ANNALS OF CLINICAL AND LABORATORY SCIENCE, Vol. 7, No. 5

Clinical Significance of the Isomorphic Pattern of the Isoenzymes of Serum Lactate Dehydrogenase

DAVID S. JACOBS, M.D.,* ROBERT A. ROBINSON, M.D.,† GARY M. CLARK, Ph.D.,‡ and JERALD M. TUCKER, B.S.*

*Providence-St. Margaret Health Center, Kansas City, KS 66112
and
†University of Missouri-Kansas City School of Medicine, Kansas City, MO 64141
and
‡University of Kansas Medical Center College of Health Sciences, Kansas City, KS 66103

ABSTRACT

Five hundred patients with the isomorphic pattern of the isoenzymes of serum lactate dehydrogenase (LDH) were surveyed. The isomorphic pattern of LDH isoenzymes is defined as a significant increase of total LDH with normal or low percentage of individual fractions, but with the $LDH_{1:2}$ ratio less than unity. Diagnoses were, in descending order of frequency, cardiorespiratory diseases, malignancy, fracture, diseases of the central nervous system, infection/inflammation, hepatic cirrhosis and/or alcoholism, trauma without fracture, infectious mononucleosis, hypothyroidism, uremia, necrosis, pseudomononucleosis, viremia and intestinal obstruction. Incidence of increased serum activity in individuals without evidence of disease or drug explanation was 3 percent. Low PaO_2 was observed in 88 percent of the 67 patients in whom it was measured.

Introduction

Serum of some patients with neoplastic disease has been known to exhibit an elevation of lactate dehydrogenase (LDH).[4,17,24] Such increases bear correlation with dissemination[36] and may fall following effective treatment.[4,7,21,29,39] Certain nonneoplastic disorders, including cardiovascular disease, cause serum total LDH to increase. Thus, although cancer patients as a group have higher LDH levels than normals, LDH is not cancer selective.[4,18,37] LDH is among a group of glycolytic enzymes which have been called ubiquitous.[6,29] LDH has been found to be higher in children than in adults, suggesting a relationship between LDH activity and growth.[4,10,11,16]

As techniques for identification of isoenzymes developed and improved, serum LDH estimations became enormously more useful. The clinical significance of the isoenzymes of LDH became

apparent when alterations of serum LDH patterns in acute infarct of myocardium were demonstrated.[35] With recognition of elevations of LDH_1 and inversion of the $LDH_{1:2}$ ratio, seen in destruction of myocardium, megaloblastic and hemolytic anemias, and of LDH_5 elevations occurring in liver and striated muscle damage, attention was turned to other patterns. The isomorphic pattern was subsequently described. Cohen et al, using agar gel, discussed diseases with elevated serum LDH levels and normal LDH isoenzyme distributions.[9] Instances were cited of metastatic carcinoma, polycythemia vera, secondary erythrocytosis, reticulum cell sarcoma, myeloma and hypothyroidism.[8]

Wright and coworkers discussed 30 patients with a variety of malignant diseases, characterized by an absolute increase of all fractions in LDH separations and relative increases in LDH_3.[40] An absolute increase in all LDH isoenzyme fractions was found in patients suffering from shock. However, these investigators, reporting in the middle 1960's with relatively small numbers of cases, did not indicate the minimum total level of LDH necessary for definition of the isomorphic pattern. Neither did Zondag and Klein, who used agar gel electrophoresis to study the sera of over 4,000 patients, including 474 cases of malignancy.[44] They reported a 'malignant pattern' in 44 instances of nonmalignant conditions, including infectious mononucleosis, acute infections, acute intoxications, extensive tissue necrosis, pulmonary embolism and hypocalcemia. Galen and Gambino, writing with particular reference to myocardial infarction, found 40 percent of their LDH patterns to be other than $LDH_{1:2}$ inversion or LDH_5 elevation.[13]

The present investigation was designed to study the clinical significance of the isomorphic distribution of LDH isoenzymes and to ascertain relationships, if any, with several other laboratory tests. By learning the types of diseases giving rise to the isomorphic pattern the present authors hoped to discover avenues for subsequent studies with appropriate control groups.

Materials and Methods

Clinical Chemistry

Total LDH was obtained from an SMA 12/60™ utilizing a 340 nm channel and a modified Wacker procedure.* Normal range of total LDH for adults by this method is usually considered to be 100 to 225 U per liter. However, in our evaluation of normal, based on 96 samples from 69 healthy adults, the mean and standard deviation were 156 and 22 U per liter, respectively, giving a normal range ($\overline{X} \pm 2$ S.D.) of 112 to 200 U per liter. A higher normal limit for infants and children diminishes to adult norms at puberty.

Electrophoretic separation was carried out on agarose plates. Identification of the LDH isoenzyme activities is based upon the conversion of lactate to pyruvate, coupled to reduction of the dye, formazan. Quantitation is achieved by estimation of the amount of formazan produced. The method is a modification of the Pfizer system.† Several modifications were introduced. After electrophoresis for 50 minutes at 90V, 14 m.a., the membrane was floated on color reagent, agarose side down, with care that the membrane was fully coated. Fixation in 10 percent acetic acid and methanol following incubation was not used; instead, three rinses of deionized water for 10 minutes each were utilized. All agarose membranes were scanned densitometrically at 520 nm and peak areas cal-

* Technicon Method No. SF4-0021F, Technicon Instruments Corporation, Tarrytown, NY.

