Z. klin. Chem. u. klin. Biochem.
10. Jg. 1972, S. 77—85

A New Method for the Determination of Serum Nucleotidase¹⁾

V (Final Part). Evaluation of the Response of Serum Nucleotidase to Bone Metastases and Therapy in Cancer Patients

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(Eingegangen am 24. August 1971)

This paper deals with comparative studies on the levels of alkaline phosphatase, the presence of bone phosphatase as measured electrophoretically, and levels of 5'-nucleotidase, as measured in the presence of phenylphosphate in sera of patients suffering from bone metastases.

Secondly, the levels of 5'-nucleotidase and other serum enzymes were studied in cancer patients during various kinds of therapy.

A classification of serum enzymes is proposed, based on their preferential response to either liver metastases or toxic liver injury or both.

Es wird über vergleichende Untersuchungen der Aktivität der alkalischen Phosphatase, das Vorkommen von Knochenphosphatase durch elektrophoretischen Nachweis und der Aktivität der 5'-Nucleotidase, bestimmt in Gegenwart von Phenylphosphat, im Serum von Patienten mit Knochenmetastasen berichtet.

Die Aktivität der 5'-Nucleotidase und anderer Enzyme im Serum wurden bei Karzinom-Patienten während verschiedener therapeutischer Verfahren bestimmt. Eine Einteilung der Serumenzyme entsprechend ihrem bevorzugten Auftreten entweder bei Lebermetastasen oder bei toxischen Leberschäden bzw. beiden wird vorgeschlagen.

Introduction

In 1968 we introduced a method for the measurement of serum 5'-nucleotidase utilizing adenosine deaminase to generate ammonia during incubation. After a certain period the BERTHELOT colour reaction for ammonia was applied which terminated the incubation without deproteinization. The extinction of the colour was found to be a sensitive measure of 5'-nucleotidase activity (1, 2).

A modification was presented in a following paper: the addition of phenylphosphate as an inhibitor of the nucleotidase effect of bone phosphatase (3).

Evidence was presented in ref. (3) for the ability of phenylphosphate to inhibit the hydrolysis of AMP by bone phosphatase. In this study, however, sera with elevated levels of alkaline phosphatase were used from patients suffering from non-malignant bone diseases. The present paper deals with comparative studies of 5'-nucleotidase and alkaline phosphatase of patients with malignant diseases affecting bone or bone and liver. For this purpose sequential studies of the enzymes were made and a greater insight was obtained into the clinical significance of consecutive 5'-nucleotidase assays using phenylphosphate as an inhibitor of interference by alkaline phosphatase. In addition, attention was paid to the influence of hepatotoxic therapy on the levels of these enzymes.

¹⁾ Enzyme. 5'-Nucleotidase = 5'-Ribonucleotide phosphohydrolase (EC 3.1.3.5); Aspartate aminotransferase = L-Aspartate: 2-oxoglutarate aminotransferase (EC 2.6.1.1); Alanine aminotransferase = L-Alanine: 2-oxoglutarate aminotransferase (EC 2.6.1.2); Alkaline phosphatase = Orthophosphoric monoester phosphohydrolase (EC 3.1.3.1); Glutamate dehydrogenase = L-Glutamate: NAD oxidoreductase (deaminating) (EC 1.4.1.2).

An attempt was made to evaluate the differential diagnostic significance of serum enzymes in the follow-up of cancer patients by comparison with the response of other enzymes such as serum aspartate-aminotransferase and alanine aminotransferase. This report concludes our series: "A Method for the Determination of Serum Nucleotidase".

Materials and Methods

5'-Nucleotidase

A complete description of the procedure for 5'-nucleotidase can be found in ref. (4). The reader can also find all the particulars by combining the procedure from ref. (1), (2) and (3). Readings were done with a Gilford 300 microspectrophotometer.

Alkaline phosphatase

The measurement was performed with the Auto-Analyzer using *p*-nitrophenyl phosphate as a substrate and 2-amino-2-methylpropanol-1 as a buffer (5). The advantage of this buffer is that a 100 U/l sample (high normal) gives a chart reading of ± 32 scale units (transmission, full scale = 100 scale units, 8 min. incubation), which is about twice as high as with glycine buffer. Dilution of serum is necessary above ± 500 U/l. In addition the substrate produces its own chromogen. Real incubation time was regularly checked and if necessary corrected for by comparison with results from the manual method. This was done particularly after renewal of the manifold.

Aspartate aminotransferase

The measurement was done with Boehringer reagent kit (non-optimal test).

