illness, and also may help to explain some of the difficulty involved in demonstrating virus in blood during the course of viral illness.

Summary. A technique to produce suspensions of human mononuclear leucocytes has been employed to demonstrate the production of interferon by these cells *in vitro* using a Sendai-Sindbis virus system. There is also evidence from the experimental data that polymorphonuclear leucocytes participate in interferon production.

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Serum Lactic Acid Dehydrogenase Isoenzyme Patterns in Tropical Sprue.* (29795)

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Marked elevations in the levels of serum lactic acid dehydrogenase (S.L.D.) have been reported in acute myocardial infarction(1), renal diseases(2), carcinomatosis(3,4), leukemia(4,5), sickle cell disease(6,7), and megaloblastic anemias such as pernicious anemia(6,7,8) and tropical sprue(9,10). The elevation in the total S.L.D. is the result of an increase in one or more of the 5 normally occurring isoenzyme fractions of L.D.H. The fractions affected vary in different pathologic states. The elevation occurring in pernicious anemia and nutritional megaloblastic anemia has been shown by Wroblewski & Gregory to consist of an increase in the LD5 isoenzyme fraction(7). This seems to be unique among hematologic disorders in which isoenzymes LD3 and LD4 are the fractions most frequently affected(7). Data are presented in this report on the serum isoenzyme pattern in patients suffering from tropical sprue.

Materials and methods. The subjects under study consisted of 6 patients with tropical sprue in its acute phase, 4 partially but inadequately treated tropical sprue cases, and 7 normal controls. The clinical diagnosis of tropical sprue was based on the following criteria: glossitis, weight loss, macrocytic anemia, megaloblastic bone marrow and laboratory evidence of malabsorption, including abnormal vitamin A, xylose and butterfat absorption tests. In some instances, determination of increased fecal fat and abnormal pattern of tissue obtained by peroral jejunal biopsies were also available.

Disc electrophoresis in acrylamide gels was

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performed by the method of Ornstein and Davis(11). The gels were prepared according to a method recommended by the manufacturer of the equipment utilized in this laboratory.† A 7½% solution of acrylamide was utilized in the lower gel and a 2.5% solution in the spacer and sample gels. The gels were cast in soft glass tubes 64 mm in length and 5 mm inside diameter. 165 μl of spacer gel solution and 100 μl of sample gel solution containing a 1-to-10 dilution of the appropriate serum were layered above a 43 mm column of lower gel. Glycine tris buffer pH 8.3 was used in the system. Columns were run at a constant current of 2.5 MA per column for one hour at room temperature in a bath assembly using the model 1400 power supply. The enzymatic reaction was accomplished using the method of Allen(7). Patterns were scanned on the Model E microdensitometer and areas were obtained by planimetry. Three sharply defined bands migrating towards the anode were noted. A very faint fourth component was seen in 2 of the sera but it did not elicit a densitometric response. Components migrating toward the cathode were not resolved by this method. In the identification of the various isoenzyme fractions, we have followed the criteria that the LD5 fraction is the one moving farthest towards the anode. Serum lactic acid dehydrogenase activity was determined by the spectrophotometric method of Wroblewski and La Due(12).

Results. Fig. 1 shows the results obtained in 7 control subjects. The mean value for LD3 was 23.6% ± 3.06;‡ for LD4, 46.3% ± 3.09; and for LD5 was 30.1 ± 1.7. A variability of ± 1.4 between duplicate samples was found. The average for the ratios of LD4/LD5 was found to be 1.54 with a range of 1.35 to 1.75.

Fig. 2 reveals the results obtained in 4 inadequately treated sprue patients, with no hematologic abnormalities, but with gastrointestinal symptoms, and definite abnormalities in the absorption tests. The mean values for LD3, LD4 and LD5 were 17% ± 7.7; 37% ± 4.7; and 46% ± 5.7 respectively. The average of the ratio of LD4/LD5 was 0.80 with a range of 0.63 to 0.93.

