

available for all the other lipid fractions to yield results of a degree of significance identical to that which would have obtained if groups of 20 test and 20 control subjects had been conventionally analysed. Furthermore, some significant results, albeit negative, were obtained for 12 months of treatment.

Similarly, although only 12 pairs of subjects who were treated soon after acute myocardial necrosis survived three months during the same period and 11 pairs for six months, a majority of the t-tests were completed, with the same probability. In short the sequential method has, as well as giving interim data and determining the trial, nearly doubled the amount of information obtained and therefore approximately halved the cost. Since there are no objective responses the differences between the replies of treated and untreated patients to questions on their well-being are probably explained by non-specific stimulating effects perhaps due to the pyridoxine content (Célice *et al.*, 1958) of the test capsules. Further, survival rates in both groups are comparable. The period of the trial is, however, as clearly shown by McMichael and Parry (1960), too short for significant conclusions on mortality to be drawn.

In view of the large amount of negative data here presented the trial has been stopped.

Summary

A total of 114 patients in three clinical groups, all of whom were suffering from results of atherosclerotic arterial occlusion, have been treated with a phospholipid preparation (lipostabil) for periods up to two years. Ten fractions of the serum lipids were unaffected by it.

The lipostabil was kindly supplied by Aspro-Nicholas of Slough, Bucks, who also made a grant towards the cost of the investigation. Many of the members of the staff of the East Ham Memorial Hospital, as well as local general medical practitioners, have co-operated willingly, and without their help the trial would not have been possible.

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The Hong Kong Government plans to open a new welfare centre on the island to house 600 old and disabled people in about two years' time. It will replace the North Point camp, which was first opened in 1938 to house refugees of the Sino-Japanese War. The proposed welfare centre will occupy an area of 188,500 square feet and will consist of eleven buildings, mostly of two or three stories, providing accommodation for 600 people. It will serve as a transit centre for old people awaiting admission to a home for the aged and as a place of refuge for destitutes and disabled people pending a more permanent solution to their problems. There will also be sheltered workshops for those less capable of open employment.

SERUM GLYCOLYTIC ENZYMES AND ACID PHOSPHATASES IN MAMMARY CARCINOMATOSIS

BY

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Assessment of the effect of endocrine treatment on the progress of breast cancer is sometimes difficult, and an addition to the usual parameters would be clinically valuable. Bodansky (1954a) has shown that serum phosphoglucose isomerase is a good index of tumour growth in metastatic breast cancer, and Lemon and Reynolds (1956) have reported a correlation between the fall in serum copper-resistant acid phosphatase and regression of osseous metastases in oophorectomized or elderly (>70 years) women with advanced breast cancer treated with cortisone.

In the present study the clinical effects of pituitary destruction on breast carcinomatosis were followed and compared with serial measurements of the following serum enzymes: total (T.A.P.), formol-stable (F.S.A.P.), and tartrate-labile (T.L.A.P.) acid phosphatases, phosphoglucose isomerase (P.G.I.), and phosphoglucomutase (P.G.M.). A few determinations were made of serum aldolase (ALD) and lactic dehydrogenase (L.D.H.). Enzyme determinations were also made in patients who had not been treated by pituitary destruction. From these data an assessment of the clinical value of these serum enzymes as an index of cancer growth has been made. In an accompanying paper (Jegatheesan and Joplin, 1962) elevations of the different serum enzymes have been correlated with sites of metastases.

Patients Studied

Serial enzyme determinations were made in 17 women with advanced mammary carcinomatosis who were treated by pituitary destruction by needle implantation of yttrium-90 seeds (Fraser *et al.*, 1959). The age range was 36 to 72, with a mean of 49 years. Serum levels of the various enzymes were measured just before pituitary destruction and followed for 6 weeks to 18 months thereafter.