Alanine aminotransferase

This enzyme was assayed with Boehringer reagent kit (non-optimal test).

Both transferases were assayed either with the aid of a mechanical cuvet changer (at 25°) with ten minutes intermittent registration or with an LKB reaction rate analyzer (at 35°). In the latter case the automatic dispenser was set for 100 μ l 2-oxoglutarate so-

lution, since smaller volumes occasionally gave erratic results. The 2-oxoglutarate solution from the reagent kit was diluted so as to obtain the prescribed final concentration. The values were regularly checked by comparison with data obtained from measurements at 340 nm with the Zeiss PMQ II.

Electrophoretic serum phosphatase fractionation

Patterns of iso-enzymes of alkaline phosphatase were obtained with agar gel electrophoresis according to HAYE and DE JONG (6), with the exception that a solution of naphthylphosphate and Fast Blue BB (Dajac) and MgSO_4 (0.07M) in borate buffer (pH 9.0) were used for the demonstration of enzyme activities (30 to 60 min. incubation at 37°). The HAYE and DE JONG technique was found to be superior to other methods tried and to give very reliable results. As a control each slide with the serum to be treated electrophoretically was placed between two slides, treated identically, on which serum from patients suffering unmistakably from liver diseases had been applied. These latter specimens were selected on the basis of elevated alkaline phosphatase levels from clear-cut cases of liver disease (gallstone etc.) without bone being involved. After electrophoresis samples and controls were simultaneously subjected to the procedure to locate the iso-enzymes. By dilution experiments it was found that below approximately 200–150 U/l bone phosphatase could not be detected clearly in the iso-enzyme pattern.

Serum glutamate dehydrogenase

This enzyme was assayed according to SCHMIDT (7) or to PERSIJN and Coworkers (8).

Note on automation of 5'-nucleotidase assay

In view of the increasing interest in 5'-nucleotidase as a clinical test a procedure for automated assay would be useful. Attempts to realise a sensitive automated procedure for our method using either the Auto-Analyzer system or the LKB reaction rate analyzer have failed. With the latter design the BERTHELOT colour reaction was replaced by the reductive amination of 2-oxoglutarate catalysed by bovine glutamate dehydrogenase in the presence of NADH. It was assumed that the concentration of 5'-nucleotidase was rate determining and that the rate of decrease in absorbance at 340 nm caused by the conversion of NADH to NAD would be a measure of 5'-nucleotidase. To stabilize glutamate dehydrogenase L-leucine was added (8). It was striking that without serum a decrease in absorbance was observed as soon as AMP was added to initiate the reaction.

The rate of decrease was markedly lower in the absence of L-leucine and strongly suppressed by diethylstilboestrol. Since

these substances are known respectively to enhance and to suppress bovine glutamate dehydrogenase (9) it seems reasonable to assume that bovine glutamate dehydrogenase and not a contamination is able to catalyse some kind of reaction between the above mentioned constituents while transforming NADH to NAD. This would make an accurate measurement of a kinetic glutamate dehydrogenase linked 5'-nucleotidase assay impossible. Using glutamate dehydrogenase from *Clostridium* (known to have a much lower K_m for ammonia (10)) gives an identical reaction. The main problem when using the Auto-Analyzer was the long incubation time necessary to produce sensitive recordings.

Results

5'-Nucleotidase and alkaline phosphatase

It seemed interesting to accumulate data which would allow us to make statements about the amount of bone phosphatase present in sera with elevated alkaline phosphatase levels. With this in view the following was done.

- The iso-enzyme pattern of an arbitrarily chosen number of sera with elevated alkaline phosphatase and normal 5'-nucleotidase was studied.
- A number of sera, in which the 5'-nucleotidase was less elevated than the alkaline phosphatase, was examined electrophoretically for the presence of bone phosphatase.

The results of these studies are given in Tables 1 and 2. In these tables pregnant female patients are not included. Table 1 shows that in practically all cases bone phosphatase is present if 5'-nucleotidase level is normal. As a typical illustration case no. 16 is described here in detail.

Figure 1 represents a patient suffering from breast carcinoma with osteolytic metastases confirmed by bone biopsy. During the course of time, as indicated on the abscissa no clinical indication of the existence of liver metastases was found.

