirubin (mg %)	SGOT (u/ml)	SGPT (u/ml)	T.T. (u%)	Serum Alkaline Phosphatase (K-A u%)	Percentages of Alkaline Phosphatase in			Actual Amounts of Alkaline Phosphatase (K-A u%) in		
											Liver	Bone	Gut	Liver	Bone	Gut
31	22	F	Treated hypophosphataemia	9·4	1·9					9·0	10	90	0	1	8	None
32	77	M	Bone pain, cancer of prostate	8·1	2·4					76	10	90	0	8	68	None
33	68	M	Paraplegia, cancer of prostate, sclerotic bone deposits							80	5	95	0	4	76	None
34	65	M	Bone pain, hypercalcaemia	10·8						65	10	90	0	7	58	None
35	60	M	Back pain, cancer of prostate	9·0	3·3					45	0	100	0	None	45	None
36		F	Radiological Looser zones	9·3	3·1					16	55	45	0	9	7	None
37		M	Osteomalacia							30	50	50	0	15	15	None
38	78	F	Osteomalacia	9·9						22	60	40	0	13	9	None
39	67	M	Bone pain, cancer of prostate, proximal myopathy	9·1						385	10	90	0	39	346	None
40	84	M	Bone pain, waddling gait, osteomalacia	7·2	4·3					15	65	35	0	9	5	None
41	85	M	Sclerotic bone deposits, cancer of prostate	8·8	2·2					13	80	20	0	10	4	None
42	67	M	Carcinomatosis							24	85	15	0	22	3	None
43	45	F	Hyperparathyroidism	11·4	3·6					44	100	0	0	44	None	None
44	89	F	Osteoarthritis radiotranslucent bones, cardiac failure	9·4	3·5					29	85	15	0	25	4	None
45	85	F	Gross osteoarthritis	9·4	3·1					15	100	0	0	15	None	None
46	5	F	Hereditary vitamin D-deficient rickets	13·2	3·6					11	0	100	0	None	11	None
47	7	M	Hereditary vitamin D-deficient rickets	9·5	2·5					25	0	100	0	None	25	None
48	3	M	Hereditary vitamin D-deficient rickets	9·8	2·0					24	0	100	0	None	24	None
49	85	F	Bone pain	9·3	2·7					13	85	15	0	11	2	None
50	73	M	Congestive cardiac failure, gynaecomastia			1·3	21	12		27	80	20	0	22	5	None
51	85	F	Paraplegia, cancer of renal pelvis, bone and liver secondaries							12	80	20	0	9·5	2·5	None
52	81	M	Kyphotic, demented	8·6	3·0					16	90	10	0	14	2	None
53	70	M	Fractured hip, post-operative jaundice			7·2			1	37	100	0	0	37	None	None
54	60	F	Right hypochondrial pain, pleural effusion			0·8			1	12	100	0	0	12	None	None
55	66	M	Crohn's disease							20	100	0	0	20	None	None
56	44	F	Recurrent melaena, varices, splenomegaly			0·6		15	2	16	100	0	0	16	None	None
57	67	M	Acute colitis, toxic megacolon, bowel obstruction			0·5		24	1	15	100	0	0	15	None	None
58	49	M	Orchitis, septicaemia, polyarteritis nodosa			0·3		29		46	100	0	0	46	None	None
59	36	F	Pyrexia of unknown origin, splenomegaly			0·5		71		38	100	0	0	38	None	None
60	71	F	Thyrotoxic, treated hypercalcaemia	9·6	3·2					14	5	95	0	1	13	None
61	71	F	Right hypochondrial pain, pneumonia			0·9	22	47	2	32	75	25	0	24	8	None
62	76	M	Kyphotic	10·4	3·0					28	50	50	0	14	14	None

Table X *Patients for diagnosis*—continued

to that seen in rickets (cases 12 to 16, and 7 to 11, respectively, Table III). Several workers have claimed that such 'disease-specific' as distinct from 'tissue-specific' diagnoses can be based on electrophoretic appearances (Hill and Sammons, 1967; Newton, 1967). Secondary zones have been sometimes described with the technique used above, but we would put no diagnostic reliance on them.

The electrophoretic results from our adult normal series contrast, in part, with those of other workers. This statement must be qualified, however, as the different electrophoretic media and enzyme localization techniques used in different reports makes comparison between them difficult. Specifically, the presence of detectable amounts of bone alkaline phosphatase in normal serum has been in question. Yong (1967) found a liver alkaline phosphatase zone present in all sera from a series of 50 normals, and an intestinal band in a lesser number of these people. Our findings support these results but, in addi-

tion, a zone of bone alkaline phosphatase activity was seen in all sera from our normal adult series (Fig. 2).

The significance of serum alkaline phosphatase activities depends, in part, on the age of the subject. Like earlier workers we find that the total serum alkaline phosphatase activity of children is significantly higher than that of normal adults (Clark and Beck, 1950). Electrophoretic fractionation shows that this difference is due entirely to the higher bone activity in childhood. Our findings in a somewhat heterogenous series of sick children suggest that in this age group fractionation of serum alkaline phosphatase might prove particularly useful in revealing an abnormally low bone alkaline phosphatase activity. This appears to be a common accompaniment of a variety of acute systemic illnesses, and it may prove a sensitive indication of arrest of osteoblastic activity and bone growth. Such an index of decreasing osteoblastic activity might be particularly useful in assessing the effect of steroid treatment on children. A fall in bone alkaline phosphatase activity can be masked by a rise in liver activity if only the total serum activity is measured (cases 7 to 9, Table VIII). Conversely, a significant rise in liver alkaline phosphatase activity can be missed because of a synchronous fall in bone activity (cases 12 to 14, Table VIII). This fact has been used in the past to question the usefulness of estimations of serum alkaline phosphatase activity in children (Hobbs, Campbell, and Scheuer, 1968). We believe that, when coupled with electrophoretic fractionation, such studies can, in fact, be of value.

