Blood Pyruvate in Malignant Neoplastic Disorders

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A moderate and statistically significant elevation of blood pyruvate levels, determined by an enzymatic method, was found in 60 patients with various malignant neoplastic disorders as compared with 20 control subjects. Studied were 42 cases of metastatic carcinoma, including 14 in which there were hepatic metastases and 18 cases of leukemia and lymphoma. No significant statistical difference was found when the values for the patients with malignant disease were compared with those found for a group of patients with non-neoplastic liver disorders.

Pyruvic acid is an important intermediary product in the metabolism of carbohydrates just as it is in the metabolism of proteins and fats. Increased blood pyruvate levels are reported to occur in a number of disorders including liver disease, congestive heart failure, diabetes mellitus, thiamine deficiency, drug intoxications, and neoplastic conditions (3, 9, 15-17). The present study was primarily undertaken to investigate blood pyruvate levels in malignant disorders with or without hepatic involvement.

Until recently, methods commonly used for determination of pyruvic acid were based on the formation of a hydrazone with 2,4-dinitro-phenyl-hydrazine (6,14). This reaction is by no means specific for pyruvic acid. The presence of other keto acids such as acetoacetic, α-ketoglutaric, oxaloacetic, and levulinic is a common source of error (4, 10).

The chromatographic methods (5,12), although more specific, appear too complicated and laborious for routine use.

A specific method based upon conversion of pyruvate to lactate by lactic dehydrogenase (LDH) in the presence of reduced nicotinamide-adenine dinucleotide (NADH), has been increasing in use in recent years (13). This enzymatic method was found to yield 30-40% lower results than the hydrazone method (1). The reaction is catalyzed by LDH and the equilibrium is strongly in the direction of pyruvate-to-

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lactate. NADH has a maximum absorbance at 340 m\(\mu\), and since its oxidation or decrease in absorbance at 340 m\(\mu\) is stoichiometrically related to the conversion of pyruvate to lactate, the concentration of pyruvate may be easily calculated.

\[
\text{CH}_3\text{CO-COOH} + \text{NAD} + \text{H}^+ \xrightarrow{\text{LDH}} \text{CH}_3\text{CHOH-COOH} + \text{NAD}^+ 
\]

Gloster and Harris (7) found that only 75% of added pyruvate is recovered in perchloric acid filtrates of whole blood, whereas 97% is recovered when trichloracetic acid filtrates are used. This result was attributed to incomplete inactivation of LDH in the red cells by perchloric acid. However, recoveries as high as 85–97% were obtained by Henry et al. (8) in perchloric acid filtrates.

**Method**

The method described by Gloster and Harris (7) was used in the present study.

**Collection of Blood**

All blood samples were obtained from fasting subjects at rest. Approximately 6 ml. of blood was collected by venipuncture in a heparinized Vacutainer* and immediately transferred to a 5-ml. calibrated Krogh-type syringe pipet. Great care was exercised in transferring the blood from the "Vacutainer" to the syringe immediately, avoiding the entry of air bubbles. The tourniquet was not applied more than 1 min.; the blood was immediately injected in a test tube containing 5 ml. of trichloracetic acid (TCA) solution, kept at 0°, and mixed well.

**Materials**

The control group consisted of 20 healthy subjects whose ages ranged from 22 to 77 years (8 males and 12 females). In order to determine the individual variations, 3 blood samples at 45-min. intervals were collected from 8 additional healthy subjects, ages 22–40 years (5 males and 3 females).

A total of 84 patients was studied. A single blood sample was obtained from each. For all patients the diagnosis was established clinically and histologically. Each of them appeared to suffer from only one condition known to alter the blood pyruvate level. The patients were classified as follows.

Group 1 consisted of 14 patients (5 males and 9 females)—10 with cholecystitis and cholecystolithiasis and 4 with cholecystitis and cholelithiasis. Ages ranged from 37 to 80 years.

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*Becton, Dickinson and Company, Rutherford, N. J.
Group 2 consisted of 10 patients (5 males and 5 females) with non-neoplastic hepatic parenchymal disorders—6 with cirrhosis, 2 with acute viral hepatitis, 1 with chronic hepatitis, and 1 with unclassified granulomatous lesions of the liver. Ages ranged from 20 to 58 years.

Group 3 consisted of 14 patients (7 males and 7 females) with metastatic carcinoma and metastatic disease of the liver—4 with pancreatic adenocarcinoma, 3 with colonic adenocarcinoma, 2 with bronchogenic carcinoma, 1 with mammary ductal carcinoma, 1 with malignant melanoma, 1 with adenocarcinoma of the gallbladder, 1 with adenocarcinoma of the cervix, and 1 with squamous cell carcinoma of the bladder. Ages ranged from 37 to 94 years.

Group 4 consisted of 28 patients (10 males and 18 females) with metastatic carcinoma and no conclusive laboratory (enzyme studies, liver function tests, and liver scans) or histologic (liver biopsy or postmortem examination) evidence of metastatic liver disease—3 with ovarian cystadenocarcinoma, 3 with mammary ductal carcinoma, 2 with bronchogenic carcinoma, 2 with pancreatic adenocarcinoma, 2 with carcinoma of the thyroid, 2 with colonic adenocarcinoma, 2 with renal adenocarcinoma, 2 with unclassified adenocarcinoma, 3 with squamous cell carcinomas of mouth, esophagus, and vulva, 1 with gastric adenocarcinoma, 1 with prostatic adenocarcinoma, 1 with uterine adenocarcinoma, 1 with uterine stromal sarcoma, and 1 with malignant melanoma. One patient with chondrosarcoma and 1 with neurofibrosarcoma, both with significant local spread, were also included in this group. Ages ranged from 20 to 77 years.

