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CORRELATION OF SERUM GLYCOLYTIC ENZYMES AND ACID PHOSPHATASES WITH SITES OF METASTASES IN MAMMARY CARCINOMATOSIS

BY

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In the foregoing paper (Joplin and Jegatheesan, 1962) it was shown that total and formol-stable serum acid phosphatases (T.A.P. and F.S.A.P.) and the glycolytic enzymes, phosphoglucose isomerase (P.G.I.), phosphoglucomutase (P.G.M.), aldolase (ALD), and lactic dehydrogenase (L.D.H.), were elevated in a proportion of patients with metastatic breast cancer. In the present study an attempt has been made to determine the source of these raised serum enzymes by correlating serum enzyme levels with sites of metastases and with enzyme concentrations in the tissues concerned.

Methods

Patients Studied.—Thirty-six patients were investigated, many being from the previous study. These were classified according to the sites of their metastases, as determined clinically, radiologically, or at necropsy. The methods used for determining the various serum enzymes have been given in the foregoing paper (see also footnote to Table I).

Methods for Determination of Enzyme Levels in Tissues.—Tissues were obtained from normal subjects and from some of the cases of breast cancer either at necropsy or at mastectomy. In the cases of breast cancer, malignant tissue was obtained from the involved breast and from metastases in various sites. The bone specimen with a breast-cancer deposit (from Case 1, Table I) was checked histologically. The tissues were washed, blotted, weighed, and ground or homogenized. Extraction was with water, by standing one to two days in the cold room. Enzyme determinations were made by the methods described for serum. The acid phosphatases are expressed as K.A. units/100 g./hour, the isomerase and mutase as Bodansky kilounits (one hour incubation for mutase).

Differential Centrifugation Study on Bone Deposit.—Part of the bone deposit from Case 1 was submitted to differential centrifugation. The tissue was homogenized in a Potter-Elvehjem glass homogenizer in cold isotonic sucrose, and centrifuged at the speeds and durations given by de Duve and Berthet (1954), in order

to separate the various cell fractions; 40 ml. of the homogenate, containing 1 g. of the tissue, was taken for the centrifugation. An M.S.E. high-speed refrigerated centrifuge was used for speeds up to 12,000 g and a Spinco (model L) for the separation of microsomes, at 100,000 g. The temperature was maintained between 0 and 4° C. throughout this procedure. Each cell fraction was then rehomogenized in small measured volumes of citrate buffer, pH 4.9. These, as well as the supernatant, and a portion of the original homogenate not submitted to centrifugation, were left in the cold room overnight for complete extraction of the enzymes and the acid phosphatase activities estimated.

Results

In Tables I, II, and III are given the serum levels of the various enzymes. In patients in whom enzyme determinations were made more than once the highest value is quoted.

Table I gives the levels of the various serum enzymes in 11 patients in whom only bone metastases were known to be present, Table II the levels in 13 patients with metastases in bone and other tissues, and Table III the levels in 12 patients with metastases in tissues other

TABLE I.—Serum Acid Phosphatases and Glycolytic Enzymes in Breast-cancer Patients with Metastases Only in Bone

Case No.	Age	T.A.P.	F.S.A.P.	T.L.A.P.	P.G.I.	P.G.M.	ALD	L.D.H.
1*	52	20.5	17.1	1.2	51	41	12.0	204
2*	72	13.9	8.9	1.2	41	82	—	—
3	58	6.0	5.0	0	38	—	—	—
4	41	5.5	4.5	0.4	48	108	9.3	310
5	49	4.9	3.4	0.3	51	129	—	—
6	65	4.5	3.5	0.6	37	60	—	—
7*	45	4.4	2.7	0	20	—	—	—
8	43	3.8	2.5	0.7	70	107	13.5	327
9	49	3.3	2.4	0.4	24	—	6.5	197
10	46	3.5	2.6	0.4	86	65	12.3	204
11	74	3.5	2.3	0.4	54	—	—	—
(Upper limits of normal)		(3.1)	(2.4)	(0.7)	(25)	(82)	(8)	(143)

*Part of this work is from a thesis submitted by K. A. J. (present address: Biochemistry Laboratory, General Hospital, Colombo, Ceylon) for the Doctorate of Philosophy of the University of London.

In cases where an enzyme determination was made more than once, the highest level recorded for the enzyme is given in this Table and in Tables II and III. Units: T.A.P., F.S.A.P., and T.L.A.P.—K.A. units P.G.I.—units according to Bodansky (1954). P.G.M.—units according to Bodansky (1957a). ALD—units according to Sibley and Lehninger (1949). L.D.H.—units according to White (1956).

