Elevation of a Serum Component in Neoplastic Disease

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Although a number of enzymes have been demonstrated in blood serum, only a few have been shown to have a consistent relationship to neoplastic disease. Warburg and Christian (10) observed an elevation of aldolase activity in the serum of tumor-bearing rats. Sibley and Lehninger (7, 8) confirmed this observation with an independent analytical technic, and although they found the aldolase activity regularly elevated in rats with neoplastic growths it was elevated in only 20 per cent of the sera of human cancer patients tested. The clinical usefulness of the determination of serum alkaline phosphatase in osteogenic sarcoma (3) and of serum acid phosphatase in disseminated prostatic carcinoma (5) suggests the possibility that other types of tumors may yield specific catalytic constituents. The detection and quantitative measurement of such specific blood components would be of practical value, not only for diagnostic purposes, but for insight into the etiology and growth of cancer.

The present report deals with the investigation of a serum component which catalyzes the transfer of hydrogen from reduced diphosphopyridine nucleotide to pyruvic acid. This catalytic component has been found to be elevated in neoplastic disease.

EXPERIMENTAL

It was found that the addition of blood serum to a buffered mixture of pyruvic acid and reduced diphosphopyridine nucleotide (DPN · H_2) resulted in an oxidation of the latter, which was slow when serum from an apparently healthy individual was added but greatly accelerated by serum from an individual with neoplastic disease. To study the activity in various sera it was necessary to formulate an accurate, reproducible assay for its quantitation. A method has been devised which fulfills the requirements for accuracy and reproducibility.

The following solutions were utilized for the assay: 0.1 M pH 7.8 phosphate buffer, 0.01 M sodium pyruvate in 0.1 M pH 7.8 phosphate buffer, a solution of 1 mg. DPN · H_2 (Sigma Chemical Co., St. Louis, Mo.) per milliliter distilled water (prepared just before use), 0.1 M sodium bicarbonate solution, and blood serum diluted in the ratio of 1 part of serum to 9 parts of water (prepared just before use). The assay solution was prepared daily in the following proportion of constituents: 1.0 ml. sodium pyruvate solution, 25 ml. phosphate buffer, 1.0 ml. sodium bicarbonate solution, and 3.0 ml. DPN · H_2 solution.

The rate of oxidation of DPN · H_2 was determined by the decrease in optical density at 340 mμ in 30 minutes at 37.5° C. The Beckman Model B spectrophotometer is well adapted for this assay.

Into four cuvettes the following were pipetted: cuvette #1 (control), 0.1 ml. of distilled water and 3.0 ml. of assay solution; cuvettes #2, 3, 4, 0.1 ml. of diluted sera and 3.0 ml. of assay solution. Each was stirred immediately, and the control cuvette was used to set the spectrophotometer at 0.500 optical density. The 0 min. optical densities of cuvettes #2, 3, and 4 were then recorded. It was important that the 0 time reading be made immediately after the addition of assay solution to each cuvette, because of the high activity of some cancer sera. The cuvettes were then incubated at 37.5° C. for 30 min., and solutions #2, 3, and 4 were again read with the control cuvette set at 0.500 optical density. The Δ optical density was calculated as follows: Δ optical density = optical density at 0 min. − optical density at 30 min. If the reaction had been greatly accelerated, the optical density reading occasionally dropped below zero. In this case the slit width was readjusted so that the control cuvette read 1.00. The sample was then read; this reading was subtracted from the initial (0 time) reading, and 0.500 was added to the difference. The calculation for Δ optical density in such assays was Δ optical density = optical density at 0 min. − optical density 30 min. + 0.500.

The serum to be assayed must be free from hemolysis, since hemolyzed red cells cause an increase in the rate of oxidation of DPN · H_2. The presence of fat globules or a high icteric index have no significant effect. However, a high icteric index can mask hemolysis. No attempt was made to control conditions under which blood was drawn. If

Received for publication March 27, 1954.

the serum could not be assayed immediately it was frozen and assayed within 3 days. Loss of activity was insignificant if undiluted serum was kept at 4° C. for 3 days or in the frozen state for 10 days.

In Chart 1 are the Δ optical density values for a number of assays determined by this method. Repeat assays were run on many of these sera the following day with excellent reproducibility. Race, age, or sex produced no observable influence on the Δ optical density. The miscellaneous diseases and the number of patients with each pathologic condition are as follows: arthritis, 1; peptic ulcer and pancreatitis, 1; amebiasis, 1; pneumonia, 1; chron-

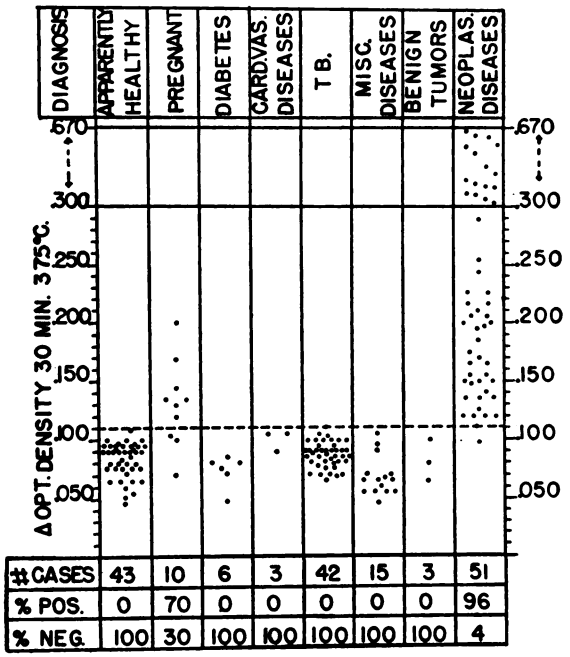

CHART 1.—Serum component activity in malignant and nonmalignant disease.

ic brucellosis, 2; glomerulo-nephritis and uremia, 2; bronchiectasis, 2; psoriasis, 1; fever of unknown origin, 1; duodenal ulcer, 2; and paraplegia (traumatic), 1. In all cases of neoplastic disease the diagnosis was confirmed by biopsy.