† Pfizer Diagnostics Divsion, New York, NY.

culated from automatic integration. An LDH_5 fraction is consistently generated from the method. No serum was frozen during any part of the study.

Total LDH and its isoenzymes were run upon the sera of 22 normal females and 20 normal males. Normal limits for each isoenzyme fraction based on percentages were established. The normal range is given as the mean plus or minus 2 S.D. Normal ranges for total LDH and each isoenzyme are expressed in table I. By definition, patterns with elevation of any fraction above its limits of normal or with inversion of the $LDH_{1:2}$ ratio excludes the pattern as one having an isomorphic configuration for the purposes of the study.

Case Collection

An arbitrary number of 275 U per liter was selected as the minimal adult level to be considered as an isomorphic pattern. Higher values were required for children. The range of LDH in pediatric study cases was 305 to 2400 U per liter. Unselected consecutive cases filling these criteria were used in order to evaluate the significance of the isomorphic pattern in a community hospital setting. During the study period from March 1972 to September 1975, these patients constituted 21 percent of all LDH isoenzyme studies and 1.4 percent of adult non-obstetric admissions. Some had multiple admissions. They had a total of 587 isomorphic patterns.

First and second diagnostic categories were entered on data cards from which information was encoded into an IBM 370/145 computer. Age, sex and percentage of each LDH isoenzyme fraction were recorded. The SMA tests of total LDH, aspartate aminotransferase (GOT or ASAT, 340 nm channel), alkaline phosphatase (Alk.P.) and total bilirubin were entered (table I). Leucine aminopeptidase (LAP), gamma glutamyl transpeptidase (GGTP, normal for females to 18 and for males to 28 U per liter at 30°), $alpha_2$ globulin and PaO_2 with or without

TABLE I

Means with One Standard Deviation for Measured Parameters

*Major Diagnostic Category**	*Number of Patients*	*Age*	*Male/ Female Ratio*	*Total LDH U/l*	*LDH_1*	*LDH_2*	*LDH_3*	*LDH_4*	*LDH_5*	*GOT U/l*	*Alkaline Phosphatase U/l*	*Total Bilirubin mg/dl*
					----------*Percent*----------							
Cardio-respiratory	194	68±17	78/116	360±206	21±4	30±4	24±4	13±3	11±3	55±52	124±83	1.1±1.9
Cancer	124	65±16	58/66	554±415	17±5	31±4	26±4	15±4	10±3	64±65	228±259	1.2±3.1
Fracture	40	60±26	14/26	322±51	19±3	29±4	26±3	14±3	12±3	54±42	113±74	0.8±0.6
Central nervous system	33	67±16	12/21	344±60	21±4	30±3	24±4	13±3	11±3	65±64	105±50	1.1±1.0
Infection/inflammation	33	55±21	12/21	357±132	20±4	31±4	25±3	13±3	11±2	55±59	118±106	0.6±0.3
Cirrhosis/alcoholism	26	60±10	12/14	334±50	22±3	33±4	24±3	12±2	10±3	92±61	176±100	2.7±4.4
Trauma without fracture	24	46±25	8/16	327±42	17±4	29±4	26±3	15±4	13±3	75±51	98±96	0.8±0.3
Mononucleosis	22	17±4	13/9	442±168	17±3	29±3	29±3	15±2	10±3	119±79	241±134	1.6±2.5
Hypothyroidism	19	60±18	2/17	347±65	21±4	32±4	24±4	13±3	11±3	51±27	89±36	0.8±1.2
Uremia	14	66±15	4/10	408±172	20±5	30±4	25±4	14±3	12±2	50±76	109±51	0.6±0.3
Necrosis	11	51±21	1/10	319±38	18±4	31±3	27±3	14±3	11±3	22±7	82±18	0.6±0.3
Pseudomononucleosis	4	23±7	1/3	307±12	16±7	30±4	29±5	14±2	11±2	60±16	96±36	0.5±0.1
Viremia	4	67±13	3/1	351±22	19±6	29±3	25±3	15±4	12±2	58±29	90±10	0.8±0.5
Intestinal obstruction	3	53±10	0/3	283±11	18±2	29±3	27±4	13±2	13±4	38±10	115±30	1.5±0.8
Apparent false positives	23	54±20	7/16	345±118	20±4	31±3	25±3	13±3	11±3	57±47	107±59	1.0±1.4
All categories	500	61±21	195/305	399±253	20±5	31±4	25±4	14±3	11±3	61±57	148±155	1.1±2.1
Normal range†				112-200	6-27	21-41	22-37	7-23	2-16	7-40	30-100	0-1.0

*Of the 500 patients, 74 had diagnoses in two different major categories. Thus, a single patient may be included in more than one of the categories.