*Cases where site of metastases was confirmed at necropsy.

than bone. In three patients in Table I, in seven in Table II, and in three in Table III the sites of metastases were confirmed at necropsy.

Table IV gives the enzyme concentrations of normal tissues as well as of some cancerous tissues obtained from some of the patients with breast cancer.

Table V gives the acid phosphatase activities of the various cell fractions of a bone metastasis from Case 1.

Discussion

Acid Phosphatase

In several breast-cancer patients without known metastases in whom we had determined serum enzymes we did not come across any instance in which the total and formol-stable serum acid phosphatases were raised. This suggests that, although tissue analysis shows that cancerous breast tissue appears to contain greater acid phosphatase activity than normal breast tissue (Table IV), the primary tumour by itself may not liberate sufficient acid phosphatase to raise the serum enzyme

levels. Furthermore, in several of the cases in which highly raised serum acid phosphatase levels were obtained—for example, Cases 1, 2, 12, and 13—the breast-tumour tissue had already been removed by radical mastectomy. From this it is concluded that raised serum acid phosphatase in breast-cancer patients is predominantly, if not always, due to the presence of metastases.

TABLE V.—*Acid Phosphatases of Cell Fractions of a Bone Metastasis from Breast Cancer*

Fraction	T.A.P.	F.S.A.P.	T.L.A.P.
Original homogenate	15.9	12.0	7.6
Nuclei (and debris)	2.7	2.1	0.6
Mitochondria	1.3	1.2	0.8
Lysosomes	1.4	1.3	1.0
Microsomes	2.6	2.3	2.1
Supernatant	4.2	2.3	2.3
Recovery	12.2	9.2	6.8

The results are in K.A. units calculated for a volume of original homogenate containing 1 g. of bone metastasis : as such, the values give the activities in 1 g. of the tissue and the fractions arising from 1 g.

TABLE II.—*Serum Acid Phosphatases and Glycolytic Enzymes in Breast-cancer Patients with Metastases in Bone and Other Tissues (the First 10 Cases have Liver Metastases)*

Case No.	Age	Site of Metastases	T.A.P.	F.S.A.P.	T.L.A.P.	P.G.I.	P.G.M.	ALD	L.D.H.
12*	48	Bone, liver	19.3	15.8	1.0	161	163	24.4	—
13*	52	"	15.2	12.4	0.4	436	258	—	—
14	53	"	8.0	6.4	0.7	141	176	14	—
15	64	" .. skin	7.2	4.4	0.1	128	165	—	—
16*	37	" .. lung, pericardium	5.8	4.2	0.2	170	—	—	321
17*	52	" .. kidney	5.0	3.9	0.2	70	—	—	240
18*	42	"	4.3	3.7	0	245	—	—	—
19	46	" .. lung, skin	4.0	3.1	0.1	153	82	8.1	—
20	40	"	3.2	1.6	0	60	—	—	—
21*	25	" .. lymph nodes	2.1	1.4	0.2	252	—	48.6	—
22	58	Bone, C.N.S.	4.3	3.8	0.3	28	72	11.5	146
23	42	" .. lymph nodes	3.5	2.5	0.5	42	26	—	—
24*	56	"	2.6	2.4	0	69	—	—	—
(Upper limits of normal)			(3.1)	(2.4)	(0.7)	(25)	(82)	(8)	(143)

*Cases where site of metastases was confirmed at necropsy.

TABLE III.—*Serum Acid Phosphatases and Glycolytic Enzymes in Breast-cancer Patients with Metastases in Tissues Other than Bone (the First Three Cases have Liver Metastases)*

Case No.	Age	Site of Metastases	T.A.P.	F.S.A.P.	T.L.A.P.	P.G.I.	P.G.M.	ALD	L.D.H.
25	59	Liver	4.5	3.0	0.8	155	—	22.4	—
26	46	" .. lymph nodes	2.0	—	0.2	56	—	—	—
27*	36	" .. lungs, lymph nodes, skin	2.8	1.7	0.3	86	135	7.2	—
28	55	Lungs, skin	3.6	2.7	0.2	72	80	—	—
29	38	Skin	3.5	2.4	0.4	47	90	11.7	—
30	50	Lungs, skin, lymph nodes	2.5	2.3	0.5	161	—	—	—
31	55	Chest wall, excluding ribs	2.6	1.8	0.4	36	42	1.2	—
32	48	Skin, lymph nodes	2.6	1.9	0.5	35	47	12.2	—
33	67	Lungs, skin	2.3	1.9	0.2	84	13	—	—
34*	43	"	2.0	1.4	0.2	55	32	—	—
35	63	Skin	2.1	1.6	0.4	29	—	2.8	92
36*	52	Lungs, C.N.S.	1.3	1.0	0.5	43	70	—	—
(Upper limits of normal)			(3.1)	(2.4)	(0.7)	(25)	(82)	(8)	(143)