The effect of this treatment on the carcinoma was assessed independently by measurement of visible masses, radiology of bone metastases, study of serum and urinary calcium, serum alkaline phosphatase, and haematology, as well as the general progress of the patient, thus enabling classification of the patients into three groups, according to their clinical response to pituitary destruction: (1) *responders*—those in whom a clear remission in the above parameters was obtained (followed in some cases right into terminal relapse); (2) *non-responders*—those showing no clinical response; and (3) "*mixed*" *responders*—those whose improvement

*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 the original lesions was about equivalent to the simultaneously appearing new lesions; also a few patients in whom the response was doubtful.

Serum enzymes were also determined, though not serially, in 44 further cases of metastatic breast cancer, including one male breast cancer. Most of these patients were not treated by pituitary destruction.

Enzyme Assay Methods

T.A.P., F.S.A.P., and T.L.A.P. were determined according to the methods described by King and Jegatheesan (1959), the upper limits of normal being 3.1, 2.4, and 0.7 K.A. units per 100 ml., respectively (Jegatheesan and King, 1962).

P.G.I. was estimated by the method of Bodansky (1954b), with a minor modification in that 10% trichloroacetic acid was used for precipitation of proteins. The normal range of values for this enzyme determined by us on 34 healthy adults was 6 to 20 units (mean 12.2 ± 3.5 , where 3.5 is the standard deviation). No sex difference was found. In view of the higher range of normal values for this enzyme reported by Bodansky (1954b) (8 to 40), Israels and Delory (1956) (4 to 40), and White (1958) (5 to 33), all using the same method, we have taken 25 units as the upper limit of normal, which is close to our mean plus 3 S.D. The error was determined by duplicate analyses on 21 sera. The average deviation from the mean of the duplicates for each analysis was 1 unit.

P.G.M. was determined by the method of Bodansky (1957a). Our normal range (12 healthy adults) was 17 to 82 units, with a mean and S.D. of 46 ± 18 . These normal values are similar to those of Bodansky. We take 82 units as the upper limit of normal, which is the mean plus 2 S.D. Twelve sera were estimated in duplicate: the average deviation from the mean of duplicates was 2.0 units.

Aldolase was determined by the method of Sibley and Lehninger (1949). Our normal range was 1 to 8 units, with a mean of 4.4, which is similar to that found by the authors of the method.

L.D.H. was determined by the method of Hill and Levi (1954), as modified by White (1956). Our normal range was 75 to 143 units, with a mean of 107 ± 18 , these values being somewhat higher than those reported by the above investigators.

Results

The findings in the 17 cases of breast carcinomatosis treated by pituitary ablation are summarized in Figs. 1, 2, and 3. As there are fluctuations in enzyme levels from day to day, even without the advent of therapy or any other discernible cause, we have prepared the data for the three clinical response groups as follows: for each case we have taken the mean of all the serial pre-ablation values as the pre-ablation level, and compared this with the mean of the post-ablation values. In the case of the clinical responders the post-ablation values have been further divided into the remission phase and the terminal relapse phase. The same method of presentation has been used for each enzyme.

Total Acid Phosphatase.—Seven of the 17 cases studied had raised pre-ablation levels, two as high as 12 K.A. units (Fig. 1). It is evident that striking changes occur after pituitary ablation only when pre-ablation

levels are greatly elevated. In the group of seven clinical responders, five showed a drop in enzyme levels. In two cases this was conspicuous. One patient showed a clear rise during remission: she soon developed new deposits, although the old ones continued to heal conspicuously and remission dominated the clinical picture. Three of the responders were followed into relapse, and all of them showed rises in acid phosphatase. In the group of six clinical non-responders, two showed rises in T.A.P. to clearly abnormal levels and three showed small rises. One showed a small fall. (The F.S.A.P., however, in this case rose from a pre-ablation level of 1.5 to a post-ablation level of 2.1 K.A. units, consistent with the clinical picture.) In the "mixed" response group of four cases, the only case showing a marked change was one in whom a fall in T.A.P. was associated with marked subjective improvement, with slight radiological sclerosis of old deposits, although the picture was dominated by the appearance of new deposits. In all, including the above 17 cases serially studied, T.A.P. was measured in 58 patients, all with metastases: 62% showed raised levels—that is, above 3.1 K.A. units/100 ml. The highest value recorded was 20.5 K.A. units.