In this limited study no attempt was made to correlate the period of gestation with the activity of sera from pregnant individuals; however, a comprehensive study of activity in pregnancy is contemplated.

To determine the fate of hydrogen lost from DPN · H_2 on oxidation, pyruvic and lactic acid determinations were made on the assay mixtures at 0 min. and after 60 min. incubation at 37.5° C. The reactions were stopped by addition of freshly prepared trichloroacetic acid. Pyruvic acid was determined by the direct method of Friedemann and Haugen (4). Lactic acid was determined by the method of Barker and Summerson (1). With serum from patients with neoplastic disease the pyruvate which disappeared was accounted for quantitatively by the formation of lactate. Similar results were obtained with serum from apparently healthy individuals and with crystalline lactic dehydrogenase (LDH) (Worthington Biochemical Sales Co., Freehold, N.J.) from rabbit muscle (Table 1). To determine the per cent conversion of pyruvate to lactate, it was necessary to incubate for 60 min. so that sufficient lactate would be formed to permit determinations within the limits of measurement of the lactate method. In the cases of LDH and one cancer serum the reaction went to equilibrium in less than 60 minutes, DPN·H_2

TABLE 1

CONVERSION OF PYRUVATE TO LACTATE

The test solutions contained the following ratio of constituents: 1×10^{-3} mM sodium pyruvate, 0.1 ml. serum (1 : 10 dilution) or 1.7 μg. lactic dehydrogenase (LDH), 3.72×10^{-4} mM DPN·H_2 (0.3 mg. 82.5 per cent pure DPN·H_2). Incubated 60 min. at 37.5° C.

	Pyruvate disappearance (mM × 10^{-4})	Lactate formed (mM × 10^{-4})	Per cent conversion pyruvate to lactate
Normal serum	1.72	1.70	99
Normal serum	1.40	1.30	93
Cancer serum	3.68	3.60	98
Cancer serum	3.36	3.40	101
Pregnancy serum	2.40	2.54	106
Crystalline LDH	3.68	3.68	100

being the limiting factor. Therefore, the relation between pyruvate disappearance or lactate appearance of the various sera as shown in Table 1 does not represent their relative activities.

Other compounds were tested for their ability to replace pyruvate as the hydrogen acceptor in the presence of the serum component and crystalline LDH (1.7 μg/test). Under the conditions of the assay, 0.1 ml. of 0.01 M solutions of D,L-glyceraldehyde, α-ketoglutaric and α-ketobutyric acids, cystine, and blue tetrazolium did not act as hydrogen acceptors. Oxalacetic acid acted as a hydrogen acceptor with about 25 per cent of the activity of pyruvic acid. However, spontaneous decarboxylation of the oxalacetic acid to form pyruvic acid appeared to account for this, since lactate was found after incubation. Malic acid, the product of reduction of oxalacetic acid, did not give the lactate test in the concentration in which it would have been present in the experiment.

DISCUSSION

It should be emphasized that this survey is of a preliminary nature, and the number of individuals tested with each particular disease is, in most

cases, insufficient. Also, there are many diseases which have not been investigated.

In over half of the sera assayed the diagnosis of the individual was unknown until the assay was completed. In a number of cases of cancer the elevated value of the serum was noted before the suspected diagnosis was confirmed by biopsy. In all cases of benign tumors the assays were run before biopsy and later confirmed.

In assays run to date the Δ optical density values have been greater than 0.110 for 96 per cent of the cancer sera tested. Of the two sera which did not show increased activity, one was a mucous-cell adenocarcinoma of the stomach (Δ optical density value of 0.110) and the other a mucous-cell adenocarcinoma of the sigmoid colon (Δ optical density of 0.097). The latter was not confirmed by reassay because of removal of the tumor by surgery. To date there have been no values above Δ optical density 0.110 in sera from noncancerous individuals except in pregnancy.

The authors have failed to find any report of a study of lactic dehydrogenase activity in human blood serum, although the enzyme has been reported in red cells and serum of the rabbit (9), in human blood cells in anemia (2), and in red cells of the monkey (6).

Methods for isolation and identification of the serum component and for comparing this material with lactic dehydrogenase and the red cell component are in preparation.

SUMMARY

1. A procedure has been formulated for the assay of a serum component which catalyzes the oxidation of reduced diphosphopyridine nucleotide and the simultaneous reduction of pyruvic acid to lactic acid.

2. The activity of the sera producing this oxidation-reduction change has been found to be low in healthy individuals and in patients with a number of pathological conditions. It is elevated in neoplastic disease and in pregnancy.

ACKNOWLEDGMENTS

The authors wish to express their appreciation and indebtedness to Dr. Riojun Kinosita for his help and encouragement as well as for his efforts to facilitate this work.

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