

THE ACTIVITY OF SERUM DEOXYCYTIDYLATE DEAMINASE IN VARIOUS DISEASES*

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SUMMARY

A sensitive method has been utilized for the determination and demonstration of activity of deoxycytidylate deaminase in human sera. Variations in different diseases were also observed. Elevated values were found in all cases of acute liver diseases and acute infections. Serial studies indicated that the enzyme levels were correlated with the stages of the diseases. Elevations were also consistently observed in lupus erythematosus, and to a lesser extent, in rheumatoid arthritis and other collagen diseases. In malignant conditions, about half of the values were elevated. Normal results were obtained in most of the other diseases studied. The enzyme levels were also measured in some human tissues. The possible significance of the results has been discussed in terms of the role that deoxycytidylate deaminase may play in DNA synthesis.

Deoxycytidylate (dCMP) deaminase, which catalyzes the conversion of deoxycytidylate to deoxyuridylate, is believed to play an important role in the de-novo synthesis of DNA. After unilateral nephrectomy of the rat, for example, dCMP deaminase activity is markedly enhanced just prior to an increase in DNA synthesis and mitotic activity in the surviving kidney¹. Similar increases in dCMP deaminase activity have been observed when DNA synthesis and mitosis in liver were stimulated by partial hepatectomy²⁻⁴. Evidence has also been obtained that the activity of dCMP deaminase is regulated by allosteric control through an interplay of end products of pyrimidine metabolism^{5,6}.

The assay of dCMP deaminase in normal adult tissues has been difficult, due to the low enzyme activity. A sensitive method has been developed recently in our laboratory, which permits the determination of dCMP deaminase activity of human tissues and serum⁷. Since the variations of dCMP deaminase activity in different pathological conditions could be of interest in regard to the function of this enzyme and to a more complete understanding of abnormal processes, a number of sera and tissues have been assayed. The results are described below.

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MATERIALS AND METHODS

The major requirement for patient selection was that the patient's diagnosis be well established. Patients with two or more diagnoses which might affect enzyme activity were excluded.

Twelve ml of venous blood were obtained by aseptic technique. The blood was allowed to clot at room temperature. The specimen was centrifuged at 3° for 15–20 min and the serum removed. $1.5 \mu\text{moles}$ of MgCl_2 per 1 ml of serum was added, and the specimen stored at -20° within 2 h after drawing the blood, in order to prevent significant loss of enzyme activity. Specimens were assayed in duplicate according to the method described by Ressler⁷.

The method is based upon the measurement of ammonia formation by means of the Berthelot reaction. Saturating concentrations of substrate are used, resulting in linearity between enzyme activity and time for several hours permitting use of an extended incubated period. The higher substrate concentration, compared to concentrations commonly used, also results in a four to five fold increase in enzyme activity. These factors and the high sensitivity of the Berthelot reaction combine to make the assay of deoxycytidylate deaminase activity in serum feasible.

The coefficient of variation of the analysis of serum by this methods is 6%. All tests in this study were performed in duplicate and when any of the duplicates were different by more than 6%, the test was repeated. Most of the results in this study are related to the normal range, approximately $0.8-2.6 \times 10^{-4} \mu\text{moles}$ of ammonia produced per minute per ml, at 23° . The correlation between a given disease and the test value can, perhaps, best be evaluated by a comparison of the frequency distribution of values of patients with a given disease to the distribution of values of other individuals (normals and other patients with different diseases).

A limited number of necropsy specimens were obtained and activity of the enzyme was determined in various tissues.

RESULTS

Figs. 1 A and B present the results of serum levels of dCMP deaminase in various conditions. The "normal" values, from individuals who were apparently in good health, were within a relatively narrow range. Five determinations of serum activity in one normal individual done on 5 different days did not indicate a large daily variation (Fig. 1 A).

Collagen diseases

Twelve patients with classical rheumatoid arthritis were studied. Most of these patients demonstrated higher activity of serum deoxycytidylate deaminase than control patients (see Fig. 1 A). No correlation could be established between the level of enzyme activity, the extent of the rheumatic process, the degree of alteration of serum proteins, or the chronicity of the disease.

All four patients with systemic lupus erythematosus studied demonstrated definitely increased activity of the enzyme. One patient with scleroderma, and one patient with polyarteritis were studied. Each of these 2 patients had increased serum activity of deoxycytidylate deaminase.