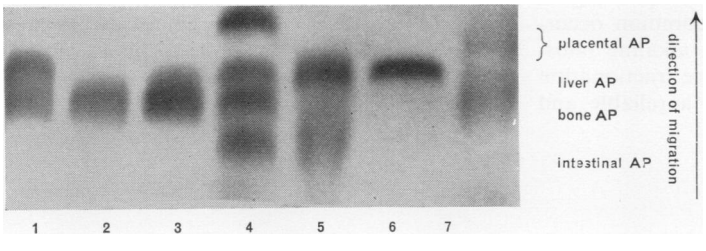

Fig. 3 *Serum alkaline phosphatase patterns in different disease states related to a normal child and to a mixture of tissue extracts.*

1 Paget's disease with infectious hepatitis (AP = 76 K-A u%) in case 23 (Table X).

2 Osteomalacia (AP = 31 K-A u%) in case 4 (Table III).

3 Normal child (AP = 24 K-A u%) in case 5 (Table VII).

4 Mixture of placental, liver, bone, and intestinal tissue extracts.

5 Cirrhosis of the liver (AP = 24 K-A u%) in case 26 (Table X).

6 Obstructive jaundice (AP = 40 K-A u%) in case 12 (Table IV).

7 Late pregnancy (AP = 18 K-A u%) in case 5 (Table VI).

At the other extreme of life, we were unable to confirm reports by earlier workers that old people, and in particular old women, have significantly higher serum alkaline phosphatase activities than normal adults (Heino and Jokipii, 1962; Klaassen and Siertsema, 1964). Our series of old people were all chosen because of their having clinical or radiological evidence of osteoporosis. We could find no evidence for any change in serum bone alkaline phosphatase activity in this group.

Between childhood and old age we have found serum alkaline phosphatase fractionation useful in dealing with a variety of clinical problems. Not only could it establish the cause of a raised serum alkaline phosphatase activity when this was in doubt but it could also reveal a mixed origin when this had not been previously suspected. For example, case 24 (Table X) was a patient with carcinoma of the breast with jaundice. Abdominal radiographs showed opacities compatible with gallstones. Bony secondaries were not suspected until the raised serum alkaline phosphatase was shown to be due, in part, to an increase in the serum bone fraction.

A number of patients presented with known chronic bone disease and a raised serum alkaline phosphatase which had been ascribed to osteoblastic hyperactivity. Electrophoretic fractionation revealed an abnormal increase in serum liver alkaline phosphatase activity (with or without an increase in the bone fraction), and further investigation showed the presence of liver disease (cases 36 to 45, Table X). In other cases (for example, nos. 61 and 62, Table X) previously unsuspected osteoblastic bone involvement was indicated by the electrophoretic results.

Routine fractionation of alkaline phosphatase has also raised a number of questions which we had not previously envisaged and which require further study. Four patients with known or suspected liver disease (cases 25 to 28, Table X) showed, in addition to a liver band, a marked intestinal band of alkaline phosphatase. All proved on subsequent investigation to be cases of liver cirrhosis. Since an intestinal band is apparently not a feature of other types of liver disease, this may prove to be an exceptional instance of disease as well as organ specificity. The Lewis blood group and secretor status of these patients was not determined.

In agreement with other workers (Gutman, Tyson, and Gutman, 1936) we find that the level of serum bone alkaline phosphatase activity in cases where there are secondary osteolytic carcinomatous deposits bears little relation to the degree of bone involvement as assessed radiologically or clinically.

It is well known that total serum alkaline phosphatase activity is raised in the later stages of pregnancy and that this increase is due to the

appearance of placental alkaline phosphatase in the serum. This placental component (Fig. 3) can be readily demonstrated by electrophoresis (Boyer, 1961). Our results support this theory although we could not detect a placental component in all the cases studied. It has also been shown that the increase in total serum alkaline phosphatase activity is greater in twin pregnancies than in single ones (unpublished results). The clinical significance of the amount of placental alkaline phosphatase appearing in the serum as a guide to placental function remains to be explored.

Instances where the electrophoretic technique described here is of little use are demonstrated by cases 46 to 48 (Table X). These children had clinical evidence of bone disease but their total serum alkaline phosphatase levels were within normal limits for their age. The electrophoretic patterns seen in these cases were indistinguishable from those of normal children.

It might be argued that, whatever the intrinsic academic interest of serum enzyme fractionation, the technique is an unnecessary refinement for routine day-to-day diagnosis. From our experience we would disagree. As a group of diagnostic procedures, serum enzyme estimations have many disadvantages compared with older, more conventional chemical tests. These disadvantages are offset by two advantages. First, the estimations are potentially highly specific. Secondly, they can be extremely sensitive indicators of disease activity. Total serum alkaline phosphatase estimations lack both specificity and sensitivity: the results may not only fail to provide the required information, they may even mislead. This is particularly so when a raised serum alkaline phosphatase level is the only abnormal laboratory finding in a patient complaining of ill defined symptoms—a not uncommon occurrence. Coupling the total serum alkaline phosphatase estimation with a sensitive fractionation technique makes the estimation a reliable and specific indicator of disease.

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