†Normal ranges for total LDH and its fractions are expressed as mean ± 2 standard deviations.

oxygen therapy were also included when available. It is customary in the present author's institution for LDH isoenzymes to be performed on admission serum if total LDH exceeds 275 U per liter and for LAP (later changed to GGTP) to be tested if admission Alk.P. is above the range of normal.

Summary statistics were obtained for each diagnostic category. Statistical procedures included Student's t-test, analysis of variance and chi-square test. Most of these calculations were performed using the Biomedical Computer Programs of the University of California (BMDP series, 1975).

Results

Patterns in which LDH_3 was less than its lower limit of normal were seen in 102 persons. Such patients were older,—age 66 compared to age 60 for those with LDH_3 within normal limits ($p = 0.001$). The LDH_5 remained relatively constant, and the differences were taken up in the first four fractions. No significant differences in sex ratios, GOT, Alk.P. or bilirubin were detected.

The patients appeared to cluster into 15 categories (table I). There were 195 males and 305 females. The mean age was 61 with range of 5 to 94 years. There were 11 patients aged 14 and under. Seventy-four of 146 patients with two diagnoses were classified in two entirely different categories. The remainder had double diagnoses in the same major category. Only four of the 500 individuals had two very strong disparate diagnostic explanations for the isomorphic pattern.

No consistent significant sex differences were observed in various diagnostic categories, although the males as a group did have higher total LDH, GGTP, GOT, Alk.P. and total bilirubin than females.

Expression of results in absolute isoenzyme activity did not provide additional useful information with regard to the isomorphic pattern.

Cardiorespiratory Disease

One hundred ninety-four patients had 225 diagnoses pertinent to cardiorespiratory diseases, the largest diagnostic category. PaO_2 was measured in 55 patients. Their mean PaO_2 was 63 mm Hg. Only one of the 25 patients not on oxygen therapy when gases were drawn had PaO_2 greater than 80, and the mean PaO_2 of the remaining 24 was 55 mm Hg.

The largest subgroup was heart disease: 132 patients had 160 cardiac diagnoses. Congestive heart failure (CHF), the most common diagnosis in this group, was seen in 86 persons.

Sixteen patients in the study had acute infarct of myocardium (MI). No isomorphic pattern was seen in the period from 48 to 72 hours following the onset of MI. Duration of the infarct in 12 cases was 24 hours or less, in two cases less than 48 hours, and in two cases four to 15 days. Four of the 16 cases had measured PaO_2. Of these, only one was not on oxygen and that patient's PaO_2 was 55 mm Hg. Those on oxygen had PaO_2 of 76, 82, and 94 mm Hg. The mean GOT of the MI group was 95, compared to 47 U per liter for the remaining cardiac patients ($p = 0.0004$).

Other cardiac diagnoses included arteriosclerotic heart disease, pulmonary edema, arrhythmia, rheumatic heart disease, and one instance each of collagen disease and of prosthetic valve malfunction.

Twenty-five of the respiratory patients had chronic obstructive pulmonary disease (COPD) without bronchitis. Seven had bronchitis with and three without COPD. There was one case of bronchial tuberculosis and one necropsy-proven instance of lipid pneumonia among the 31 pneumonias. Asthma, viral pneumonia, pulmonary embolism, atelectasis

and pneumothorax were seen in descending order of frequency. A number of the cases had other disorders co-existing such as sickle crisis, CHF, azotemia and cerebrovascular accident.

Excluding patients who had both cardiac and respiratory diagnoses, 105 cardiac patients had a mean age of 73 years, in contrast to 61 respiratory cases with a mean age of 59 years ($p \leq 0.0001$). Mean LDH was 325 in the cardiac group, 426 U per liter in the respiratory patients ($p = 0.0037$).

Polycythemia

All eight patients were placed within the cardiorespiratory group. Two had chronic lung disease, one had pneumonia, and three had heart disease, of whom one also was classified as having chronic lung disease. Two of the heart disease patients were in congestive failure. One well-defined case of stress polycythemia had a seizure shortly before the isozyme sample was drawn. One patient had strong evidence for polycythemia vera, and another reasonable evidence for that diagnosis. PaO_2 was measured in two patients, neither of whom had polycythemia vera and each of whom was hypoxic.

Cancer

Almost all of the neoplastic diagnoses had biopsy, bone marrow or necropsy evidence. In order to avoid bias in the series, very occasional cases were accepted into the cancer group without tissue diagnosis, if other evidence was reasonably strong, e.g., clinical and X-ray findings of carcinoma of prostate with marked elevation of acid and alkaline phosphatase. Although patients with not more than moderately strong clinical evidence for neoplasia were excluded, there were only two such individuals.