In the 6 patients with active sprue (Fig. 3), mean values obtained for the 3 fractions were as follows: LD3 was 12% ± 2.8, LD4 was 36% ± 2.6 and LD5 52% ± 3.1. The average of the ratio LD4/LD5 was 0.70 with a range of 0.58 to 0.80.

Discussion. Marked elevation of serum lactic dehydrogenase levels has been observed in megaloblastic anemias(6,7,8), including tropical sprue in relapse(9,10). The pathogenesis of this marked elevation of SLD is not well understood, although several possibilities have been postulated including intravascular hemolysis, increased production of the enzyme by the immature cells, increased activity of the rapidly proliferating abnormal cells in the bone marrow and others. Intravascular hemolysis as primary cause for this abnormality is excluded by Zimmerman and associates(6), while suggesting that the second possibility most likely accounts for this observation. Since the work of Sheehy and co-workers(13) on the pathogenesis of the anemia in tropical sprue showed conclusively that the severe anemia in this condition is largely due to defective erythrocyte production with erythrocyte destruction playing a secondary role, the hemolytic factor as a cause for the increased LDH activity in the serum seems to be of less importance. Whether there is increased production of the enzyme by the megaloblasts or by other cells in the bone marrow, or whether there are other contributing factors resulting in this derangement, is still a moot question.

Serum lactic dehydrogenase has been separated into a number of components by heat stability(7), chromatography(7), continuous flow electrophoresis(7), paper electrophoresis (15), starch gel electrophoresis(7), and by gradient elution procedures(14,15). Wroblewski(7) has reported on the isoenzyme patterns obtained in various hematologic conditions. With the exception of the megaloblastic anemias, all others revealed an increase in the LD3 and LD4 fractions. On the other hand, pernicious and other related anemias

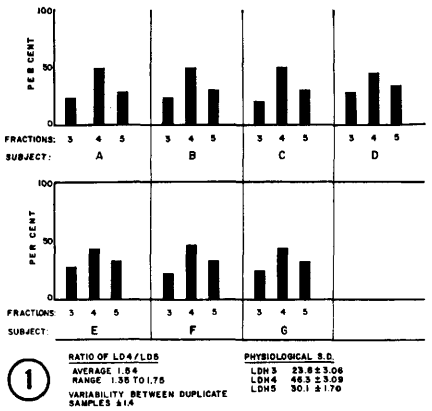
† Canalco Corp., Bethesda, Md.

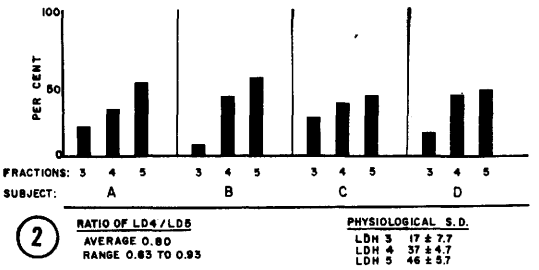
‡ In this report the standard deviation reflects the variability due to physiological difference between individual patients.

had a higher LD5 component. As is to be expected, cases of tropical sprue in its active form showed a similar isoenzyme pattern. This abnormality reverted to normal following the institution of specific therapy.

It is of interest that although the serum LDH activity may return to normal after specific therapy, the isoenzyme pattern remains abnormal. Slight change may be noted 24-48 hours after initiation of therapy; however the isoenzyme pattern does not revert completely to normal until about 2-3 weeks post treatment (Fig. 4). Patients who have been inadequately treated or those who have stopped therapy and start to relapse show varying degrees of abnormality as compared to those in complete relapse (Fig. 2). This may be an indication of the patient's need for further active specific therapy for the disease. Similarly, in a treated case, an abnormal increase in LD5 fraction may indicate early relapse or the need for more careful therapy.