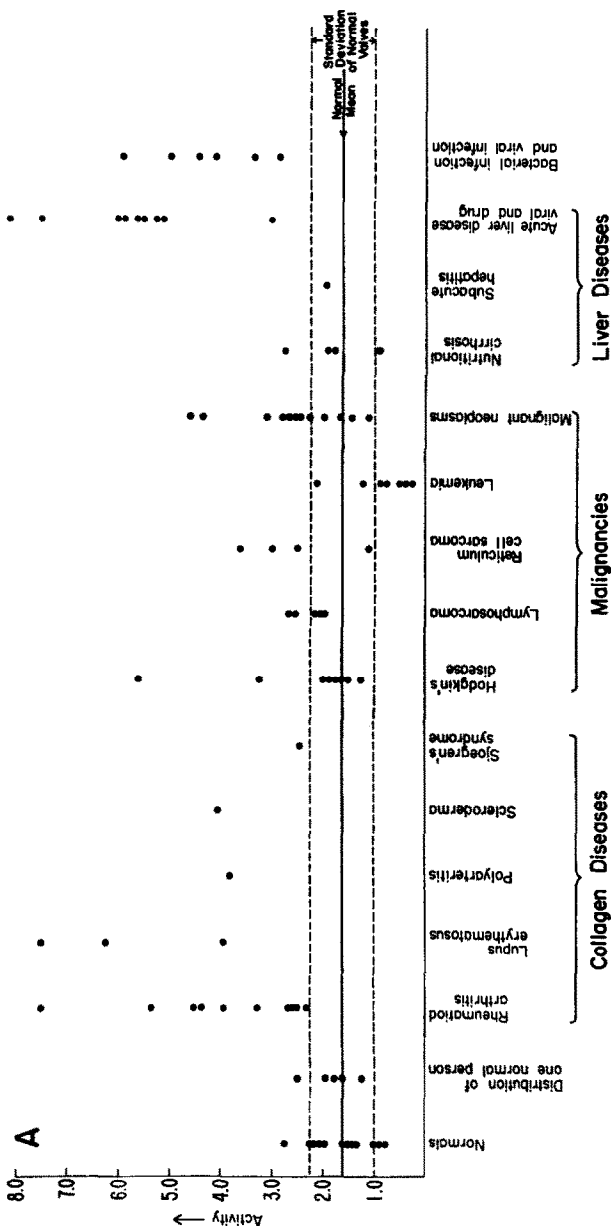
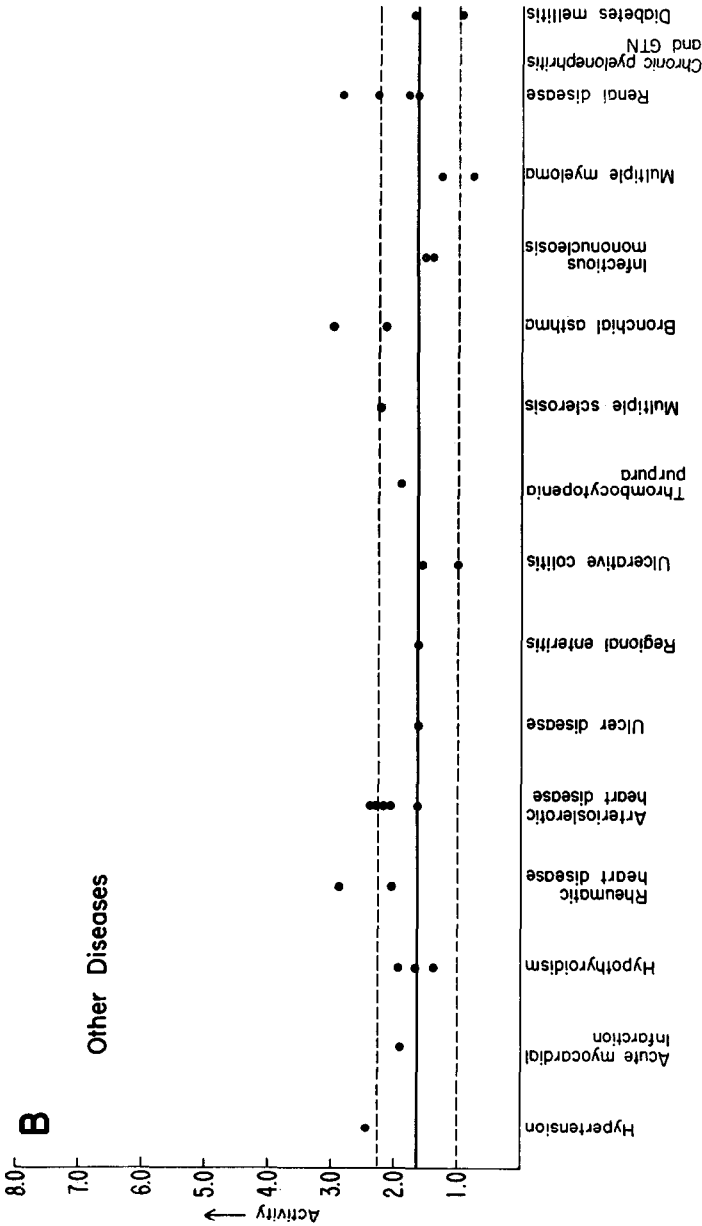