The 124 patients with cancer represent the second largest group. The average age was 65 years, with a range of six to 89 years. Their mean LDH of 554 U per liter is the highest in the study. Although only two of 376 noncancer patients had total LDH higher than 1000 U per liter, 14 of the 124 cancer patients had total LDH of 1000 U per liter or more. Of these, the highest value found was in a six year old child with neuroblastoma who had a total LDH of 2400 U per liter. The highest adult value was 2090 U per liter in a 64 year old female with papillary serous cystadenocarcinoma of the ovary with metastases. The cancer group included 95 instances of carcinoma among 100 solid tumors. In descending order, primary tumors were in lung, breast, large intestine, prostate, gonads, stomach, endometrium, pancreas, esophagus, cervix uteri, larynx, kidney and urinary bladder. The source of the primary tumor was uncertain in seven patients, of whom four had adenocarcinoma.

Among 27 hematologic malignant diseases, there were 15 lymphoproliferative disorders, including seven leukemic cases, four solid lymphomas, one myeloma and one case of Hodgkin's disease. There were 12 myeloproliferative diseases, including five instances of chronic granulocytic leukemia, two of acute myelogenous leukemia and two instances of myelofibrosis. Cases of polycythemia were categorized elsewhere.

Total bilirubin and Alk.P. were evaluated in 26 (21 percent) and 81 (65 percent), respectively, of the 124 cancer patients. Nineteen of 21 patients in whom GGTP was measured had elevated values (90 percent), while only 14 of 34 individuals with LAP determinations had increases (41 percent). $Alpha_2$ globulin was high in 14 of 41 patients (34 percent).

Significant differences were found between the cancer and noncancer patients.

Age, total LDH, LDH_3, LDH_4 and Alk.P. were higher in the cancer group, while LDH_1 and LDH_5 were lower (table II). Noncancer cases included 22 instances of infectious mononucleosis (IM) which, with the exception of patient age, are very similar to the cancer cases in regard to those variables tabulated. The ratio LDH_{3+4}/LDH_1 was significantly different between cancer and all noncancer patients, but it was not different between cancer and IM. Other comparisons between cancer and IM parameters include the following: LDH_2 is higher in the cancer group ($p = 0.05$) and LDH_3 is lower ($p = 0.01$). Mean GOT was 64 in the cancer group, 119 U per liter in the IM group ($p = 0.0005$). There were no significant differences in Alk.P., bilirubin or $alpha_2$ globulin. Mean Alk.P. of 228 in the cancer patients, varying from 25 to 2120 U per liter, was exceeded by only one other group, IM. Although five of the 22 IM patients were 14 years or younger, only one of the cancer patients was pediatric.

Infectious Mononucleosis and Pseudomononucleosis

Positive mononucleosis serology with clinical and hematologic evidence was required for the diagnosis of IM. There were 22 such patients. Four cases with clinical and peripheral blood smear findings suggestive of IM, but with negative serology, were classified as pseudomononucleosis (PMN). Mean age of these groups was 17 and 23 years, respectively, in strong contrast to all other diagnostic categories. Age ranges were 5 to 23 and 15 to 31 years for IM and PMN, respectively. P values for the mean age of the IM group were less than 0.0001 compared to all groups, save PMN, in which $p = 0.025$. The only group characterized by mean higher total LDH was the malignant disease category ($p = 0.0053$). Mean Alk.P. for IM was 241 (range 75 to 575) and mean Alk.P. for PMN was 96 (range 45 to 130) U per liter. LAP was measured in nine IM patients and was elevated in all. GGTP was measured in one IM patient (96 U per liter) and in one PMN patient (26 U per liter, high). Mean bilirubin was 1.6 mg per dl with range 0.2 to 11.1 mg per dl for IM patients. Mean bilirubin was 0.5 mg per dl with range 0.3 to 0.5 mg per dl for PMN patients.

Fracture, Trauma and Shock

The age range for the 40 fracture patients was 14 to 92 years. Age range of the 24 trauma without fracture cases was 13 to 94 years. Of the cumulative patient charts examined for evidence of shock, such evi-

TABLE II

Variables Between 124 Cancer and 376 Non-cancer Patients

	Cancer	*Non-cancer*	*p-value**
Age	65 (6 - 89)†	59 (5 - 94)†	0.001
Total LDH	554 U/l (275 - 2400)	347 U/l (275 - 2160)	0.001
LDH_1	17.4% (7.5 - 26.8)	20.3% (10.1 - 27.0)	0.001
LDH_2	31.2% (23.6 - 40.7)	30.5% (20.2 - 40.8)	0.074
LDH_3	26.5% (15.6 - 36.8)	24.7% (14.8 - 36.8)	0.001
LDH_4	14.7% (6.5 - 22.5)	13.4% (6.5 - 21.8)	0.001
LDH_5	10.2% (2.4 - 15.9)	11.2% (2.0 - 15.9)	0.005
LDH_{3+4}/LDH_1	2.7 (1.0 - 7.3)	2.0 (0.8 - 5.0)	0.001
Alkaline phosphatase	228 U/l (25 - 2120)	122 U/l (36 - 670)	0.001

*p-values refer to Student's t-test. †Ranges for each variable are shown in parentheses.

dence was found in only two instances. Eight of the nine fracture patients in whom PaO_2 was measured were hypoxic. PaO_2 in the five patients on oxygen ranged from 39 to 77 mm Hg; in the three who were not, PaO_2 was 35, 42 and 77 mm Hg. Both patients who suffered shock were hypoxic, with PaO_2 of 49 and 67 mm Hg, respectively. Only one trauma patient without fracture had blood gases measured.