*Cases where site of metastases was confirmed at necropsy.

TABLE IV.—*Tissue Enzyme Concentrations*

Tissue	Acid Phosphatases (K.A. units/100 g./hr.)							Phosphoglucose Isomerase (Bodansky Kilounits)			Phosphoglucomutase (Bodansky Kilounits hour ⁻¹)		
	Total		Formol-stable		Tartrate-labile		No. of Cases	Phosphoglucose Isomerase		No. of Cases	Phosphoglucomutase		No. of Cases
	Mean	Range	Mean	Range	Mean	Range		Mean	Range		Mean	Range	
Normal :													
Breast	31	4-6-77	22	2-66	8	0-27	6	0.02	0.004 and 0.03	2	—	—	—
Bone	142	103 and 181	102	64 and 140	85	49 and 120	2	0.3	—	1	3.1	1.6 and 4.6	2
Liver	291	270 and 312	129	108 and 150	105	84 and 125	2	30	21 and 40	2	109	101 and 117	2
Lung	221	195 and 246	134	117 and 151	78	35 and 120	2	6.1	—	1	7.5	3.9 and 11	2
Skin	45	24-75	40	20-66	36	15-57	3	—	—	—	—	—	—
Skeletal muscle	93	70 and 115	8	5 and 10	8	5 and 10	2	—	—	—	—	—	—
Breast cancer in :													
Breast (primary)	232	182-265	140	92-166	91	30-160	3	0.17	—	1	—	—	—
Bone (Case 1)	1,594	—	1,200	—	759	—	1	2.5	1.4 and 3.5	1	1.1	—	1
Liver	337	306-400	182	165-207	138	115-178	3	15	11 and 19	2	134	—	1
Skin	42	—	42	—	42	—	1	—	—	—	—	—	—

Examination of Tables I, II, and III shows that in all the 10 cases where acid phosphatase (T.A.P.) was 5 K.A. units and above, bone metastases were present. In some of these cases metastases were present in bone only (Table I), while in others metastases were present in bone as well as in other tissues (Table II). Metastases in soft tissues alone (Table III), although occasionally giving values slightly above normal limits, did not show as high values as those found in cases where bone is involved. Taken along with the fact that a metastatic bone deposit from Case 1 (Table IV) showed a very high acid phosphatase activity, it may be concluded that appreciably raised serum acid phosphatase activity in breast cancer is generally due to active bone metastases. It does not, however, follow that all cases with bone metastases show appreciably raised serum acid phosphatase levels. As is seen from Tables I and II, 10 out of 24 cases with bone deposits had T.A.P. values of 5 K.A. units and above, while two of these cases had normal values.

Examination of the Tables also shows that serum T.L.A.P. levels have always remained within, or close to, normal limits irrespective of the presence of metastases. This finding is most surprising in view of the tissue T.L.A.P. concentrations (Table IV). Study of Table IV shows that all the tissues commonly involved in breast-cancer metastases (excepting muscle) contain tartrate-labile enzyme in relatively high concentration approaching that of F.S.A.P. For example, of the T.A.P. activity of bone, whether normal or cancerous, 60 to 80% is formol-stable whereas 50 to 55% is tartrate-labile. Again, in the case of liver, about 50% of the total activity is formol-stable while 35 to 40% is tartrate-labile. In the skin almost all (80 to 100%) of the total activity is formol-stable as well as tartrate-labile. From these considerations it should follow that if the T.A.P. activity of serum is raised by the lysis of tumour tissue from any of these sites, with consequent leakage of the enzymes into the blood-stream, and if both formol-stable and tartrate-labile serum enzymes are cleared at the same rate, both should be increased to levels of similar magnitude. This, as is seen from Tables I, II, and III, especially considering the cases where T.A.P. is appreciably elevated, is not what is found. For example, in Cases 1 and 12, where serum T.A.P. is raised to the order of 20 K.A. units, F.S.A.P. is raised to about 16 K.A. units while tartrate-labile enzyme remains at about 1 K.A. unit only. In other words, of the extra 17 K.A. units of T.A.P. which has entered the blood-stream due to the cancer, about 75% is formol-stable while only 2 to 5% is tartrate-labile.

