Excessively High Levels of Lactic Acid Dehydrogenase Activity in Pernicious Anemia*

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ELEVATED serum levels of lactic acid dehydrogenase (LDH) activity have been described in many diseases, such as myocardial infarction, homologous serum hepatitis, carcinoma of various sites, metastatic carcinoma to the liver, pulmonary infarction, myelogenous leukemia and various anemias [*1–4*]. While elevated levels of LDH activity cannot be construed to be specific for the many diseases in which it has been described, some studies have indicated that (1) serial changes of levels of LDH activity in serum and other body fluids "may serve to reflect the presence, or the proliferative behavior, of malignant tumors" [*3*], and (2) that the breakdown of LDH to its various isozyme components may reveal a specific pattern for a given disease process [*1,5,6*], thus greatly enhancing the specificity of this laboratory determination.

We shall present a case of pernicious anemia in which the serum LDH activity was elevated to an excessive and unexpected level.

CASE REPORT

The patient, a sixty-six year old white American lawyer, was admitted with the chief complaints of syncope and chest pain, suspected to be due to myocardial infarction. He gave a five year history of paresthesias involving the upper extremities. Five years previously studies revealed gastric achlorhydria which did not respond to histamine stimulation. In view of this, he was treated with vitamin B_{12} for a short period of time with subsequent diminution in symptoms. No other findings compatible with pernicious anemia were elicited. The patient did well until one year prior to the present admission, when he noted the onset of lightheadedness, dizziness, occasional syncope and substernal pains. He was hospitalized, and the diagnoses of cerebral arteriosclerosis with transitory attacks of ischemia and arteriosclerotic heart disease (compensated, class IIB) with angina pectoris were made. Anticoagulant therapy was instituted. He again did well (with only occasional attacks of angina and fleeting paresthesias) until two weeks prior to the present admission when he noted an increase in the frequency of anginal attacks. On the morning of admission the patient experienced an episode of lightheadedness, dizziness and syncope. Following this he was well until that evening when he became aware of moderately severe, substernal non-radiating pains on the left side of the chest.

Physical examination at the time of admission revealed a well developed, well nourished, adult Caucasian man in no acute distress but with evidence of slowed cerebration. The blood pressure was 130/76 mm. Hg, the pulse 76 beats per minute, full and regular. Positive findings were limited to grade 2 arteriosclerotic retinopathy and a few facial telangiectases. Cardiograms taken on admission and subsequently were within normal limits. Chest roentgenograms and electroencephalogram showed no abnormalities. The hemogram at this time revealed a white blood cell count of 4,840 per cu. mm. with some increase in eosinophils, a hemoglobin of 7.8 gm. per cent and a hematocrit of 25 per cent. Blood sugar, blood urea nitrogen, serum cholesterol and prothrombin time were all within normal limits. Thymol turbidity was 0.75 units, and cephalin flocculation test results were negative. There was 7.7 per cent bromsulphalein retention in thirty minutes. Serum iron was essentially normal, with 39 per cent saturation. Blood indices revealed a mean corpuscular volume of 125 cu. μ, a mean corpuscular hemoglobin concentration of 32 gm. per 100 ml. and a mean corpuscular hemoglobin of 40 $\mu\mu$g. Table 1 shows the hemoglobin, hematocrit, transaminase, and LDH values and the reticulocyte count. Sigmoidoscopy to 17 cm. was not revealing, and stools were repeatedly negative for blood. Studies of the upper gastro-

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TABLE I

PERTINENT LABORATORY DETERMINATIONS

Hospital Day	Hemoglobin (gm./100 ml.)	Hematocrit (%)	Lactic Acid Hydrogenase (B.B. units)	Reticulocytes (%)	Transaminase (S-F units)
1	7.8	25.0	...	...	89.0
2	...	...	...	...	84.0
3	8.0	25.0	...	...	89.0
4	...	...	...	...	...
5	7.5	22.1	500	...	...
6	...	...	...	...	89.0
7	...	...	...	...	...
8	...	...	...	...	...
9	...	...	19,800	...	...
10	...	...	...	...	...
11	...	...	...	...	...
12	7.5	21.0	...	...	100.0
13	...	...	28,125	1.3	...
14	...	...	...	2.1	...
15	...	...	18,000	9.7	...
16	...	...	...	...	...
17	...	...	...	8.2	...
18	...	...	...	9.7	...
19	7.9	27.0	...	14.6	...
20	...	...	...	8.4	...
21	...	...	...	7.2	...
22	9.4	30.0	9,600	...	32.0
23	...	...	...	...	...
24	...	...	...	...	...
25	...	...	...	...	...
26	9.8	34.0	...	...	...
27	...	...	...	...	...
28	10.0	36.0	...	...	...
29	...	...	6,400	...	...