Central Nervous System Disorder

Age range of 33 patients was 18 to 88 years. Seizures occurred in six. Twenty-one had intracranial hemorrhages and/or infarcts. Seven others had diagnoses including cerebrovascular insufficiency and chronic brain damage secondary to alcoholism. All five patients whose blood gases were measured were on oxygen therapy. PaO_2 was 64, 64, 69, 78 and 94 mm Hg.

Infection/Inflammation

This diverse group included 33 patients. There were two instances of polymyositis, three of phlebitis, two of pancreatitis, four of pharyngitis and three of cholecystitis. One case each of ureteral empyema, leg ulcer, pyoderma gangrenosa, endometritis, sarcoidosis, Shigellosis, septicemia and urinary tract infection was found.

Hepatic Cirrhosis and Alcoholism

Two patients with these diagnoses also had congestive heart failure and were classified in the latter group, leaving 26 patients. Biopsy proof was available in four. The clinical charts did not offer alternate diagnoses which could have better explained the isomorphic pattern. Age range was 40 to 83 years. Mean Alk.P. was 176 with range of 66 to 420 U per liter. LAP was done in 12 patients; six were high. GGTP was performed in only two patients, both abnormal (82 and 409 U per liter, respectively). Mean value of GOT was 92 with range 30 to 297 U per liter. Mean total bilirubin was 2.7 mg per dl with range of 0.5 to 23.1 mg per dl. Only one patient had blood gases measured; PaO_2 was 59 mm Hg without oxygen therapy.

Hypothyroidism

All but one of the 19 hypothyroid patients had low T3 (resin)/T4 values recorded, with low adjusted free thyroxine index. The one patient whose T3/T4 was normal was on levothyroxine; the isomorphic LDH pattern was recorded 16 days subsequent to discontinuation of the drug. The age range was 20 to 85 years, mean LDH 347, range 280 to 500 U per liter. Mean LDH_3 of 23.5 percent was lowest of any diagnostic category, but with no statistical significance. PaO_2 was measured in only one individual and was 78 mm Hg.

Uremia

The age range of 14 patients in this group was 33 to 89 years. Uremia was a secondary diagnosis in nine patients; six of these had a primary cardiorespiratory diagnosis and three had cancer. Of the five patients with no explanation for LDH elevation save uremia, creatinine ranged from 4.0 to 21.5 mg per dl, and serum urea nitrogen from 39 to 190 mg per dl.

Necrosis

The age range of 11 patients in this group was 18 to 82 years. Areas of necrosis were present in each of five benign tumors found. Two apparently benign tumors had a possible metastatic potential: an adrenal cortical adenoma in a 39 year old woman, and a cellular and atypical uterine leiomyoma in a 50 year old female. There were three other cases of

necrotic uterine leiomyomas, two instances of necrosis in lower extremities and a third with fracture and gangrene. There was one instance each of macerated stillbirth, infarct of prostate documented by needle biopsy and fat necrosis of body wall.

Viremia

Since infectious mononucleosis, pseudomononucleosis and viral pneumonia were classified elsewhere, only four patients comprise this group. No blood gases were obtained.

Intestinal Obstruction

All of the three cases were primary diagnoses, and all were females. Ages were 41, 57 and 60. None had blood gases.

Apparent False Positives

Twenty-three of the 500 patients originally did not seem to have a clinical explanation for an isomorphic pattern. The mean age of these patients was 54 years with a range of 15 to 79 years. Upon reexamination, one 43 year old male had hypertension treated with methyldopa, his urine was loaded with red cells and he may well have had hypertensive heart disease. A 26 year old male with a hemoglobinopathy (sickle cell disease with high HbF) and a white blood count (WBC) of 23,900 probably had an infection. A 15 year old male had a WBC of 4100 with 62 percent lymphs, occasional virocytes but negative mononucleosis serology. His case could well be classified as pseudomononucleosis. A 79 year old female had GOT of 105 U per liter. She was on a diuretic and digitalis and was probably correctly diagnosed as having arteriosclerotic heart disease. Of the remaining 19 patients, one was on levodopa, two on aspirin and one on aspirin and sodium warfarin.