During the first two weeks of therapy, only 5'-nucleotidase and aspartate and alanine transaminase levels were elevated. Prednison therapy was especially successful,

Tab. 1

Results of electrophoretic examination of sera on the presence (+) or absence (—) of alkaline bone phosphatase fraction in the case of normal 5'-nucleotidase levels

Case No.	Alkaline phosphatase [U/l]	5'-Nucleotidase [U/l]	Bone phosphatase fraction	Diagnosis	X Ray evidence for osteolytic metastases	Therapy	Clinical evidence for liver metastases
1	257	7.7	—	mamma ca.	+	estrogen	no
2	357	9.8	+	mamma ca.	+, recalcification	endoxan, prednison	no
3	225	9.3	+	mamma ca.	—, osteoplastic metastases	radiotherapy	yes
4	440	10.5	+	mamma ca.	+	endoxan, prednison	no
5	630	9.1	+	PAGET and Palatum ca.	—, osteoplastic metastases	no surgery	no
6	685	8.8	+	prostate ca. ¹⁾	—, osteoplastic metastases	surgery	no
7	338	10.2	+	mamma ca.	+	no	no
8	500	4.3	+	mamma ca.	+	prednison	no
9	750	5.9	+	mamma ca.	+	no	no
10	530	8.0	+	PAGET	PAGET	no	no
11	270	9.8	+	mamma ca.	+	prednison	no
12	217	10.5	+	mamma ca.	+	surgery	yes
13	560	10.3	+	ca. of antrum	+	no	no
14	255	9.3	+	parotis ca.	+, lung metastases	no	no
15	267	3.6	+	ca. suspect ²⁾	no metastases	no	no
16	1330	8.0	+	mamma ca.	+	prednison	no
17	267	8.7	+	mamma ca.	+	prednison	no

¹⁾ Acid phosphatase level elevated (41 U/l).

²⁾ Young girl suspect for malignant disease but found to have no malignancy.

Tab. 2

Results of electrophoretic examination of sera on the presence (+) or absence (—) of alkaline bone phosphatase fraction in case of elevated 5'-nucleotidase levels

Case No.	Alkaline phosphatase [U/l]	5'-Nucleotidase [U/l]	Bone phosphatase fraction	Diagnosis	X Ray evidence for osteolytic metastases	Therapy	Clinical evidence for liver metastases
1	412	13.7	+	mamma ca.	—, osteoplastic metastases	radiotherapy	no
2	625	17.9	+	mamma ca.	+, recalcification	androgens	yes
3	568	49.0	+	bronchus ca.	+	no	yes
4	245	17.4	+	M. HODGKIN	+	radiotherapy	no
5	344	26.6	—	mamma ca.	—	surgery	no
6	1720	11.3	+	mamma ca.	osteoplastic metastases	estrogen	no
7	660	13.4	+	rhabdomyosarcoma	—	radiotherapy	no
8	3100	193.0	+	mamma ca.	+, recalcification	estrogen	yes
9	1140	32.8	+	mamma ca.	—, osteoplastic metastases	radiotherapy	yes
10	595	15.4	+	mamma ca.	—, osteoplastic metastases	5-fluoruracil	no
11	880	69.4	—	chordoma	—	leukeran	yes
12	470	18.8	+	bronchus ca.	—	no	yes
13	375	21.6	+	oesophagus ca.	—	no	yes
14	630	36.8	+	bronchus ca.	—	radiotherapy	yes
15	388	12.6	+	mamma ca.	+	prednison	yes ¹⁾
16	540	19.1	+	ca. of the bladder	—	actinomycin	no
17	1720	64.8	+	ca. of the bladder	+	no	no
18	2150	25.5	+	mamma ca.	+	androgens	no
19	339	23.0	+	mamma ca.	+	prednison	no
20	485	12.8	+	osteosarcoma	—, osteoplastic metastases	no	no
21	398	23.6	—	mamma ca.	+	androgens	yes

¹⁾Elevated 5'-nucleotidase 1 month before.

Fig. 1

Variation, with time, of alkaline phosphatase (o—o) and 5'-nucleotidase (•—•) levels. Patient Ze. Q. The dotted line represents upper normal level of alkaline phosphatase and 5'-nucleotidase. The sign b. f. + indicates, that a bone fraction could be demonstrated

since it resulted in normalization of the osteolytic areas. This was revealed by

- X ray investigation showing recalcification;
- a rise of alkaline phosphatase level in the serum, which was shown electrophoretically to contain bone phosphatase (right arrow).

This graph illustrates that even if alkaline phosphatase level rises to a value 28 times its mean normal level, 5'-nucleotidase can remain in the normal range.