Formol-stable Acid Phosphatase.—In every blood specimen on which T.A.P. was measured, both the F.S. and T.L. fractions were also measured. In the 17 patients serially studied it was found that the formol-stable paralleled the total, with one exception. An almost identical figure to Fig. 1 was obtained when the mean F.S.A.P. levels were plotted before and after pituitary ablation. Of a total of 58 patients in whom F.S.A.P. was measured 52% showed elevations. The highest value for this enzyme was 17.1 K.A. units.

Tartrate-labile Acid Phosphatase.—In the 17 patients serially studied the mean levels of this enzyme remained within normal limits—that is, below 0.7 K.A. units—throughout the study. Nearly all the individual readings were within the normal range. A few determinations showed a slight elevation. Of a total of 58 patients in whom T.L.A.P. was determined 8% showed slight elevation. The highest value was 1.2 units—that is, about 0.5 K.A. unit above the upper limit of normal

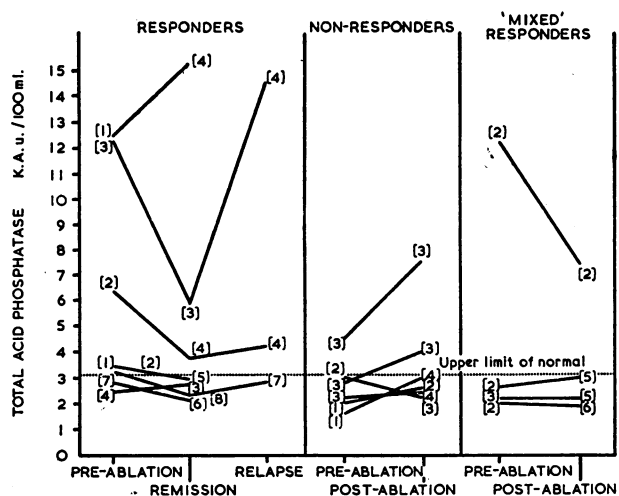


FIG. 1.—Clinical effect of pituitary ablation in breast carcinomatosis, correlated with total serum acid phosphatase. Patients are independently classified into three clinical cancer-response groups. For each case the value shown is the mean of all the observations made while in a given status. The numbers of observations from which the means are derived are given in parentheses.

of 0.7 K.A. unit (cf. T.A.P.: highest value 20.5 K.A. units, upper limit of normal 3.1 K.A. units).

Phosphoglucose Isomerase.—The data with regard to the 17 patients serially studied are shown in Fig. 2. In the group of seven clinical responders, all had pre-ablation levels well above normal, and all showed clear falls during remission: furthermore, in all except one patient (who was exceptional in having an extremely high initial level) the mean remission value fell to within the normal range. In the four responders who were subsequently followed into terminal relapse, the enzyme levels rose again. Omitting the case whose levels were exceptionally high throughout, the mean values for the group, \pm S.E.M., were for the pre-ablation, remission, and relapse phases 43 ± 3 , 21 ± 2 , and 38 units respectively. The changes are obviously significant. In the group of six clinical non-responders all had raised initial values; three showed rises after ablation and two showed slight falls. None reached normal levels. Altogether, including the above 17 cases serially studied, P.G.I.

