THE DIAGNOSTIC PATTERNS OF THE ISO-ENZYMES OF SERUM NON-SPECIFIC ALKALINE PHOSPHATASE*

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Various authors have recently studied the diagnostic value of the iso-enzymes of serum alkaline phosphatase.[1-8] Results obtained from these studies vary and are not comparable to one another due to the different techniques employed, such as cellulose chromatography,[1] immunological procedures,[2] starch-block electrophoresis,[3] starch-gel electrophoresis[4-7] and agar-gel electrophoresis.[8]

The number and location of the iso-enzyme fractions vary according to the technique used, and according to the experimental conditions. The relationship between one or more iso-enzymes in serum to those in certain tissues, mainly liver and bone, has been demonstrated. It is therefore not unreasonable to predict that the iso-enzyme pattern of serum alkaline phosphatase might be an additional aid in the differential diagnosis of metastatic hepatobiliary and skeletal diseases.

The object of the present study was to investigate the alkaline phosphatase iso-enzyme patterns in patients with metastatic hepatobiliary and skeletal diseases.

MATERIALS AND METHODS

Venous blood specimens were obtained from 48 patients (Table I). The White series consisted of adult male and female patients with malignant tumours and metastases to either the liver, skeletal tissues or other tissue. The Bantu series consisted of adult male patients with primary liver carcinoma. Bantu female patients were omitted. The diagnosis in individual cases was obtained by a combination of clinical, surgical (with histopathological confirmation), chemical and radiological methods.

The serum was separated from the rest of the blood and stored at 4°C to prevent loss of enzyme activity. Serum samples were subjected to starch-gel electrophoresis according to the method described by Smithies[9,10] using the boric acid sodium hydroxide buffer (average pH bridge buffer = 8·34 and average pH gel buffer = 8·816), voltage = 200 V at 50 m.a. and with an electrophoretic run of 18 hours. The whole apparatus was installed in a refrigerator, and connected to a power pack on the outside. This procedure and the average time of 3½ hours between the collection of the specimen and the onset of the electrophoresis, resulted in a minimum loss of enzyme activity.

The gel was sliced in half after completion of the separation, and the upper half stained for proteins and the lower half for alkaline phosphatase. The protein was stained with amido black 10 B as described by Smithies,[10] and Smith.[11] The alkaline phosphatase was stained by the diazo-dye method described by Taswell and Jeffers.[5]

The total alkaline phosphatase value was determined within 24 hours on every specimen by the method of King and Armstrong as described by King and Wootton.[12] The normal value for adults according to this method is 4·5 - 9·5 KA units. The results are listed in Table I, together with a summary of the series and the number of iso-enzyme bands in every individual case. In Fig. 1 an example of the iso-enzyme pattern

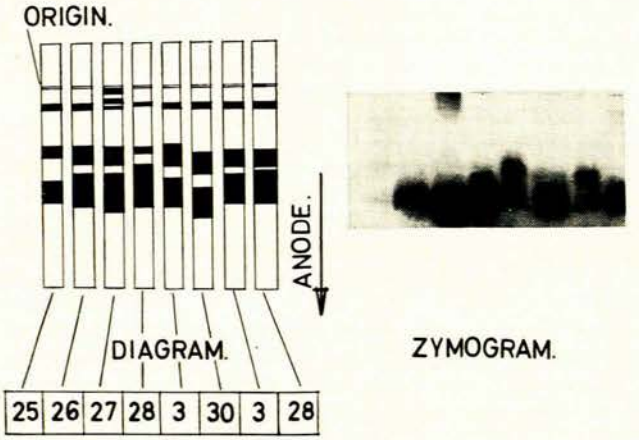

Fig. 1. Example of iso-enzyme separation.

is illustrated and Fig. 2 is a schematic presentation of the iso-enzyme patterns in relation to the protein fractions. The protein fractions obtained correspond to those of Smithies.[10]

DISCUSSION

Kay[13] suggested that the alkaline phosphatase located in the different tissues was identical. Bodansky[14] disagreed