Table 2 shows that in the majority of cases a bone phosphatase fraction is present if 5'-nucleotidase levels are elevated, but to a lesser degree than alkaline phosphatase. A typical illustration (case No. 18) has been given in Figure 2. Patient Ni (fig. 2) and patient Ze (fig. 1) pre-

Fig. 2

Variation, with time, of alkaline phosphatase (o—o) and 5'-nucleotidase (•—•) levels. Patient Ni. Q. The dotted line represents upper normal level of alkaline phosphatase and 5'-nucleotidase. The sign b. f. + indicates, that a bone fraction could be demonstrated

sented similar clinical pictures. Estrogen therapy was stopped because of hypercalcaemia and replaced by prednison therapy. It is interesting to note that amino-

transferases fluctuated closely parallel to the 5'-nucleotidase level during the first month indicated. The fraction from bone could be demonstrated electrophoretically (see arrow) in a serum sample with a 5'-nucleotidase level of 25.5 U/l. A repeated assay of this sample for 5'-nucleotidase but omitting phenylphosphate gave a 5'-nucleotidase value of 45.9 U/l.

There is no indication that the sharp increase of alkaline phosphatase would elevate the level of 5'-nucleotidase. In fact 5'-nucleotidase level starts to rise just when alkaline phosphatase begins to fall.

From this point liver phosphatase may be expected to increase at some time or other. Apparently this increase is not sufficient to neutralize the decrease of alkaline phosphatase to an amount of 1700 U/l within 35 days. In this respect it should be noted that return of increased alkaline phosphatase levels due to bone repair usually takes several months (see for example fig. 5 ref. 11).

In a previous paper (11) we reported evidence that the 5'-nucleotidase level rises before the alkaline phosphatase level if the liver is primarily involved. After studying this phenomenon more extensively in many hundreds of patients the following characteristics of 5'-nucleotidase and alkaline phosphatase can be described: serum 5'-nucleotidase level will rise to a greater degree than the alkaline phosphatase level and before the rise of the alkaline phosphatase level. A typical illustration is given in Figure 3. This figure shows plots of serum alkaline

Fig. 3

Variation, with time, of alkaline phosphatase (o—o) and 5'-nucleotidase (•—•) levels. Patient De. Q. The dotted line represents the upper normal limits

phosphatase and 5'-nucleotidase levels in a patient suffering from M. HODGKIN. Aminotransferase levels were hardly elevated. X ray examination detected no bone metastases. The data of Figure 3 indicate that the 5'-nucleotidase level is increasing before that of alkaline phosphatase.

On the other hand it can occur that the alkaline phosphatase level rises before that of 5'-nucleotidase and even more than the latter. Such a phenomenon occurs in

clear-cut cases of osteoblastic processes such as recalcification of osteolytic areas or the presence of osteoblastic metastases. Two cases will serve as an illustration. Patient M (fig. 4) was suffering from breast carcinoma. The liver was found to be enlarged on palpation three weeks before onset of therapy. Evidence for the recalcification of osteolytic areas was found at the time indicated by arrow A. Subsequently progression of osteolytic areas took place. Note the transient increase of alkaline phosphatase level above that of 5'-nucleotidase during a period in which there was evidence for recalcification. At the time indicated by arrow B a bone phosphatase fraction could not be detected electrophoretically. Aminotransferases rose parallel to the increase of 5'-nucleotidase.

Figure 5 shows serum enzyme levels of a patient suffering from breast carcinoma with osteolytic metastases. At the

Fig. 4

Variation, with time, of alkaline phosphatase (o—o) and 5'-nucleotidase (•—•) levels. Patient M. Q. The dotted line represents the upper normal limits.

Fig. 5

Variation, with time, of alkaline phosphatase (o—o), 5'-nucleotidase (•—•), aspartate (25°, ▲—▲) and alanine (25°, △—△) transaminase levels. Patient S. Q. The dotted lines represent the normal limits of aspartate and alanine transaminase (upper) and alkaline phosphatase and 5'-nucleotidase (lower).

time indicated by arrow A there was progression of the osteolytic metastases. A report of X ray investigation mentions the cessation of progression and recalcification at time B.

Recalcification was confirmed at time C. Evidence for progression of osteolytic metastases in bone and liver indicating the end of the remission phase was found at the time indicated by arrow D. Note that alkaline phosphatase increases above 5'-nucleotidase only during recalcification and that 5'-nucleotidase level rises prior to the level of alkaline phosphatase before therapy started and at the end of the remission phase.