Muscle-wasting, which was suggested by White (1958) as a probable cause of elevation of serum glycolytic enzyme in cancer, would appear unlikely to be an important factor causing raised acid phosphatase levels. For from Table IV it is seen that, although muscle contains very little tartrate-labile enzyme, its F.S.A.P., too, is negligible. Thus, muscle cannot account for the raised levels of serum F.S.A.P. Furthermore, the acid phosphatase of muscle is similar to that of the red cell in that it is formol-labile while being tartrate-stable, whereas the increased acid phosphatase activity in patients with breast cancer is formol-stable and tartrate-stable. (It may be mentioned here that the acid phosphatase of the prostate and that found in the serum of patients with prostatic cancer is of a completely different type to the above in that it is formol-stable and tartrate-labile.)

Consideration of Table IV also shows that most of the tissues studied contain appreciable proportions of T.S.A.P. (Total minus tartrate-labile = tartrate-stable.) The problem, then, is whether the formol-stable and tartrate-stable enzyme selectively enters or remains in the blood-stream to the exclusion of the tartrate-labile enzyme found in most tissues. From the fact that the bone metastasis contained much higher activity than normal bone (Table IV) and from consideration of Tables I, II, and III, it was suggested earlier that bone metastases may be the source of significantly raised serum acid phosphatase levels. Hence a differential centrifugation of a homogenate from a bone metastasis was carried out (Table V) to see if any of the cell fractions could account for the type of acid phosphatase present in the serum of patients with breast-cancer metastases. Allowing for the fact that a separation of cell debris and contaminating mitochondria from nuclei was not made in the procedure, examination of Table V shows that the acid phosphatase of the nuclei of this bone metastasis is mainly formol-stable and tartrate-stable, whereas that of the other cellular fractions is mainly formol-stable and tartrate-labile.

The increased acid phosphatase found in serum of our patients with breast cancer, being formol-stable and tartrate-stable, corresponds to that of the nuclei studied, and therefore possibly arises from the increased nuclear activity and breakdown that occurs in cancer. It would appear that the cytoplasmic acid phosphatase either does not leak into the blood in this process or if it does is rapidly inactivated or eliminated. The acid phosphatase present in normal serum is mainly formol-stable and tartrate-stable, so it, too, may arise from nuclear activity or breakdown when occurring at a normal rate.

The picture presented in prostatic cancer is quite different from that in breast cancer. The prostatic cytoplasm actively secretes massive amounts of formol-stable, tartrate-labile acid phosphatase (Seligman and Manheimer, 1949), and when metastases appear in prostatic cancer the cells of the affected tissues seem to carry on the same function (Gutman, Sproul, and Gutman, 1936). This may account for the increased levels of formol-stable but tartrate-labile acid phosphatase that is found in serum in this condition.

The different types of acid phosphatases are classified on their response to formaldehyde and tartrate inhibition in Table VI.

TABLE VI.—*Different Types of Acid Phosphatases (Based on Formol and Tartrate Inhibition)*

Acid Phosphatase	Formol	(+) Tartrate
Serum, metastatic breast cancer ..	(Mainly) stable	(Mainly) stable
Normal serum ..	"	"
Nuclei in bone metastasis from breast cancer ..	"	"
Cytoplasm in bone metastasis from breast cancer ..	Stable	Labile
Prostatic cytoplasm ..	"	"
Serum, metastatic prostatic cancer ..	Labile	Stable
Red cell ..	"	"
Skeletal muscle ..	"	"

Glycolytic Enzymes

From an examination of the enzyme levels of the 36 patients in Tables I, II, and III, it may be stated in general that highly raised values for P.G.I., P.G.M., and aldolase are obtained only when liver metastases are present. The first 10 cases in Table II and the first three in Table III had liver metastases. None of the other 23 were known to have liver metastases. It is

seen that in the (probable) absence of liver metastases there is only one value out of 23 above 100 units for serum isomerase, none out of 16 above 150 units for P.G.M., and none out of 10 above 15 units for aldolase. On the other hand, in the cases with liver metastases there are 9 values out of 13 above 100 units for isomerase, four out of six above 150 units for mutase, and three out of six above 15 units for aldolase.