*Alkaline phosphatase in the text denotes non-specific alkaline phosphatase.

with this view and originated the theory of the existence of multiple molecular forms of a specific enzyme. Markert and Möller[15] first defined the present term 'iso-enzyme'. Of all the different techniques, electrophoresis in starch gel possesses the highest resolving power. Moss[7] made butanol extracts from bone, liver, kidney and small intestine, separated them by starch-gel electrophoresis, and compared the alkaline phosphatase fractions to those of the protein fractions of blood serum. All these tissues showed either 2 or 3 iso-enzyme bands divided in 2 zones corresponding to the beta-lipoproteins and the beta-globulin.

TABLE I. SUMMARY OF CASES

Case No.	*Age*	*Race*	*Sex*	*No. of iso-enzymes*	*Total alkaline phosphatase (KA units)*	*State of health*
1	39	W	F	3	5·10	Healthy
2	30	W	F	3	4·875	Healthy
3	27	W	M	3	7·170	Healthy
4	31	W	M	3	6·45	Healthy
5	63	W	M	3	4·5	Primary stomach carcinoma Metastases liver
6	70	W	F	3	4·70	Primary myelomatosis
7	56	W	F	4	10·5	Primary breast carcinoma Metastases liver
8	60	W	F	4	12·0	Primary carcinoma of ovary Metastases liver
9	40	W	F	3	6·56	Primary carcinoma of ovary Metastases liver
10	47	W	M	4	13·69	Primary mesothelioma Metastases liver
11	55	W	M	3	4·50	Healthy
12	38	W	M	3	5·25	Healthy
13	±40	B	M	4	21·75	Primary liver carcinoma
14	±40	B	M	4	15·15	Primary liver carcinoma
15	±36	B	M	4	12·00	Primary liver carcinoma Metastases lung and rib
16	37	W	M	2	4·65	Healthy
17	53	W	M	4	10·025	Primary oesophagus carcinoma Metastases liver and lung
18	50	W	M	4	27·00	Primary colon carcinoma Metastases liver
19	45	W	F	3	4·950	Primary breast carcinoma Metastases brain
20	40	B	M	6	64·05	Primary liver carcinoma
21	66	W	M	3	4·88	Primary prostate carcinoma Metastases in soft tissue of pelvis
22	45	W	M	5	25·20	Primary mesothelioma Metastases liver
23	55	W	M	2	4·875	Primary carcinoma of the stomach Metastases lymph glands
24	36	W	F	2	16·125	Primary breast carcinoma Metastases liver
25	45	W	F	4	30·00	Primary carcinoma of the stomach Metastases liver
26	56	W	F	3	25·025	Primary breast carcinoma Metastases bone
27	65	W	M	5	45·889	Primary carcinoma of gallbladder Metastases liver
28	55	W	M	3	28·325	Primary ? with metastases in bone
29	20	W	M	3	4·567	Healthy
30	56	W	F	3	9·6	Primary suprarenal carcinoma Metastases liver
31	50	W	F	4	14·60	Primary colon carcinoma Metastases soft tissue of pelvis
32	64	W	F	2	20·95	Primary breast carcinoma Metastases bone
33	±60	B	M	4	62·5	Primary liver carcinoma
34	±28	B	M	4	58·0	Primary liver carcinoma
35	80	B	M	4	58·0	Primary liver carcinoma
36	24	B	M	4	30·5	Primary liver carcinoma
37	29	B	M	4	38·0	Primary liver carcinoma
38	38	B	M	3	4·6	Healthy
39	35	B	M	3	11·5	Healthy
40	46	B	M	3	6·430	Healthy
41	46	B	M	3	6·00	Healthy
42	58	W	M	2	16·00	Wide spread follicular lymphoblastoma
43	39	W	F	4	80·00	Primary breast carcinoma Metastases liver
44	53	W	F	4	12·00	Primary malignant carcinoid of small gut. Metastases liver
45	52	W	F	2	20·10	Primary breast carcinoma Metastases bone and liver
46	58	W	F	4	45·97	Primary carcinoma of gallbladder Metastases liver
47	57	W	M	4	13·95	Primary mesothelioma Metastases liver
48	52	B	M	2	19·3	Primary liver carcinoma

W = White, B = Bantu.