5'-Nucleotidase and transaminases

The sequential course of 5'-nucleotidases and aminotransferases was studied in 50 patients suffering from malignant diseases known to give liver metastases. Care was taken to exclude patients with apparent toxic liver damage as described below. We cannot provide proof that among these patients some may not have an elevated 5'-nucleotidase from a nonmalignant cause, such as biliary obstruction or cirrhosis. Study of the variation in serum enzymes over a period and their response to therapy made the occurrence of non-malignant liver disfunctions improbable. In 39 cases the 5'-nucleotidase level rose prior to the rise of the aspartate aminotransferase level and generally to a much higher value. In 2 cases 5'-nucleotidase and aspartate aminotransferase activity rose equally. Examples have been given in a previous paper (ref. (11), fig. 8, 12 and 13) and in this paper (fig. 5).

Additional information on the response of aspartate aminotransferase in comparison with 5'-nucleotidase was obtained by assembling data from autopsy or surgery reports confirming the existence of liver metastases (tab. 3). The reports were selected at random. Each case in table 3 is used only once in this paper. The highest

level of each serum enzyme mentioned was chosen for use in table 3. This was done because in the final stage transaminases probably tend to fall. Since the table in addition records 5'-nucleotidase and alkaline phosphatase elevations, a fair comparison of transaminases to these enzymes would not be possible if the last values available before death had been given. From the data of table 3 it seems evident that aspartate aminotransferase is a less sensitive parameter for the existence of liver metastases than 5'-nucleotidase, but that omitting aspartate aminotransferase from routine examination is not advisable.

Alanine aminotransferase is a less sensitive monitor of liver metastases, which agrees with data from many reports in the literature (see ref. (11)). The foregoing studies provided a basis for evaluating the significance of assays of 5'-nucleotidase in relation to some other serum enzymes in cases of metastatic liver and bone. Cancer patients however often receive therapy which would be reflected by changes in the pattern of serum enzymes. It seemed therefore interesting to study the response of 5'-nucleotidase to such therapy in relation to other serum enzymes. While these studies were being carried out it became evident that there is no "standard" response to a given therapy. In fact a therapy may be without measurable effect on the levels of any enzyme in one case while effecting a variety of transient rises of serum enzymes in other cases.

Studies were carried with about 40 patients whose serum enzymes showed at least a partial response to therapy. In some cases these patients received several subsequent therapeutic treatments which permitted us to study the effect of possible accumulation of the hepato-toxic effects of serum enzymes. The results can be summarized as follows:

a) If there is a response, the transient rise of 5'-nucleotidase is generally between a few U/l and about 20 U/l.

Tab. 3

Classification of aspartate and alanine aminotransferase, 5'-nucleotidase and alkaline phosphatase in cases of confirmed metastatic liver¹⁾

Case No.	5'-Nucleotidase	Alkaline phosphatase	Aspartate aminotransferase	Alanine aminotransferase	Diagnosis	Metastatic liver confirmed by
1	++++	+	++++	+	mamma ca.	autopsy
2	+	±	++++	++	liver ca.	autopsy
3	normal	normal	normal	normal	reticulosarcoma	autopsy
4	+	++++ ²⁾	+++	+	mamma ca.	autopsy
5	++++	++++ ²⁾	++	+	mamma ca.	autopsy
6	++	+++	+	normal	rectum ca.	autopsy
7	+	±	normal	normal	cardia ca.	laparotomy
8	++++	++	+++	+	mamma ca.	autopsy
9	±	±	±	normal	mamma ca.	laparotomy
10	++	+++ ³⁾	+++	+	mamma ca.	autopsy
11	++	++	+	±	mamma ca.	autopsy
12	+++	+++	++	+	seminoma	liver scintigraphy
13	++	+	+	+	ca. of the bladder	autopsy ⁴⁾
14	normal	+	normal	normal	lung ca.	autopsy ⁵⁾
15	+++	++	+	+	mamma ca.	autopsy
16	+++	++	normal	normal	ovarium ca.	autopsy
17	++	+	+	±	mamma ca.	laparotomy
18	normal	normal	+++	+	parotis ca.	autopsy
19	normal	normal	±	normal	mamma ca.	autopsy ⁶⁾
20	++	±	+	±	mamma ca.	laparotomy ⁷⁾

¹⁾ Notations:

± = between upper limit normal to twice elevated
 + = between twice elevated to 4 × elevated
 ++ = between 4 × elevated to 8 × elevated
 +++ = between 8 × elevated to 16 × elevated
 ++++ = more than 16 times elevated.

²⁾ Recalcification of osteolytic areas (X Ray evidence).

³⁾ Osteoplastic metastases (X Ray evidence).