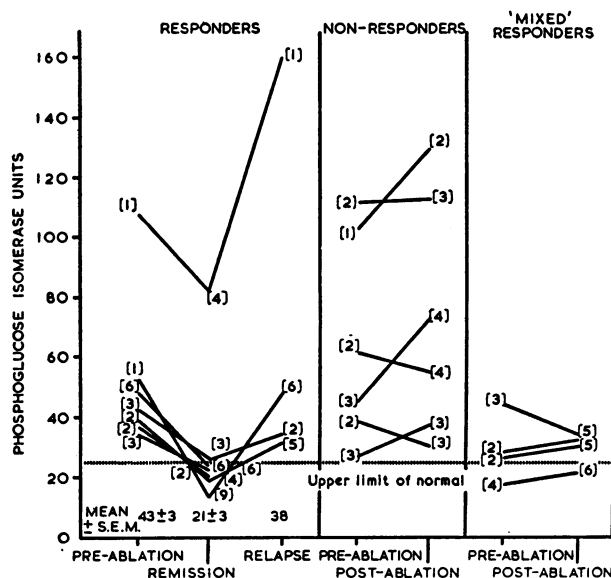


FIG. 2.—Clinical effect of pituitary ablation on breast carcinoma correlated with serum phosphoglucose isomerase. (See remarks under Fig. 1.)

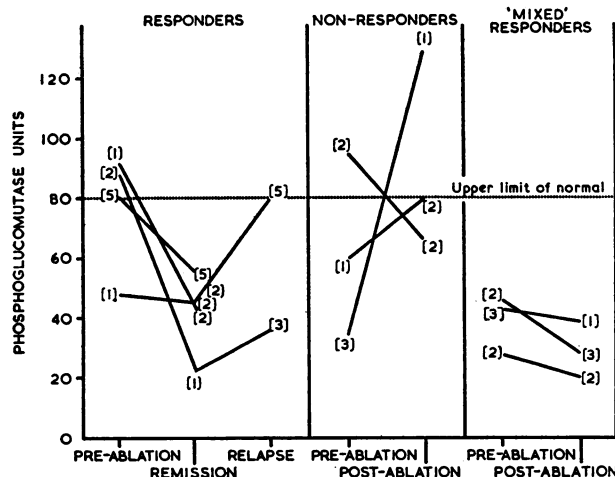


FIG. 3.—Clinical effect of pituitary ablation on breast carcinoma correlated with serum phosphoglucose mutase. (See remarks under Fig. 1.)

was measured in 58 cases. The range of values obtained was from 9 to 864 units. It was found to be above the normal limit of 25 units in 85% of the cases. The highest value recorded (864 units) was in a case of male breast cancer with liver and bone secondaries.

Phosphoglucose mutase.—Results for the cases serially studied are shown in Fig. 3. Although there is a tendency for P.G.M. levels to follow clinical response, with the exception of one isolated determination all the figures were within, or only slightly above, the normal range whether during the pre-ablation, remission, or relapse phase. Single determinations were made on further cases of breast cancer: out of a total of 29 cases, the level was well within the normal range in 17. As the level of this enzyme seems to fluctuate very widely from day to day (for example, Fig. 4), the significance of the changes in P.G.M. in Fig. 3 is doubtful. In view of this, and because of the difficult analytical technique, P.G.M. determinations were made in only a proportion of the sera studied for the previous enzymes. The mean value for all the 29 cases studied was 70 units and the range 9 to 320 units, the highest value, as in the case of isomerase, being from the case of male breast cancer.

Aldolase.—Pre-ablation determinations were made in seven cases, and were above the upper limit of normal of 8 units in four. Post-ablation measurements were made on two responders when in the phase of final relapse, and three "mixed" responders: all but one showed raised levels. Day-to-day fluctuations were wide, as in the case of mutase. Aldolase was measured altogether in a total of 19 cases: it was above 8 units in 12 (63%). The mean value was 11 units and the range 1 to 49 units.

Lactic Dehydrogenase.—Raised levels—that is, above the normal limit of 143 units—were found in 8 out of 11 cases in which measurements were made. The mean value was 179 units and the range 92 to 327 units. Serial study was not made.