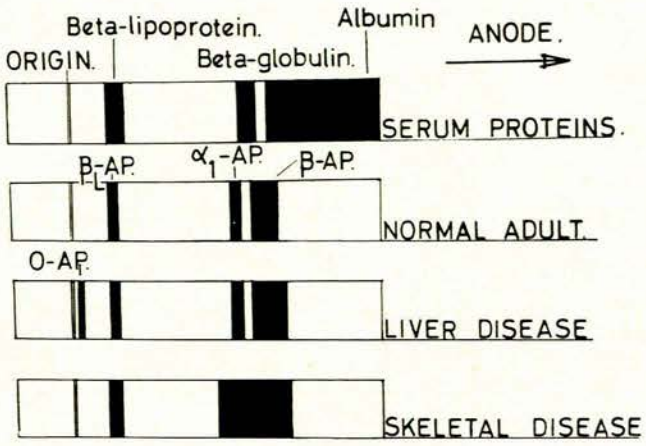

Fig. 2. Serum alkaline phosphatase iso-enzyme patterns.

Fahey *et al.*[1] used chromatography to separate the serum alkaline phosphatase in a patient with carcinoma of the breast and an increased total serum alkaline phosphatase value, due to liver and bone metastases. They found 2 fractions not knowing that they were iso-enzymes.

Keiding[3] separated serum alkaline phosphatase into 3 fractions with starch-block electrophoresis. The betaphosphatase corresponded to the beta-globulin and increased with skeletal disease. The alpha$_1$- and alpha$_2$-phosphatase were in the distribution of the alpha$_1$- and alpha$_2$-globulins and originated from the liver. Rosenburg[16] also found the alpha$_1$- and alpha$_2$-fractions and observed that bile alkaline phosphatase corresponded to the alpha-fractions.

Boyer[4] conducted an extensive study on the iso-enzymes of alkaline phosphatase. He divided the fractions into 6 zones, A, B, C, D, E and F. Zone A was cathodic to the transferrin C-band and zone D to the haptoglobulin H area. Each zone was subdivided into different bands with not more than 4 zones and 8 bands in each case. The normal adult showed 1 - 2 bands in the C-zone and occasionally 1 band in the F-zone. He examined 120 sick adults and came to the conclusion that the iso-enzyme patterns of serum alkaline phosphatase were of no value in the differential diagnosis of disease.

Taswell and Jeffers[5] separated the iso-enzymes of alkaline phosphatase in a series of patients with hepatobiliary and skeletal disease and suggested a new nomenclature for their 8 bands. The beta-alkaline phosphatase is situated anodal to the beta-globulin and was increased in all cases with hepatobiliary disease. The alpha-AP band was cathodal to the beta-globulins. The beta-lipoprotein-AP band was in the vicinity of the beta-lipoproteins. Other bands were interspersed between these and named according to the corresponding protein fractions. The normal adult showed a weak beta-AP band and a B_L-AP band. The 'adult

variant' presented an extra band, the alpha$_1$-AP band. Patients with obstructive metastatic and infiltrating hepatobiliary disease presented the normal band distribution, with an increase in the beta-AP and a prominent band in the origin (O-AP). They also differentiated between the different hepatobiliary disease on the width of the O-AP and B_L-AP bands. Osteoblastic disease presented a broad band in the alpha-beta region but no O-AP band. Patients with both liver and bone metastases showed a combined picture.

Haije and De Jong[8] separated the serum iso-enzymes on agar-gel and found 3 fractions. Fraction 1 in the vicinity of the beta-lipoproteins originated from the bone and fractions II and III in the beta-globulin area from the liver.

Gordon[6] separated the iso-enzymes on starch-gel and used a nomenclature similar to that of Boyer.