⁴⁾ A few metastases of ± 0.5 cm.

⁵⁾ One liver metastase of ± 0.3 cm.

⁶⁾ γ-glutamyltranspeptidase = ++.

⁷⁾ γ-glutamyltranspeptidase: +.

Higher elevations seldom occur but can be found e. g. following splenectomy (one case has been mentioned in ref. (3)). Elevations of alkaline phosphatase are of the same order up to the upper normal limit.

b) In contrast alanine aminotransferase and aspartate aminotransferase levels showed a much greater variety in response. Extensive elevations could be found, whereas 5'-nucleotidase and alkaline phosphatase did not show significant alterations.

For 5'-nucleotidase, alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase the length of time from the onset of therapy till the return of serum enzymes to their previous values was approximately equal, i. e. 20–40 days.

Figure 6 describes 4 cases where the response of 5'-nucleotidase, alkaline phosphatase and aminotransferases was weak to moderate. Figure 6a concerns a patient

Fig. 6

Variation, with time, of alkaline phosphatase (○—○), 5'-nucleotidase (●—●), aspartate (35°, ▲—▲) and alanine (35°, △—△) transaminase levels

a = Patient G. ♀ b = Patient Wa. ♂ c = Patient St. ♂ d = Patient K. ♂

The upper dotted line represents upper normal limit of aspartate and alanine transaminase, the lower dotted line those of 5'-nucleotidase and alkaline phosphatase

suffering from M. HODGKIN who had been treated by splenectomy (left arrow). During splenectomy a biopsy was taken from the liver which did not reveal the presence of any metastatic tissue. At the time indicated by the right arrow the patient received intravenous chemotherapy involving vincristine, natulan and prednison; alanine aminotransferase only shows a clear reaction after the second treatment. Figure 6b concerns a patient suffering from a carcinoma of the stomach. Examination of the liver during partial gastrectomy (arrow) failed to detect the presence of metastases.

Figure 6c describes the variation of the serum enzymes following surgical therapy of lung metastases from

a testis tumour. Two days after the resection aspartate aminotransferase value rose to 58 U/l while alanine aminotransferase was only reached the upper normal value. Figure 6d shows the levels of serum enzymes of a patient with liposarcoma in the leg, who received intravenous injections of both vincristine and actinomycin (see arrows). It would appear that alanine aminotransferase attains higher values than aspartate aminotransferase. Additional evidence is presented in figures 7,

Fig. 7

Variation, with time, of alkaline phosphatase (○—○), 5'-nucleotidase (●—●), aspartate (35°, ▲—▲) and alanine (35°, △—△) transaminase levels

a = Patient R. ♂ b = Patient Z. ♀

The upper dotted line represents upper normal limit of aspartate and alanine transaminase, the lower dotted line those of 5'-nucleotidase and alkaline phosphatase

Fig. 8

Changes of 5'-nucleotidase (●—●), alkaline phosphatase (○—○), aspartate (35°, ▲—▲) and alanine (35°, △—△) transaminase levels during several courses of intermittent metothrexate treatment. Patient Be. ♀

The dotted lines represent the upper normal limits

Fig. 9

Variation, with time, of aspartate (35°, ▲—▲) and alanine (35°, △—△) transaminase (top). The dotted line represents the upper normal limits. Variation, with time, of alkaline phosphatase (o—o), 5'-nucleotidase (•—•) and glutamate dehydrogenase (x—x) (bottom). The upper dotted line represents the upper normal limits of alkaline phosphatase and 5'-nucleotidase, the lower dotted line the upper normal limit of glutamate dehydrogenase. Patient No. ♀

8 and 9. Patient R (fig. 7a) was suffering from a tumour in the lower jaw. A intraarterial catheter (for infusion therapy) was placed during general anaesthesia.

Figure 7a shows changes in the level of enzymes in the blood as a response to infusions of ^3H -thymidine and metothrexate at the time indicated by the arrow, plus metothrexate over 7 days. Patient Z. (fig. 7b) received five injections of androgens. The treatment was stopped because of clinical evidence of toxicity. Ovarectomy was performed two months before, when examination of the liver did not reveal the presence of metastases. Note the weak response of 5'-nucleotidase in both cases.