Discussion

Emergence of cardiorespiratory patients as the largest group generating the isomorphic pattern would appear to be a new finding. Acute MI of 48 hours duration, and usually for a number of days thereafter, is accompanied by elevated LDH, flipped $LDH_{1:2}$ ratio and elevation of LDH_1. The proportionally tiny fraction of MI patients with isomorphic patterns would not have come as a surprise to those who have advocated serial enzyme determinations in workup for infarct myocardium.[31]

Our experience with over 3,000 LDH separations has confirmed prior observations that not all patients with diffuse neoplastic disease have an increase of total LDH,[35] or that those with an elevated LDH necessarily have an isomorphic LDH abnormality.[8,38,44] In our review of cases, normal LDH levels with known primary and/or metastatic disease were not infrequently seen, and there was some shifting of isoenzyme patterns, not uncommonly from or to an LDH_5 elevation. Elevations of $LDH_{3,4}$ and/or $_5$ are reported in cancer patients.[24,38,39,40] and the three cathodal fractions have been reported to diminish in children with malignant diseases treated with prednisone.[33]

LDH is operative in glycolysis, which is accelerated in tumor cells. Blood flow through tumors has been reported to range from 1 to 15 percent that of the adjacent tissue.[20] A 3 percent oxygen environment induced significant increases of LDH_4 and LDH_5 and decreases in LDH_2 and LDH_3 in incubated fibroblast and epithelial cell lines.[28] Cathodal shift would expedite reduction of pyruvate to lactate in the presence of high pyruvate concentration, sustaining glycolytic activity by replenishing NAD.[14] Increased total LDH with cathodal shift has been described in malignant tumor tissue in a variety of organs.[15,22,30]

Although cancer patients with LDH_5 over its limit of normal were not infrequently seen, LDH_5 elevation is not apparent in cancer patients in the framework of this study. Mean LDH_5 is, in fact, lower in cancer patients than in noncancer patients with isomorphic patterns (table II). Validity of comparison of tissue and serum isoenzyme concentration may be diminished by a number of factors. They include variation of rates of liberation of isoenzymes into the circulation from damaged cells, cell permeability, isoenzyme stability under a variety of physiologic conditions and relative rates of metabolism, inactivation and excretion.[5,41,44] When isoenzymes are expressed as percentages, diminution of LDH_1 bears an arithmetic inverse relationship to midzone and/or cathodal dominance, a phenomenon previously reported.[24]

In the present study, a midzone pattern of dominance of LDH_3 and LDH_4 is seen in the cancer patients with decreased LDH_1 and may be expressed LDH_{3+4}/LDH_1 (table II). Although Zondag and Klein reported an incidence of midzone elevations with or without LDH_5 elevations 4.75 times higher than their incidence of elevation in all five fractions in instances of malignancy,[44] the present authors have found fewer than a dozen such cases. The upper limits of normal for our methods are higher for fractions LDH_3 and LDH_4 than are the upper limits for those fractions published by some others.[8,39,40,44] By virtue of our limits of normal, it would be difficult for fractions 2 and 3 or 3 and 4 to exceed simultaneously their upper limits when expressed as percentages, and they have never done so in this laboratory.

Emergence of the cirrhosis/alcoholism group, although a new finding, should have been anticipated by virtue of presently established knowledge of frequency of arterial oxygen unsaturation and vascular shunting in hepatic cirrhosis.[43]

It comes as a surprise to these investigators that substantial numbers of individuals with trauma, with and without fractures, appeared in the study groups. Although shock has been previously proven to cause the isomorphic pattern in dogs,[34] it was not a major factor in the present study, probably by virtue of the time of sampling of most of our patients on admission. The authors suspected but have not proven instances of fat embolism in at least some of the fracture cases. Correlation exists between hypoxia and fat embolism in fracture patients.[23] Fractures were found in many bones throughout the body, with no special predilection for the thorax, and occurred in a wide age span. The authors feel that additional studies are needed to establish relationships, if any, between fracture and other trauma, the isomorphic pattern and fat embolism.

Results of a number of prior studies have been confirmed by our observations. Elevation of LDH with IM is well established, and isoenzyme patterns of the type presently described have been previously recorded in cases of IM.[24,44] Some but not all patients with hypothyroidism have elevations of serum LDH activity.[12] Correlation of hypothyroidism with the isomorphic pattern has been reported.[8] Necrosis as a cause of the isomorphic pattern has been previously documented.[44] Necrosis in benign tumors, as occurred in five of our cases, including four uterine leiomyomas, has not to the best of our knowledge been previously reported.

Four patients among the apparent false positives were known to have been on drug therapy, including levodopa, sodium warfarin and aspirin. These agents might cause elevation of LDH.[1,2,19,25,26,27,42] Thus, 15 patients remaining in this group may be considered false positives, an incidence of 3 percent.

Although clinical error exists in the diagnosis of cancer,[3] none of the apparent

false positive cases in this study has so far emerged with a cancer diagnosis. Since development of the isomorphic pattern is usually not an early finding in neoplasia, evolution of subsequent cancer is not anticipated in these individuals.

Because pulmonary embolism may be missed as a clinical diagnosis, the infrequency of clinical diagnosis of pulmonary embolism in the present study may represent misleading information. All patients with pulmonary embolism, however, do not have elevation of LDH.[9,32]

Conclusions

A large group of patients in a community hospital setting was surveyed to learn the types of disease giving rise to the isomorphic pattern of LDH. Widely divergent groups of clinical diagnoses emerged, segregated by several chemical variables. Cardiorespiratory and malignant disease accounted for over half of all patients. Very high total LDH (over 1000 U per liter) was seen in 14 of 124 cancer patients and in only two of 376 noncancer cases with the isomorphic pattern. Necrosis was present in each of five benign tumors found. Fifty-nine of 67 patients in whom PaO_2 was measured were hypoxic. Most had cardiorespiratory or central nervous system disease or fracture.