It is impossible to compare the results of the above authors due to differences in electrophoretic techniques used, but certain deductions can nevertheless be made from their information and that obtained from our 48 cases.

With the exception of 1 case, all 8 adult White controls showed 3 bands of alkaline phosphatase iso-enzymes. The most distinct band could be seen in the beta-globulin area. Slightly cathodal to the latter another band was present and in contrast to the findings of Tasswell and Jeffers[5] this seems to be a constant finding. They preferred to call this band the 'adult variant'. The third band was found in the region of the beta-lipoproteins. The same 3 bands were found in the control Bantu males and according to their positions relative to the protein bands they may be labelled as beta-AP, alpha$_1$-AP and beta$_L$-AP bands.

The cases with metastatic lesions in the liver and those with primary liver carcinoma had an interesting iso-enzyme pattern. Twelve of the 16 White patients with metastatic liver lesions showed the normal distribution of bands and an additional one near the origin. There was also an increased concentration of the beta-AP and alpha$_1$-AP bands. This increase was parallel with the plasma concentration of the enzyme. Interestingly enough 1 of the 12 cases showed 3 bands at the origin with a total of 5 iso-enzyme fractions. Of the remaining 4 cases, 3 had a normal distribution and 1 showed only 2 bands. Of the 10 Bantu cases with primary liver carcinoma, 9 showed the 3 normal bands and an additional 1 near the origin, and in the remaining 1 the zymogram pattern was that of 6 iso-enzymes with 4 of these near the origin. It is therefore quite clear that the vast majority of cases with primary or metastatic lesions and an increased serum phosphatase have an additional band near the origin and also show widening of the other bands corresponding to the increase in the serum value of the total enzyme.

The 4 White cases with bone metastases showed a marked increase in the alpha$_1$-AP and beta-AP bands with resultant overlapping, so that the normal bands were replaced by one broad band, the so-called alpha-beta band.

The remaining 6 cases, with either metastases to soft tissue and/or liver, showed a mixed pattern as could be expected.

CONCLUSIONS

1. The normal adult White and Bantu contains 3 and occasionally 2 iso-enzymes in the serum on starch-gel electrophoresis. They are the beta-AP, the alpha$_1$-AP and the beta$_L$-AP bands corresponding to the serum protein bands.

2. The iso-enzyme pattern can be of help in the differential diagnosis of liver and skeletal metastases provided that the following is borne in mind:

(*a*) The iso-enzyme pattern in any case with a normal total serum alkaline phosphatase value will be of little value. The reason for this is understandable because liver function can be normal in an early metastasis and if the patient presents with an early bone metastasis, osteoblastic reaction might not be well enough advanced to increase the serum value and the iso-enzyme pattern.
The first step, therefore, is to do a total serum alkaline phosphatase determination, before proceeding to iso-enzyme separation.

(*b*) An increase in the total serum alkaline phosphatase value with the normal iso-enzyme pattern but with an increase in the width of the alpha-beta region could mean either hepatocellular or osteoblastic activity (the latter being the more probable).

(*c*) An increase in the total serum alkaline phosphatase value with a normal iso-enzyme pattern and with an increase in the alpha-beta-AP region and with one or more bands in the origin indicates liver metastases. This is a sensitive indicator.

SUMMARY

Starch-gel electrophoresis was used to separate the iso-enzymes of alkaline phosphatase in 48 adult male and female White and Bantu patients. The total serum alkaline phosphatase value was determined in every case. Normal adult male and female persons contained 3 (or 2) iso-enzymes in their serum. Separation of the iso-enzymes in patients with liver or skeletal metastases of a malignant tumour is of no value, if the total serum alkaline phosphatase is normal. Patients with liver metastases and an increase in the total serum alkaline phosphatase value, present the usual 3 bands, with an increase in the alpha-beta band proportional to the serum value, and one or more bands in the origin. Patients with bone metastases present the 3 bands with an increase in the alpha-beta-AP band.

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