Figure 8 is an example of variations of serum enzymes during intermittent courses of therapy. Note the variety in response of the transaminases and the weak response of 5'-nucleotidase and alkaline phosphatase. This figure concerns a patient suffering from chorionepithelioma. An interesting illustration of the weak response of 5'-

nucleotidase and alkaline phosphatase to toxic effects of therapy as compared to aspartate aminotransferase and alanine aminotransferase is given in Figure 9. This figure concerns a patient suffering from melanoma. At the time indicated by the arrow inguinal lymph nodes were excised under general anesthesia using halothane (fluothane). The pronounced response of aspartate aminotransferase, alanine aminotransferase and glutamate dehydrogenase immediately after surgery is apparently a hypersensitivity reaction to this hepatotoxin, which is known to cause histological changes in a few cases indistinguishable from viral hepatitis (12). The serum enzyme with the highest response was aspartate aminotransferase (3000 U/l, 35°). It will be noted that the 5'-nucleotidase level did not rise more than 12 U/l at most. Note that glutamate dehydrogenase, a liver specific enzyme, increases to about twenty times the normal limit. Nineteen days later the patient received prednison therapy which evoked again a small rise of 5'-nucleotidase. It would appear that the response of the aminotransferases to therapy is overshadowed by their response to halothane. It is of interest to note that serum bilirubin did increase to 170 mg/l (283 $\mu\text{mol/l}$) at the 19th day after surgery. Fever was noted during the five postoperative days. The data of the other cases are illustrated in table 4. The greatest changes of serum enzymes as a response to therapy are classified, using the same symbols as in table 3.

Some responses of alkaline phosphatase are not given since their rapid change, caused by a process of recalcification, overshadowed any change due to toxicity.

Discussion

The serum enzymes studied in this investigation are characterised by a variety of response to changes in liver due to malignant growth or therapy. It is therefore of great importance to know what kind of a change in a serum enzyme level each of these two factors may produce if laboratory data are used for evaluation of the therapy applied. Such knowledge can be made complete only if the results of consecutive assays of sera enzymes for a given period are studied in relation to clinical data from the patients involved. Reliable results are not obtained by plotting levels of one enzyme against levels of a second enzyme for a number of patients and concluding which enzyme corresponds to the greatest number of plots on a side of the tangent. This procedure, which masks potential-hepatotoxic effects, is occasionally followed (see e. g. ref. (13)). One conclusion to be drawn from our data is that application of phenylphosphate in the assay of 5'-nucleotidase in sera of cancer patients leads to 5'-nucleotidase values unaffected even at high levels of bone phosphatase. Further evidence for this phenomenon presented here and in ref. (11) combined with the data about the presence of bone phosphatase in cases of elevated alkaline phosphatase (tab. 1, 2; fig. 1, 2 and 4) allow the following conclusions to be drawn in the case of patients suffering from malignant disease known to affect bone.

Tab. 4

Classification of elevations of alkaline phosphatase, 5'-nucleotidase, aspartate and alanine aminotransferase as a response to therapy. For explanation of symbols see table 3. Thus an elevation of 15 U/l for aspartate aminotransferase and 5'-nucleotidase is noted as normal and $\pm$ respectively. If no change occurred this has been indicated by "no"

Case No.	Diagnosis	5'-Nucleotidase	Alkaline phosphatase	Aspartate aminotransferase	Alanine aminotransferase	Therapy	Period of response (days)
1	ca. of tongue	no	no	normal	normal	surgery	15
2	ca. of testis	normal	no	$\pm$	++	actinomycin	7
3	mamma ca.	no	no	$\pm$	++	metothrexate	?
4	ca. of testis	+	normal	++	+++	radiotherapy	60
5	mamma ca.	normal	normal	normal	normal	estrogens	15
6	mamma ca.	$\pm$	normal	normal	$\pm$	surgery	17
7	M. HODGKIN	normal	normal	normal	$\pm$	bleiomycin	21
8	chorionepithelioma	no	normal	$\pm$	$\pm$	metothrexate	6
9	ca. of nasal cavity	no	no	$\pm$	++	metothrexate	?
10	mamma ca.	$\pm$	normal	+++	+++	surgery	?
11	ca. of the jaw	no	no	++	+++	metothrexate	23
12	ca. of tongue	normal	normal	$\pm$	++	metothrexate	24
13	ca. of the bladder	no	no	+++	+++	surgery	?
14	mamma ca.	normal	normal	normal	normal	endoxan	?
15	ca. of oesophagus	normal	normal	$\pm$	+	radiotherapy	24
16	ca. of antrum	normal	normal	+	++	metothrexate	16
17	ca. of the cheek	no	no	+	++	5-fluoruracil	18
18	M. HODGKIN	+	+	++	++	natulan	15
19	M. HODGKIN	+	$\pm$	+++	+++	radiotherapy	60
20	ca. of the cheek	normal	normal	$\pm$	$\pm$	5-fluoruracil	13
21	chorionepithelioma	++	normal	+	$\pm$	metothrexate	15
22	chorionepithelioma	normal	no	++	+++	metothrexate	13
23	melanoma	$\pm$	normal	normal	+	surgery	30
24	mamma ca.	+	$\pm$	normal	normal	estrogen	30
25	chorionepithelioma	$\pm$	normal	$\pm$	$\pm$	actinomycin	12
26	chorionepithelioma	normal	no	$\pm$	+	metothrexate	11
27	mamma ca.	$\pm$	—	+	+	estrogen	50
28	M. HODGKIN	normal	—	normal	normal	surgery	22
29	reticulosarcoma	normal	normal	+++	+++	metothrexate	12
30	ca. of the bladder	$\pm$	+	+++	+++	surgery	23
31	ca. of the jaw	normal	no	++	++	metothrexate	21
32	palatum ca.	normal	normal	+++	+++	metothrexate	19
33	mamma ca.	no	no	+	$\pm$	radiotherapy	?
34	mamma ca.	$\pm$	—	normal	++	radiotherapy	60
35	ca. of the cheek	normal	no	normal	+	metothrexate	12
36	chorionepithelioma	normal	normal	+	++	surgery	13