Fig. 1. Serum deoxyctidylate deaminase activity in various clinical conditions. The ordinate represents serum activity in $\mu\text{moles} \times 10^{-4}$ of ammonia produced per min per ml at 23° . The normal mean and ± 1 standard deviation of the normal values are projected across the graph for purposes of comparison.

Leukemia, lymphomas and carcinomas

Sera, of 7 leukemic patients tested, tended to have less enzyme activity than any other group. All of these patients, however, were receiving treatment with at least one cytotoxic agent at the time the deoxyctidylate deaminase activity was measured.



except one. This latter patient had a value within normal limits and it did not change appreciably after cytotoxic agents were administered. Included in this group were patients with diagnoses of acute myelomonocytic, acute stem cell, acute and chronic granulocytic and chronic lymphocytic leukemia; there was no apparent correlation between the serum enzyme activity and the types of leukemia studied.

Twelve patients with malignant neoplasms demonstrated variable serum enzyme activity. About half the values were above the normal range. The enzyme

activities in the sera of patients with lymphomas were also variable; 7 of 17 patients had increased enzyme activity (see Fig. 1A). There was no correlation in these patients between enzyme activity and serum uric acid, or the presence of metastatic lesions, or liver involvement, with or without jaundice.

Liver disease

Sixteen patients with liver disease were studied. Six of the patients had either chronic or subacute liver disease. All of the latter six had normal serum activity of deoxycytidylate deaminase and activity remained normal in the presence of an exacerbation of their disease. These six patients consisted of 3 with nutritional cirrhosis, 2 with chronic active hepatitis, and one with subacute viral hepatitis.

All of the 10 patients with acute liver disease had marked increases in serum activity of the enzyme. Nine of these patients had infectious, or serum hepatitis; one patient had drug-induced hepatitis. The most persistent elevation of serum activity of deoxycytidylate deaminase was observed in a patient with acute liver disease who also developed renal failure.

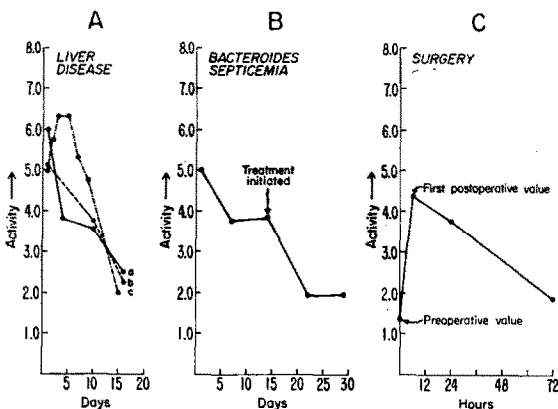


Fig. 2. Serial study of serum deoxycytidylate deaminase activity during the courses of hospitalization. A: Three patients with acute viral hepatitis. B. *Bacteroides* septicemia. C. Surgery.

Unfortunately we were able to evaluate only a few of the patients with acute liver disease for enzyme activity early in the course of their illness (Fig. 2A). Serum enzyme activity in most patients with viral hepatitis approached normal values approximately two weeks after hospitalization.

Infectious disease

Patients with bacterial infections demonstrated high serum activity of dCMP deaminase. Following treatment of the infection, enzyme activity became normal in all patients. To some degree the level of activity was related to the extent of the infection, *e.g.*, urinary tract infections did not seem to exert as much effect on enzyme activity as pneumonia or septicemia even though the sedimentation rate and WBC were comparably elevated. A patient with *bacteroides* septicemia was followed with serial determinations of serum enzyme activity (Fig. 2B). We were able to study only one patient with a documented viral infection. This patient was diagnosed as having

varicella pneumonia, and demonstrated elevated serum enzyme activity throughout her hospitalization.