A need exists for accumulation of additional data with appropriate control groups to ascertain the association of variables, particularly the relationships between varying serum LDH levels and PaO_2. Patients with similar diseases and normal total LDH must be compared, but quantitation of the severity of such disorders as congestive heart failure can best be accomplished within studies of narrowly defined patient groups. A role for the LDH isoenzymes, among other laboratory and clinical parameters, would appear to exist in future prospective studies of particular disease entities.

References

1. AME COUNCIL ON DRUGS: AMA Drug Evaluations, 1st ed. Chicago, American Medical Association, p. 543, 1971.
2. BATSAKIS, J. G. and BRIERE, R.O.: Interpretive Enzymology. Springfield, Charles C Thomas, pp. 60–62, 1967.
3. BAUER, F. W. and ROBBINS, S. L.: An autopsy study of cancer patients. I. Accuracy of the clinical diagnoses (1955 to 1965) Boston City Hospital. J. Amer. Med. Assoc. *221*:1471–1474, 1972.
4. BIERMAN, H. R., HILL, B. R., REINHARDT, L., ET AL: Correlation of serum lactic dehydrogenase activity with the clinical status of patients with cancer, lymphomas, and the leukemias. Cancer Res. *17*:660–667, 1957.
5. BODANSKY, O.: Enzyme and chemistry determinations in the diagnosis of cancer. Oncology, 1970: Proceedings of the Tenth International Cancer Congress, volume 3. Clark, R. L., Cumley, R. W., McCay, J. E., and Copeland, M. M., eds. Chicago, Year Book Medical Publishers, pp. 106–109, 1971.
6. BODANSKY, O.: Reflections on biochemical aspects of human cancer. The Lucy Wortham James Lecture. Cancer *33*:364–370, 1974.
7. BRINDLEY, C. O. and FRANCIS, F. L.: Serum lactic dehydrogenase and glutamic-oxaloacetic transminase correlations with measurements of tumor masses during therapy. Cancer Res. *23*:112–117, 1963.
8. COHEN, L., DJORDJEVICH, J., and JACOBSEN, S.: The contribution of isozymes of serum lactic dehydrogenase (LDH) to the diagnosis of specific organ injury, with special reference to myocardial injury. Med. Clin. North. Amer. *50*:193–209, 1966.
9. COHEN, L., DJORDJEVICH, J., and ORMISTE, V.: Serum lactic dehydrogenase isozyme patterns in the cardiovascular and other diseases, with particular reference to acute myocardial infarction. J. Lab. Clin. Med. *64*:355–374, 1964.
10. EMANUEL, B., WEST, M., and ZIMMERMAN, H. J.: Serum enzymes in disease. XII. Transaminases, glycolytic and oxidative enzymes in normal infants and children. Amer. J. Dis. Child. 105:261–264, 1963.
11. ERICKSON, R. J. and MORALES, D. R.: Clinical use of lactic dehydrogenase. N. Eng. J. Med. *265*:478–534, 1961.
12. FLEISHER, G. A., MCCONAHEY, W. M., and PANKOW, M.: Serum creatine kinase, lactic dehydrogenase, and glutamic-oxalacetic transaminase in thyroid diseases and pregnancy. Mayo Clin. Proc. *40*:300–311, 1965.
13. GALEN, R. S. and GAMBINO, S. R.: Isoenzymes of CPK and LDH in myocardial infarction and certain other diseases. Pathobiol. Ann. 5:283–315, 1975.
14. GOLDMAN, R. D., KAPLAN, N. O., and HALL, T. C.: Lactic dehydrogenase in human neoplastic tissues. Cancer Res. *24*:389–399, 1964.