a) If a patient has an elevated alkaline phosphatase activity and a normal 5'-nucleotidase activity (measured in the presence of phenylphosphate) the amount of elevated alkaline phosphatase consists entirely of bone phosphatase.

b) If both alkaline phosphatase and 5'-nucleotidase are elevated, but alkaline phosphatase to a greater extent, bone phosphatase is present in the serum.

c) If alkaline phosphatase and 5'-nucleotidase are elevated, but alkaline phosphatase to a lesser degree, bone phosphatase may be present in a number of cases. A statement can be made only after a separate electrophoretic investigation.

The clinical significance of assays of 5'-nucleotidase, alkaline phosphatase and aminotransferases in cancer patients can be summarized as follows:

The 5'-nucleotidase level is a useful parameter for the existence of liver metastases. Consecutive assays enable one to evaluate the effect of therapy on liver metastases even if therapy is prolonged.

Increases of 5'-nucleotidase as a response to therapy can easily be detected, since they are small and accompanied by an increase of alanine aminotransferase that is in many cases much stronger. A normal 5'-nucleotidase value does not exclude the existence of liver metastases, which however can be ascertained with a higher degree of sensitivity by determining 5'-nucleotidase in combination with aspartate aminotransferase.

Consecutive alkaline phosphatase assays inform us about the course of osteoblastic processes even during hepa-

totoxic therapy. Using alkaline phosphatase/5'-nucleotidase ratios statements can be made about the presence of bone phosphatase in serum. Alanine aminotransferase is a useful monitor of the hepatotoxic effects of therapy especially if it is more elevated than aspartate aminotransferase.

The reverse (aspartate aminotransferase elevated more than alanine aminotransferase) can be an indicator of metastatic liver or may be a sign of hepatotoxic liver damage. A higher increase of aspartate aminotransferase compared to alanine aminotransferase could also be explained by more widespread tissue damage including the liver, such as may occur in the case of surgery. Nevertheless alanine aminotransferase and aspartate aminotransferase are useful parameters detecting early toxic effects from therapy or adapting therapy during its course to the condition of the liver of the patient.

For a discussion of data about 5'-nucleotidase and serum transaminase in the literature the reader should consult ref. (11). On the basis of evidence presented there and in this paper we propose to divide serum enzymes into three groups according to their clinical value as a liver test. Although the number of serum enzymes used as a liver test is not large, we consider a classification useful for clinical evaluation.

Group I Serum enzymes which are a reliable monitor for the existence of liver metastases, since their values rise little or not at all in cases of toxic liver damage (5'-nucleotidase, alkaline phosphatase).

Group II Serum enzymes which may increase considerably both in cases of liver metastases and toxic damage

of the liver. An example of this group is aspartate aminotransferase.

Group III Serum enzymes which react mainly to hepatotoxic therapy and which are of little or no diagnostic value in cases of metastatic liver. An example is alanine aminotransferase.

Preliminary investigations in our laboratories have produced evidence that serum γ -glutamyl-transpeptidase and glutamate dehydrogenase might belong to group II. If so, this means that γ -glutamyl-transpeptidase could replace aspartate aminotransferase, but not 5'-nucleotidase as has been suggested (13). Further investigations are in progress.

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