Illustrative Case

Fig. 4 shows serial levels of certain enzymes in a woman of 40 with massive bone deposits. She was treated with two pituitary implants, as the first failed to produce hypopituitarism (Fraser and Joplin, 1960). Although a clinical cancer remission followed both pituitary implants it was but short-lived after the first, no doubt owing to failure to produce hypopituitarism. Cancer remission was easy to follow in this case, as hypercalcaemia, hypercalciuria, and leucoerythroblastic anaemia all disappeared and radiological bone-healing occurred, as well as a very striking general clinical improvement. The cancer was still in complete remission at the time of her death from an unrelated cause, 25 months after the second implant. It is seen that of the enzymes shown isomerase correlates best with the clinical picture.

Discussion

From our results it will be seen that serum isomerase is elevated in about 85% of cases of breast carcinoma and shows a good correlation with clinical status after pituitary destruction. T.A.P. (and equivalently F.S.A.P.) is elevated in only about 50 to 60% of the cases and correlation with clinical picture is less

consistent unless initial levels are very high. These findings are in general agreement with those of Bodansky (1954a) with respect to isomerase, and of Lemon and Reynolds (1956) on serum acid phosphatase. The former made a serial study of serum isomerase in 10 cases of metastatic breast cancer and reported an impressive degree of correlation with cancer progress; the latter studied serum copper-resistant acid phosphatase in 30 cases of advanced breast cancer and showed a certain degree of correlation with clinical status. The significance of copper-resistant acid phosphatase is the same as of formol-stable, in that both copper and formaldehyde destroy or inhibit acid phosphatase arising from erythrocytes (Abul-Fadl and King, 1949). An equivalence between serum copper-resistant and formol-stable acid phosphatase values has been found by Jegatheesan (1959).

Serum T.L.A.P. is generally not raised in breast cancer. This is consistent with the claim for the greater specificity of this enzyme in prostatic cancer (Abul-Fadl and King, 1949; Fishman *et al.*, 1956; Jegatheesan and King, 1962). The P.G.M. levels, from our work, seem unlikely to provide a useful index of cancer activity. The day-to-day fluctuations were large and in most cases the values were within the normal range. The experimental procedure involved in determining the enzyme is time-consuming. Bodansky (1957b) reported increased levels in three cases.

Sibley and Fleisher (1954) found increased aldolase levels in 23% of 168 cancer cases (no classification was made regarding the presence of secondaries), as well as in various other diseases. We have not made enough observations to assess this enzyme fully, but its fluctuations were disappointingly wide.

Hill and Levi (1954) reported elevated lactic dehydrogenase in 96% of their cancer cases, but their upper limit of normal appears to have been fixed too low. If 143 units be taken as the upper limit of normal, 8 out of 11 cases of breast cancer with secondaries in which we performed determinations had raised levels.

It must be pointed out that serum glycolytic enzymes are raised in several non-malignant diseases such as hepatitis, pancreatitis, myocardial infarction, progressive muscular dystrophy, rheumatoid arthritis, etc. (Bruns and Hinsberg, 1954; Schapira *et al.*, 1955; Bing *et al.*, 1957; Jegatheesan, 1959). It appears from the work of these and several other investigators that rises in serum enzymes are to be expected in any condition where there is cell impairment and necrosis. Due allowance therefore has to be given for any complicating factors in the assessment and correlation of serum enzyme levels with cancer activity.

Summary

Serum enzymes were measured in 61 patients with breast carcinomatosis as follows. Total, formol-stable, and tartrate-labile acid phosphatase in 58, phosphoglucose isomerase in 58, phosphoglucomutase in 29, aldolase in 19, and lactic dehydrogenase in 11. Serial measurements of some of these enzymes were made in 17 patients who had been treated by pituitary destruction by needle implantation of yttrium-90 seeds.