The few determinations of serum L.D.H. show that highly raised (above 300 units) values for this enzyme may be obtained in the presence of bone metastases with or without liver involvement.

It is seen from the few determinations of glycolytic enzymes we made on tissues that normal liver and hepatic metastases contain much more isomerase and mutase activity than the other tissues. These observations are in agreement with those of Bodansky (1957b) and may explain the very high values obtained for serum isomerase and mutase in the presence of liver metastases.

Usefulness of Serum Enzyme Determination

From the foregoing it is seen that serum acid phosphatase (total or equivalently formol-stable) may be of use in detecting active bone metastases. Determination of acid phosphatase, which is a simple procedure carried out in any routine laboratory, may be employed as a useful parameter in such patients to supplement those commonly used—for example, radiology, alkaline phosphatase, calcium metabolism.

Apart from the fact that serum total and formol-stable acid phosphatases show a certain degree of correlation with cancer activity in breast-cancer patients (Joplin and Jegatheesan, 1962), it may be mentioned that patients who show appreciably raised levels (T.A.P. above 5 K.A. units) have a very poor prognosis. Such patients in our study did not survive for more than a few months.

With regard to serum isomerase it has already been shown that this enzyme correlates well with cancer activity and effect of treatment (Joplin and Jegatheesan, 1962). The procedure for the determination of this enzyme is quite simple (Bodansky, 1954) and could be set up in any laboratory. A series of estimations can be completed in a couple of hours. No expensive chemicals or equipment are needed, and the colour reaction upon which the method is based can be read in an ordinary photo-electric colorimeter.

The three glycolytic enzymes—serum isomerase, mutase, and aldolase—when greatly elevated appear to be useful in showing liver metastases. Of these, determinations of isomerase and aldolase (Sibley and Lehninger, 1949) are quite simple. However, the colours obtained for aldolase are not stable, and much care is required in timing the intervals of addition of reagents and the reading of the final colours. This difficulty does not apply to isomerase. As regards serum mutase, the procedure is time-consuming and complicated, and the coenzyme required (glucose-1:6-diphosphate) is very expensive and not easily available. Furthermore, the enzyme does not correlate well with cancer activity, as shown in the previous paper.

Summary

Appreciably raised serum total or formol-stable acid phosphatase levels (above 5 K.A. units) are generally associated with active bone metastases in breast-cancer patients.

The raised acid phosphatase found in serum of patients with breast cancer is of a different type from that of the prostate or red cells.

Differential centrifugation of a homogenate of a bone metastasis in a patient with breast cancer indicated that metastatic nuclei may be the source of the raised serum acid phosphatase.

High levels of serum phosphoglucose isomerase, phosphoglucomutase, and aldolase may indicate liver metastases.

Serum acid phosphatase (total or formol-stable) and serum isomerase provide useful and convenient additional parameters in the assessment of breast-cancer activity.

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In the *Report of the County Medical Officer of Health and Principal School Medical Officer for the Year 1960*, issued by the London County Council, it is recorded that during the year 1960 a psychiatrist and a psychologist at the County Hall dealt with 18 cases of school phobia, all previously regarded as more or less insoluble. Six of the children were found to be true cases of anxiety and their non-attendance was part of a deep psychological disorder. They were all referred to child guidance clinics and were still under treatment at the end of the year, maintaining occasional attendances at day special schools or classes. Four children were of the mildly neurotic and anxious type, unable to face up to the demands of the large classes in ordinary schools. They were said to have all settled down very well in the more sheltered environment of day special schools either for the delicate or for the maladjusted. Four of the cases—all boys—were considered to be real truants. Following court action all were now attending school regularly under probationary supervision. The remaining four cases fitted into no category and each represented an individual problem. They included a 14-year-old girl with a wig who refused to attend school because the other children pulled it off, and a girl who had not been allowed to attend school for four years and had been under a child guidance clinic for two years. Her parents disapproved of school on principle, but when attendance was arranged for her at an "open-air" school they became among the school's most vocal supporters. The most acute cases of separation anxiety under treatment at child guidance clinics were placed on home tuition for limited periods. There were some 15 such cases during the year, of whom over half were back in school or attending special classes at least part-time after a few months on home tuition. Seven intractable non-attenders were admitted during the year to the Bethlem Royal Hospital Adolescent Unit. All returned after treatment to day or boarding schools.