ADENOSINE DEAMINASE AND AMP DEAMINASE ACTIVITIES IN BLOOD OF CANCER PATIENTS

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INTRODUCTION

Many authors in recent years have reported elevated activities of various enzymes in plasma or serum of cancer patients. Particular attention has been paid to increases in certain glycolytic enzymes, notably lactic dehydrogenase, phosphohexose-isomerase and aldolase (Wroblewsky, 1959; Bodansky, 1954; Sibley and Lehninger, 1949; Gullino et al., 1960, 1961). The subject has been recently reviewed by White (1960). These authors have, however, drawn attention to the unspecific nature of these tests from a diagnostic point of view, since such abnormalities usually occur only in advanced cancer and are often associated with other diseases involving inflammation, or tissue wasting.

Adenosine deaminase activity in the blood of diseased patients was investigated in this laboratory in 1941 by Dr. Isabel Farrell (then Isabel Carey), who found the activity of this enzyme to be abnormally high in leukaemic blood. The matter was not pursued further as Miss Carey then began to study for a medical degree. More recently, attention was again drawn to the possible significance of blood adenosine deaminase activity in cancer patients in general when Straub et al. (1957) reported that the enzyme was elevated in the plasma in 92% of the 527 cancer cases studied. Similar results were obtained by Letnansky and Seelich (1958), who also found raised plasma enzyme activity in inflammatory diseases. Both groups of authors suggested the use of plasma adenosine deaminase activity as a diagnostic test for the presence of cancer, although Letnansky and Seelich mentioned the desirability of further work to ascertain whether the enzyme levels were a function of the size and nature of the tumour. With regard to the source of the increase, Straub and his colleagues showed that in the case of Ehrlich ascites tumour in mice, the increased adenosine deaminase is produced by the cancer cells. In agreement with this, Fodor et al. (1958) and de Lamirande et al. (1958) have found this enzyme to be greatly increased in tumour tissue in vitro.

In view of the findings of Straub et al. (1957) and Letnansky and Seelich (1958) it was decided to investigate further the utility of plasma adenosine deaminase levels as a diagnostic test for the presence of cancer. It was also thought of possible interest to follow alterations in activity in certain patients during treatment with a view to correlating these changes with the course of the disease.

Concurrent estimations of AMP deaminase were also carried out in many of the same subjects; very little attention has been devoted to this enzyme (muscle adenylic acid deaminase) since the work of Conway and Cooke (1939), and it was considered possible that it too might show changes in cancer.
In addition, both enzymes were estimated in laked blood cells after removal of the plasma.

Methods

Heparinised blood samples from cancer patients (and from some normal subjects) were obtained from the Research laboratory, St. Luke’s Hospital, Dublin. The blood was centrifuged as soon as possible, usually within 1 hour, since it was noted that a 24-hour delay in separation caused a considerable increase in AMP deaminase in the plasma, though no significant increase in adenosine deaminase occurred. Haemolysis, however slight, caused an increase in both enzymes in the plasma, therefore all haemolysed samples were discarded.

Both enzymes were measured in terms of ammonia liberation from the relevant substrates during a fixed period using reaction mixtures described by Conway and Cooke (1939). For plasma adenosine deaminase, this consisted of 1 ml plasma, 1 ml 0.1 M potassium phosphate buffer (pH 7.2), and 0.5 ml 0.2% adenosine (Light & Co.). For plasma AMP deaminase, 0.5 ml plasma was added to 1 ml water and 1.5 ml of a 2% (NaOH-neutralised to pH 7.0) solution of adenosine-5-monophosphate (Schwartz, Inc.). For both plasma enzymes, ammonia liberation was measured after 30 min. incubation in a water bath at 37°C. For estimation of blood cell enzymes the cells, after removal of the plasma, were laked by adding water up to the original blood volume. The reaction mixture for each enzyme was the same as that used with plasma, but due to the much higher activities in blood cells, the reactions were made to take place at room temperature and measured over a 10 min. period only. Glass-distilled water was used in making up all solutions concerned with the reaction mixtures as water passed through a deioniser was found to inhibit AMP deaminase.

Ammonia was determined by Rieff’s (1960) modification of Conway’s (1957) microdiffusion method. Conway units were prepared in advance with 1 ml 0.1% boric acid in the centre and 1 ml. saturated potassium carbonate in the outer chamber. At zero time and again after the incubation period, 0.5 ml. samples (in duplicate) of the reaction mixtures were added to the outer chambers and the units quickly sealed and rotated. The potassium carbonate stopped further enzyme activity as well as liberating the ammonia formed. After 1 hr. diffusion, titrations were carried out using 0.001 N HCl. Units of enzyme activity are defined as µg ammonia liberated from 1 ml plasma or laked blood cells per hour. Standard and blank determinations were made on each occasion.

Results

(1) Comparison of normals and cancer patients

Plasma adenosine deaminase

The range for 31 normal subjects was 0.00-11.4 units. The mean was 5.1 ± 0.48 (standard error of the mean). Seventy-six cancer patients (on first determination) ranged from 1.80 to 87.0, with a mean of 8.9 ± 1.2. The difference between the mean values for normal and cancer subjects can be considered significant (3.8 ± 1.3), but is mainly due to a small proportion of very high values in the cancer group.