*Clin. exp. Immunol.* (1976) 26, 647–649.

*BRIEF COMMUNICATION*

# Adenosine deaminase activity in thymus and other human tissues

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(*Received* 2 *August* 1976)

SUMMARY

Adenosine deaminase activity (ADA) has been estimated in human tissues. Levels in the thymus during childhood were very much higher than in any of the other 6 tissues studied. Intermediate activities were obtained from spleen and lymph nodes and also skin. Cerebral cortex, liver and kidney had relatively low levels.

ADA activity in lymphocytes from peripheral blood was significantly increased after antigenic stimulation by TAB immunization.

The available evidence appears to be consistent with T-lymphocyte growth and development in the thymus being dependant on ADA.

## INTRODUCTION

Adenosine deaminase (adenosine aminohydrolase, EC 3.5.4.4) or ADA has been found in most mammalian tissues in which it has been studied. A single genetic locus on chromosome 20 primarily controls ADA in man (Hirschhorn, 1975). Interest in this enzyme has been revived by the association between combined immunodeficiency and ADA deficiency (Meuwissen & Pollara, 1974) and further stimulated by the finding of a deficiency of the metabolically related enzyme nucleoside phosphorylase in a child with defective T-cell function (Giblett *et al.*, 1975).

The present study has revealed high ADA activity in human thymus and added estimates of ADA activity in a variety of other human tissues, to those already available for other species (Brady & O'Donovan, 1965; McGuire *et al.*, 1976). It is suggested that finding high activities in human thymus may help our understanding of the pathogenesis of combined immunodeficiency in ADA deficiency.

## MATERIALS AND METHODS

Lymphocytes were obtained from heparinized human peripheral blood by a Ficoll–Paque technique (Böyum, 1968). Samples of thymus were obtained at open heart surgery at the Royal Hospital for Sick Children, Edinburgh (Mr. W. H. Bissett). Samples of thymus and other tissues were obtained from post mortems on children aged up to 1 year, generally within 24–48 hr of death.

Tissue samples were washed, minced and homogenized in about 5 vol. of 0·1 mol/l phosphate buffer pH 7·0 using an Ultra Turrax TP18-10 (Janke & Kunkel KG Staufren-i-Breisgau, Federal Republic of Germany). The homogenates were centrifuged at 20,000 *g* for 30 min at 4°C and the supernatant used for assay.

*Adenosine deaminase.* This activity was estimated by the method of Kalckar (1947) monitoring the decrease in absorbance at 265 nm. Three ml of phosphate buffer pH 7·0 containing 100 $\mu$mol/l adenosine were preincubated at 37°C. The reaction was started by the addition of 20–100 $\mu$l of tissue extract. After a lag phase of 1–2 min the absorbance was monitored using a Unicam SP 800 with scale expansion and a coupled recorder. The reaction was linear with respect to volume of extract during the assay period. This method has been widely used and therefore comparisons with existing data can be made. However, substrate concentrations for maximal velocities are not practicable due to the high molar extinction of adenosine at 265 nm.

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Activity was expressed as nmol of adenosine deaminated per hr per mg of protein. Protein was estimated by the Miller (1959) modification of the method of Lowry *et al.*, (1951).

## RESULTS AND DISCUSSION

### *ADA activity in lymphocytes before and after immunization*

Lymphocytes were obtained from three healthy volunteers before and 14 days after the completion of TAB immunization required for their overseas travel.

The ADA activity was increased in all three individuals, respectively from 900–1115, from 324–978 and from 545–1552 nmol adenosine/hr per mg protein. The overall means were significantly different ($P<0{\cdot}001$) using the Student's *t*-test. These findings are consistent with the effects on ADA of antigenic stimulation in the rabbit (Hall, 1963) and with the results of *in vitro* lymphocyte stimulation (Hovi *et al.*, 1976) and inhibition (Carson & Seegmiller, 1976) in man.

TABLE 1. Adenosine deaminase activity in human tissues

| Tissue | nmol adenosine/hr per mg protein | |
|---|---|---|
| | mean ± s.e.m. | *n* |
| Thymus (surgical biopsies) | 22,642 ± 10,179 | (3) |
| *Post-mortem samples* | | |
| Thymus | 13,811 ± 2965 | (5) |
| Lymph node | 2098 ± 575 | (7) |
| Spleen | 1151 ± 237 | (7) |
| Skin | 2549 ± 910 | (7) |
| Cerebral cortex | 635 ± 294 | (4) |
| Liver | 132 ± 38 | (7) |
| Kidney | 237 ± 56 | (7) |

### *ADA activity in human tissues*

Our most striking finding is the very high levels of activity found in human thymus. Unfortunately very little other data is available on this tissue; during the course of our work three high activities were noted by Tung *et al.* (1976) for human tissue. The horse, however, shows unremarkable levels of activity in thymus and no detectable activity in erythrocytes despite relatively high levels in lymph nodes and spleen (McGuire *et al.*, 1976). It may also be relevant that a form of severe combined immunodeficiency found in foals was not associated with deficiencies of ADA or nucleoside phosphorylase. The spleen showed a relatively high level of activity as found in six other mammalian species, rat, guinea-pig, mouse, cat, rabbit and dog. Although lymph nodes were less widely studied, high activities have also been found in other species (Brady & O'Donovan, 1965).

The relatively high activity in our samples of human skin may be linked to the easily detectable activity in cultured skin fibroblasts and cultured amnion cells (Adams, McVie & Harkness, in press). Relatively high ADA activity has also been noted in rat skin (Block & Johnson, 1954).

The results in Table 1 provide some evidence for the instability of this enzyme in human tissues since the mean level in the surgical biopsies is almost twice that from post-mortem material. It is, however, not yet possible to exclude an age difference as the cause for the difference in the two means since cardiac surgery patients were aged 3–12 years. The relative order of the different tissue activities in post-mortem samples was similar in all individuals studied. Therefore some overall correction for post-mortem autolysis on the basis of the two means for the thymus may be justifiable despite the existence of tissue specific processing of the common structural unit of ADA (Hirschhorn, 1975).

The mechanism by which ADA deficiency may cause combined immunodeficiency is unknown. ADA activities increase during 'growth' in lymphocytes and in other tissues for example regenerating mouse liver (Rothman *et al.*, 1971). The function of ADA is unknown but the detoxication of adenosine has been frequently advanced as a possibility (Brady & O'Donovan, 1965). The enzyme ADA would be especially important where other mechanisms of adenosine removal are not available. If other enzymes for adenosine metabolism in thymus are relatively inadequate then ADA deficiency could severely inhibit T-cell growth and development in the thymus. The very high activities of ADA in human thymus may indicate an anatomical site for the biochemical effects of ADA deficiency.

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