Group 5 consisted of 18 patients (7 males and 11 females) with various types of leukemia or lymphoma—2 with chronic lymphocytic leukemia, 2 with acute myelogenous leukemia, 1 with subacute myelogenous leukemia, 1 with acute lymphoblastic leukemia, and 1 with chronic myelogenous leukemia. Five patients with Hodgkin's disease, 4 with reticulum cell lymphosarcoma, and 2 with lymphoblastic lymphosarcoma were also included in this group. Ages ranged from 25 to 73 years.

Results

In the control group the fasting blood pyruvate levels ranged from 0.40 to 0.90 mg. of pyruvic acid per 100 ml., with a mean value of 0.65 (1 S.D. = 0.12).

In 8 additional control subjects, 3 determinations at 45-min. intervals were performed. Individual variations of up to 33% were noted. However, all determinations gave results within the above range (0.40–0.90 mg./100 ml.).
The recovery of added lithium pyruvate in trichloracetic acid filtrates ranged from 89.9% to 92.3%.

In Group 1, the values ranged from 0.45 to 1.10 mg./100 ml. (Fig. 1) with a mean of 0.74 (1 S.D. = 0.17).

In Group 2, the values ranged from 0.50 to 1.10 mg./100 ml. (Fig. 1) with a mean of 0.88 (1 S.D. = 0.23).

In Group 3, the values ranged from 0.50 to 1.60 mg./100 ml. (Fig. 2) with a mean of 1.05 (1 S.D. = 0.28).

In Group 4, the values ranged from 0.60 to 1.40 mg./100 ml. (Fig. 2) with a mean of 0.92 (1 S.D. = 0.28).

In Group 5, the values ranged from 0.60 to 2.40 mg./100 ml. (Fig. 2) with a mean of 1.03 (1 S.D. = 0.44).
All the standard deviations in this study were computed from linear (not logarithmic) values. The \( t^* \) test was used for statistical analysis of the results obtained in all groups.

When compared with results for the control group (Fig. 3), the only statistically significant increases in blood pyruvate levels were noted in groups of patients with non-neoplastic liver disorders, metastatic carcinoma, metastatic carcinoma with liver involvement, and leukemia or lymphoma. The \( t \) values were 3.8, 4.05, 5.5, and 3.8, respectively (\( p < .001 \)).

Patients with cholecystitis and cholelithiasis showed no statistically significant increase when compared with the control group. The \( t \) value was 1.8.

There was no statistically significant difference between malignancy groups and the group with non-neoplastic liver disorders. When compared with results for the group with non-neoplastic liver disorders, the \( t \) values in groups with metastatic carcinoma, metastatic carcinoma with liver involvement, and leukemia or lymphoma were .42, 1.7, and 1.01, respectively.

\[ t = \frac{m_1 - m_2}{\sqrt{\left(\frac{\sum d_1^2 + \sum d_2^2}{n_1 + n_2 - 2}\right) \times \left(\frac{1}{n_1} + \frac{1}{n_2}\right)}} \]

*\( t \) values were derived from:

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**Fig. 3.** Variations of blood pyruvate level and mean values (M) for control and study groups.
Discussion

Both muscular exercise and oral or intravenous feeding are known to alter the blood pyruvate levels \((8, 11)\). Therefore, all determinations were done with the subjects fasting and at rest.

A decrease in blood pyruvate concentration of 15\% during the first 4 min. after blood withdrawal, due to the action of circulating LDH, has been reported \((7)\). In this study the time elapsed between the collection of blood and adding of TCA was kept under 30 sec.

The recovery of added pyruvate in trichloracetic acid filtrates has been reported to be as high as 97\% \((7)\). In our study the recovery ranged from 89.9\% to 92.3\%.

Venous stasis of up to 2 min. does not alter the blood pyruvate levels \((13)\). In this study the venous stasis caused by the application of the tourniquet did not exceed 1 min.

Because of the rather significant variations of normal levels, even with the enzymatic method, it has been suggested that the tentative normal limits be accepted as 0.30–0.90 mg./100 ml. with this method \((8)\). Other nonspecific methods may give higher results because of the interference of other keto acids.

In the same individual while fasting and at rest, significant variations have also been demonstrated. After serial determinations in 14 control subjects, Segal and Blair \((13)\) reported variations of up to 18\%. In our series they ranged from 4.5\% to 33\%.

Tsirimbis and Stich in 1960 reported elevated serum and blood pyruvate levels in 41 patients with malignant tumors \((16)\). Subsequently they studied 10 cases of leukemias and found significantly elevated serum and blood pyruvate levels \((17)\). There was no correlation between the white cell count or number of blast cells and blood pyruvate levels.

There seems to be no clear explanation for this elevation at the present time. In both cancer and leukemic cells, aerobic glycolysis seems predominant \((2)\). However, blood lactic acid levels appear increased in cancer cells and decreased in leukemic cells \((17)\). Beck \((2)\) has reported a decrease in activity of various glycolytic enzymes, including LDH, in leukemic cells. On the other hand, plasma LDH activity has been found to be elevated frequently in leukemia and cancer, probably due to the increased turnover of neoplastic cells \((17)\).

The present study indicates a frequent and statistically significant elevation of blood pyruvate levels in leukemias, lymphomas, and malignant tumors. Because of the wide range of normal variations and the fact that blood pyruvate levels are often only moderately elevated in
various disease states (including neoplastic disorders), some overlapping between normal values and those found in disease states is present.

References