15. HILF, R., RECTOR, W. D., and ORLANDO, R. A.: Multiple molecular forms of lactate dehydrogenase and glucose 6-phosphate dehydrogenase in normal and abnormal human breast tissues. Cancer *37*:1825–1830, 1976.
16. HILL, B. R.: Some properties of serum lactic dehydrogenase. Cancer Res. *16*:460–467, 1956.
17. HILL, B. R. and LEVI, C.: Elevation of a serum component in neoplastic disease. Cancer Res. *14*:513–515, 1954.
18. HILL, J. H.: Serum lactic dehydrogenase in cancer patients. J. Nat. Cancer Inst. *18*:307–313, 1957.
19. JANOTA, I., WINCEY, C. W., SANDIFORD, M., ET AL: Effect of salicylate on the activity of plasma enzymes in the rabbit. Nature *185*:935–936, 1960.
20. LEVEEN, H. H., WAPNICK, S., PICCONE, V., ET AL: Tumor eradication by radiofrequency therapy: response in 21 patients. J. Amer. Med. Assoc. *235*:2198–2200, 1976.
21. MUNJAL, D., CHAWLA, P. L., LOKICH, J. J., ET AL: Carcinoembryonic antigen and phosphohexose isomerase, gamma-glutamyl transpeptidase and lactate dehydrogenase levels in patients with and without liver metastases. Cancer *37*:1800–1807, 1976.
22. OLIVER, J. A. and ABDALLA, A. M.: Prostatic cancer: generalised malignant pattern of lactate dehydrogenase isoenzymes. Brit. J. Urol. *43*:321–323, 1971.
23. PAZELL, J. A. and PELTIER, L. F.: Experience with sixty-three patients with fat embolism. Surg. Gynecol. Obstet. *135*:77–80, 1972.
24. RATLIFF, C. R., CULP, T. W., and HALL, F. F.: Serum lactic dehydrogenase and other enzymes in malignant disease: comparison with an immunodiagnostic test for cancer. Amer. J. Gastroenterol. *56*:199–208, 1971.
25. RICH, R. R. and JOHNSON, J. S.: Salicylate hepatotoxicity in patients with juvenile rheumatoid arthritis. Arthritis Rheum. *16*:1–9, 1973.
26. ROUTH, J. I. and PAUL, W. D.: Assessment of interference by aspirin with some assays commonly done in the clinical laboratory. Clin. Chem. *22*:837–842, 1976.
27. RUSSELL, A. S., STURGE, R. A., and SMITH, M. A.: Serum transaminases during salicylate therapy. Brit. Med. J. *2*:428–429, 1971.
28. SCHROEDER, J., UPSON, R., and CHVAPIL, M.: LDH isoenzyme pattern, lactate/pyruvate, and ultrastructure of fibrinogenic and epithelial cell lines exposed to 3% oxygen. Exp. Cell Res. *94*:227–234, 1975.
29. SCHWARTZ, M. K.: Enzymes in cancer. Clin. Chem. *19*:10–22, 1973.
30. SCHWARTZ, M. K.: Laboratory aids to diagnosis—enzymes. Cancer *37* suppl:1:542–548, 1976.
31. STARKWEATHER, W. H., SPENCER, H. H., SCHWARZ, E. L., ET AL: The electrophoretic separation of lactate dehydrogenase isoenzymes and their evaluation in clinical medicine. J. Lab. Clin. Med. *67*:329–343, 1966.
32. TRUJILLO, N. P., NUTTER, D., and EVANS, J. M.: The isoenzymes of lactic dehydrogenase: II. Pulmonary embolism, liver disease, the postoperative state, and other medical conditions. Arch. Intern. Med. *119*:333–344, 1967.
33. VAN GENNIP, A. H., TABAK-VAN GORCUM, J. A., TAMINIAU, J. A., ET AL: The behaviour of LDH-3 in patients with malignant diseases during therapy with cytostatic drugs and prednisone, studied by LDH-isoenzyme electrophoresis on cellulose acetate. Clin. Chim. Acta *58*:85–94, 1975.
34. VESSEL, E. S.: Significance of the heterogeneity of lactic dehydrogenase activity in human tissues. Ann. N. Y. Acad. Sci. *94*:877–889, 1961.
35. VESSEL, E. S. and BEARN, A. G.: Localization of lactic acid dehydrogenase activity in serum fractions. Proc. Soc. Exp. Biol. Med. *94*:96–99, 1957.
36. WEST, M. and ZIMMERMAN, H. J.: Serum enzymes in disease. I. Lactic dehydrogenase and glutamic oxalacetic transaminase in carcinoma. Arch. Intern. Med. *102*:103–113, 1958.
37. WHITE, L. P.: Serum enzymes. II. Glycolytic enzymes in patients with cancer and other diseases. J. Nat. Cancer Inst. *21*:671–684, 1958.
38. WIDY-KIERSKA, K. and ROSZKOWSKI, I.: LDH isoenzymes in patients with uterine tumors. Obstet. Gynecol. *33*:173–176, 1969.
39. WIEME, R. J., VAN HOVE, W. Z., and VAN DER STRAETEN, M. E.: The influence of cytostatic treatment on serum LDH patterns of patients with bronchial carcinoma and its relation to tumor regression. Ann. N. Y. Acad. Sci. *151*: 213–221, 1968.
40. WRIGHT, E. J., CAWLEY, L. P., and EBERHARDT, L.: Clinical application and interpretation of the serum lactic dehydrogenase zymogram. Amer. J. Clin. Path. *45*:737–745, 1966.
41. WROBLEWSKI, F. and GREGORY, K. F.: Lactic dehydrogenase isoenzymes and their distribu tion in normal tissues and plasma and in disease states. Ann. N. Y. Acad. Sci. *94*:912–932, 1961.
42. YOUNG, D. S., PESTANER, L. C., and GIBBERMAN, V.: Effects of drugs on clinical laboratory tests. Clin. Chem. *21*:323D–324D, 1975.
43. ZIMMERMAN, H. J.: Hepatic failure. The Liver. Gall, E. A. and Mostofi, F. K., eds. Baltimore, Williams & Wilkins, p. 398, 1973.
44. ZONDAG, H. A. and KLEIN, F.: Clinical applications of lactate dehydrogenase isozymes: alterations in malignancy. Ann. N. Y. Acad. Sci. *151*:578–586, 1968.