Summary. The serum lactic acid dehydrogenase isoenzyme pattern has been studied in 7 normal subjects, 6 patients with tropical sprue in its acute phase and 4 partially, but inadequately treated cases of tropical sprue. The results reveal a marked increase in the LD5 isoenzyme fraction which returned to normal with continuous adequate specific therapy. Since the isoenzyme abnormality does not disappear until 10-14 days after the serum lactic dehydrogenase reach a





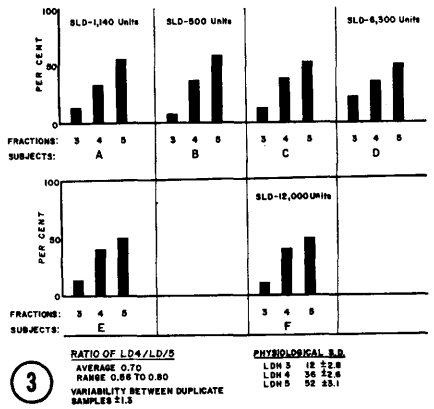


FIG. 3 SERUM LACTIC DEHYDROGENASE ISOZYME PATTERN IN ACUTE TROPICAL SPRUE.

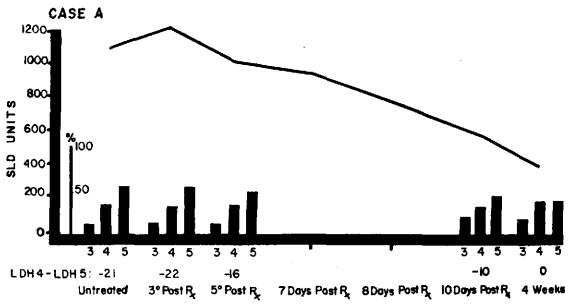


FIG. 1. Serum lactic dehydrogenase isozyme pattern in normal subjects.
FIG. 2. Serum lactic acid dehydrogenase isozyme pattern in tropical sprue in partial remission.
FIG. 3. Serum lactic dehydrogenase isozyme pattern in acute tropical sprue.
FIG. 4. Effect of specific therapy upon SLD isozyme pattern in tropical sprue.

normal level, an abnormally high LD5 isoenzyme fraction may be an indication of the lack of total correction of the abnormalities present during the acute phase of the disease. As such, this may be a sign of the need of more active therapy.

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Detection of M Protein in Colonies of Streptococcal L Forms by Immunofluorescence.* (29796)

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A major difficulty in the study of L forms of any bacterial species concerns the problem of classification. For instance, the gross appearance of L-form colonies in agar medium is not sufficiently characteristic to identify a particular L-form colony as a derivative of a bacterial species. Freimer *et al*(1) indicated that a useful characteristic of L-form colonies of Group A streptococci is the elaboration of type-specific M protein identical to that of the streptococci from which the L forms were derived. While almost all of the M protein produced by intact streptococci is confined to the cell wall, a significant portion of this antigen elaborated by L-form colonies diffuses into the surrounding agar medium. Although this special feature of streptococcal L forms has afforded a method for serological identification, in practice, appreciable growth of a subculture of the L-form colony in question is required to perform a precipitin analysis. By use of a fluorescent-antibody technique which employs fluorescein-conjugated type-specific antisera, M protein can be directly detected in the substance of a L-form colony, thus permitting rapid identification on the initial culture.

Materials and methods. Streptococci. Group A streptococci T1/114/7 (type 1), B930/24/5 (type 3), B90/3 (type 5), S43/100 (type 6), T14/46/4 (type 14), and SS132 (nontypable) were obtained from Dr. R. C. Lancefield, Rockefeller Institute.

L forms of streptococci. Types 3 and 14 L forms were obtained from Dr. E. A. Mortimer, Cleveland Metropolitan General Hospital, Cleveland, Ohio. Types 1 and 5 L forms were isolated by previously described procedures(1). Type 6 L forms were isolated by the method of Mortimer(2).

Cultivation of L forms. L forms were grown on an agar medium prepared with

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