Serum phosphoglucose isomerase was raised in about 85% of these patients. It correlates well with the effect of pituitary destruction on cancer activity. It should be useful clinically.

Total and formol-stable serum acid phosphatases were elevated in about 50–60% cases. They correlate less well with cancer activity. Serum tartrate-labile acid phosphatase was generally not raised.

Serum phosphoglucomutase, aldolase, and lactic dehydrogenase all showed marked daily fluctuations, and were less frequently raised than phosphoglucose isomerase.

We thank Professor E. J. King for constant advice on the biochemical aspects of the work, and Professor Russell Fraser and Dr. R. Morrison for their help and advice in the study of the patients under their care. One of us (K. A. J.) acknowledges a Colombo Plan Fellowship which afforded the opportunity for this work.

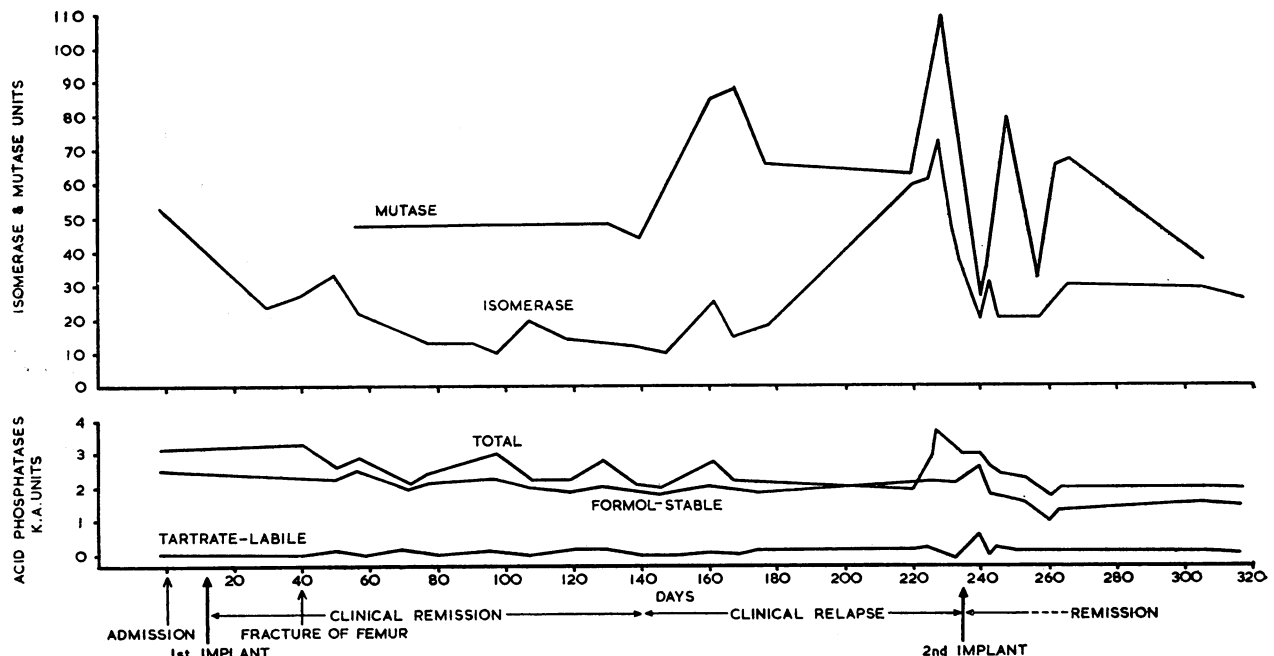


FIG. 4.—Serial estimations of enzyme levels in a case having first a partial and later a complete pituitary ablation. The first yttrium-90 pituitary implant caused an immediate clinical remission in the cancer, but failed to produce hypopituitarism. Cancer relapse soon occurred, and was again successfully treated with further pituitary destruction. Hypopituitarism resulted.

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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)

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.

*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.