Other diseases

A number of patients with other diseases were studied. In general, the results were within the normal range. Surgery, however, appeared to be associated with increased serum activity of the enzyme. Two patients followed before and after surgery included one case of a resection of an abdominal aortic aneurysm, and one case of a repair of bilateral inguinal hernias. Maximum serum enzyme activity was present within 6 h following the operative procedure, and returned to normal levels within 4 days (Fig. 2C).

The patients with hyperthyroidism were not receiving any therapy at the time of the assay. In the patients with ulcerative colitis and regional enteritis, the diseases were acute when the test was done. No abnormalities of liver function were detected in the patients with infectious mononucleosis. In the patients with renal disease, azotemia was present, and the condition was either chronic, or an acute exacerbation of a chronic renal disease.

A limited number of patients in the pediatric age group were studied. The patterns of variations of dCMP deaminase activity were similar to those observed in adult patients; but the levels of enzyme activity were higher in most of the respective conditions.

Serial studies

Serial studies were done on 3 patients with acute liver disease (Fig. 2). The enzyme levels decreased as the condition improved. The first value of curve C in Fig. 2A was obtained early in the course of the disease. Decreasing deoxycytidylate deaminase activity generally indicated improvement before other serum enzyme activities declined.

Fig. 2B represents a serial study of a patient with bacteroides septicemia. Specific therapy was initiated on the 15th hospital day. Fig. 2C shows the effect of surgery (repair of bilateral inguinal hernias), upon serum enzyme activity. The activity rapidly declined from the first post-operative value.

Tissue levels

Specimens of various tissues were obtained at post-mortem examinations and assayed for dCMP deaminase activity. Enzyme activity decreased slowly over several days when the tissues were stored at -20° . The results are presented in Table I. The highest values were generally found in liver and spleen. One spleen specimen, which had considerably less activity than any of the other spleens was from a patient who had aplastic anemia and septicemia.

DISCUSSION

The data appear to provide some consistencies between serum levels of dCMP deaminase and clinical conditions. The causes of the elevation of serum dCMP deaminase activity were not established in the present study. The possibility that the enzyme is liberated into the circulation from damaged tissue is consistent with the

TABLE I

dCMP ACTIVITY OF HUMAN TISSUES

($\mu\text{moles} \times 10^{-2}/\text{min/g}$ tissue at 37°)

<i>Autopsy tissue number</i>	<i>Diagnoses</i>	<i>Liver</i>	<i>Heart</i>	<i>Lung</i>	<i>Skeletal muscle</i>	<i>Pan- creas</i>	<i>Spleen</i>	<i>Kidney</i>	<i>Thymus</i>	<i>Thyr.</i>
1	Hodgkin's disease Stage IV	28	—	—	2.5	—	20	—	—	—
2	Cerebral abscess 8-year-old child	—	—	—	—	—	—	—	9.6	—
3	Aplastic anemia Septicemia	12	1.4	1.7	1.1	9	3.8	—	—	1.7
4	Metastatic carcinoma Congestive heart failure Pneumonia	21	4.6	4.4	1.6	17	—	—	—	—
5	Pancreatitis, hypertension Renal failure Pneumonia	20	4.6	20	4.0	—	21.5	—	—	—
6	Metastatic adeno-carci- noma (Lung primary)	12	2.4	3.2	0.3	3.8	27.1	10.8	—	—
7	Relatively normal (Died of gunshot wound)	13.5	0.72	1.3	0.6	1.3	8	3.8	—	—

elevated levels observed after surgery, as well as in pyogenic infections, acute liver disease, and collagen diseases. This explanation would appear unlikely for all of the elevations, however, since patients with chronic or subacute liver disease, who had elevated levels of other serum enzymes such as SGOT or SGPT, nevertheless had normal dCMP deaminase activity.

The results are consistent with the possibility that elevated dCMP deaminase levels are associated with cellular proliferation and increased DNA synthesis. The lack of elevated enzyme activity in chronic or subacute liver disease could be related to observations that liver damage occurring gradually over prolonged periods may be associated with minimal observable changes in DNA synthesis⁸.

Perhaps, the serum elevations are related, more specifically, to activity of the reticuloendothelial system. Thus elevations were always observed in cases of acute liver disease and acute bacterial infections, when DNA synthesis in such cells may be expected to be quite active; in cases of collagen diseases or cancer in which the response of the reticuloendothelial system to the clinical condition may be less direct, elevations of dCMP deaminase activity were less consistent; relatively low levels of dCMP deaminase activity were found in patients on cytotoxic drugs; and activity in liver and spleen was generally higher than in other tissues. At the present time no single hypothesis for elevations of dCMP deaminase activity in the plasma satisfactorily explains all of our findings.

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