intestinal tract and colon revealed no abnormalities. The results of bone marrow aspirate (Fig. 1), obtained on the ninth hospital day, showed a considerable increase in megaloblasts which was considered compatible with the diagnosis of pernicious anemia. Subsequently the results of a Schilling test showed an increase from 3 to 12 per cent in the urinary excretion of Co^{60}-B_{12} when intrinsic factor was utilized. Although therapy for his anemia was not instituted until the seventeenth hospital day, the two flushing doses of vitamin B_{12} administered on the twelfth and fifteenth hospital days must be included in determining the patient's response to medication. On each of these days he was given 1,000 μg. of vitamin B_{12} intramuscularly. On the seventeenth day, vitamin B_{12} therapy (100 μg. per day, given intramuscularly) was begun and continued for the remainder of his hospital stay.

On the twenty-second hospital day the patient noted a slight but gradual decrease in his symptoms, and by the twenty-ninth day he was essentially free of lightheadedness, dizziness, chest pain and paresthesias. He was eating well, and his attitude had improved considerably. The patient was discharged on the thirtieth hospital day.

PRINCIPLES AND METHODS

The serum LDH activity was determined by the colorimetric method of Berger and Broida [7]. In this method 1 volume of serum is diluted with 5 volumes of water, and this 1:6 dilution is used in performing the test. To a vial containing 1 mg. of $DPNH_2$, 1 ml. of the pyruvic acid substrate is added. This mixture then is brought to a temperature of 37°C. by placing the vial in a water bath for ten minutes. Of the 1:6 serum dilution 0.1 ml. then is added to the vial, and this is incubated at 37°C. for thirty minutes. At the end of the thirty minute period the reaction is stopped by adding 1 ml. of 2,4-dinitrophenylhydrazine to the vial. The mixture is allowed to stand at room temperature for twenty minutes, after which 10 ml. of 0.4 N NaOH is added. After a five minute waiting period this colored solution is read at 550 mμ. against a distilled water reference. In our laboratory a Universal Coleman, Model 14, Spectrophotometer is used. The suggested normal

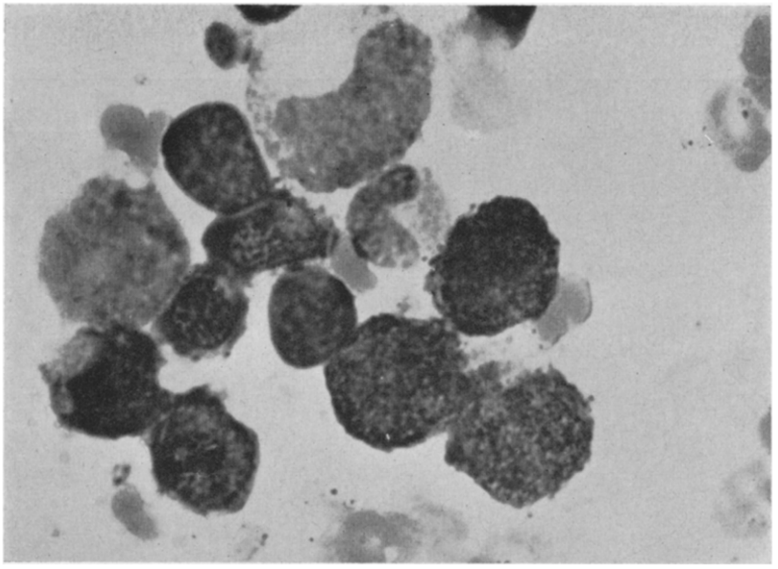

FIG. 1. Bone marrow aspirate showing numerous megaloblasts.

range is 100 to 350 B.B. (Berger and Broida) units, 350 to 550 B.B. units is considered borderline and over 550 B.B. units is elevated. The B.B. units obtained by this procedure at 37°C. are interchangeable with the Wroblewski units at 25°C. [7].

COMMENTS

Relatively little has appeared in the American literature regarding the association between high serum levels of LDH activity and pernicious anemia. LDH activity in pernicious anemia was reported first by Hess and Gehm [*8*] in the German literature in 1955. In this article they mentioned sixteen cases of pernicious anemia in which the level of serum LDH activity was elevated five to twenty-one times the normal. Further cases have been reported; both Lührs and Negelain [*9*] and Zimmerman et al. [*10*] found the activity to be increased, but less markedly. Zimmerman et al., using a modified Neiland method, reported seven cases of pernicious anemia in which the level was three to four times the upper limit of normal. Gordin and Enari [*2*] found an elevated level of activity both in pernicious anemia and in tapeworm (Diphyllobothrium) megaloblastic anemia, but the level was considerably higher in pernicious anemia. Two authors, Amelung [*11*] in Düsseldorf, Germany, and Grönvall [*12*] in Malmö, Sweden, suggest from their studies that the extremely high level of serum LDH activity may be of significant diagnostic value. Both studies set up limits within their own systems over which pernicious anemia could be highly suspect. In a later paper by Heller et al. [*13*], in which they determined LDH activity in plasma, the highest level reported was over sixty times the normal mean. In a still later paper [*14*], these authors determined LDH activity in both marrow cells and marrow plasma and found it consistently increased in megaloblastic anemias.

To date, the value of 28,125 B.B. units recorded in our patient is far higher than any other value obtained in this laboratory in other various disease processes. This unusually high level was obtained by diluting the original serum 120 times. Although Berger [*15*] was aware of high levels of activity in pernicious anemia, he had not heard of one this high. The highest level previously recorded here was 3,800 B.B. units in a patient with disseminated carcinomatosis from an anaplastic carcinoma of the pancreas.

Gordin and Enari [*16*] discussed LDH activity in conjunction with therapy and its relationship to reticulocytosis and increased hemoglobin levels. They found a correlation between reticulocytosis and decreasing LDH activity, followed later by a rise in hemoglobin levels. This was reported also by Amelung [*10*]. Our results (Fig. 2) parallel their findings. Shortly after the first injection of vitamin B_{12} the LDH activity decreased to 18,000 B.B. units and during the next week to a level of 9,600 B.B. units. During this interval the per-

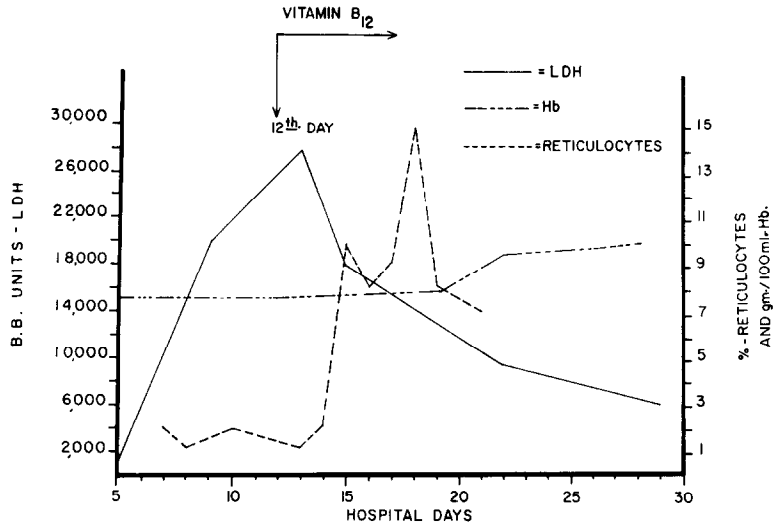

FIG. 2. Relationship between LDH, reticulocytes and hemoglobin levels.

centage of reticulocytes in the peripheral blood increased rapidly until the seventh day following the first injection of vitamin B_{12}, when it was 14.6 per cent. It will be noted that the hemoglobin level increased somewhat later, and to be sure, somewhat less remarkably.

At present we are unable to explain this patient's initial LDH activity level of 500 B.B. units. It was the consensus that this was in error, but for this we have no proof.

A brief statement should be made concerning the source of the extremely high LDH activity levels found in the serum of patients with megaloblastic anemia and particularly pernicious anemia. While irrefutable proof cannot be offered, it seems apparent from the work of Heller et al. [*13,14*] that the high serum or plasma level of LDH activity is a reflection of an increase in the marrow plasma level. Their studies suggest that initially the increase is within the individual megaloblast and that with increased intramedullary destruction rate of the abnormal cells there is a concomitant release of the enzyme into the marrow plasma and subsequently into peripheral plasma.

In all the published reports of high serum LDH activity in megaloblastic anemia the spectrophotometric methods of measuring the appearance or disappearance of an absorption peak at 340 mμ have been used. The measurement of the appearance of this peak is occasioned when the system involves the conversion of lactic acid to pyruvic acid in the presence of DPN and LDH. The appearance of this peak is due to the appearance of $DPNH_2$. Our method (Sigma) is a colorimetric method measuring the loss of pyruvic acid (which is converted to lactic acid) in the presence of $DPNH_2$ and LDH. It is our understanding that this or a similar method is the one employed in many general service hospitals. We have presented this case in the hope that it will serve as an incentive to clinicians to employ this procedure in the diagnosis and followup of patients with pernicious anemia.

SUMMARY

A sixty-six year old man with pernicious anemia is described in whom serum lactic acid dehydrogenase (LDH) activity was elevated to an extreme level of 28,125 B.B. (Berger and Broida) units, an increase of eighty times the normal upper limit for this determination. It is apparent from other reports that the highest levels of LDH activity occur in pernicious anemia. The determination of serum LDH activity may have a definite place in the diagnosis of pernicious anemia. The rate of decline of such activity could be of clinical importance in following response of the patient to vitamin B_{12} therapy.

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