Studies on enolase isoenzymes in normal and pathological human tissues

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Enolase has three immunologically distinct subunit types α, β and γ (Fletcher et al., 1976; Pearce et al., 1976). In the rat the β-subunit is essentially confined to the muscular tissues and the γ-subunit to neurons and cells of the APUD (amine precursor uptake and decarboxylation) system (Schmechel et al., 1978). Immunological titration with monospecific antisera suggests that the distribution of these subunits in human tissue extracts is rather less specific and that the occurrence of the αβ-hybrid may be widespread. These findings have important implications if enolase isoenzymes are to be used in diagnostic tests and particularly in determining damage to specific tissues (Herranz-Dominguez et al., 1975). The problem has been further investigated by immunohistolocalization studies on normal human tissues. The findings for the cellular distribution of α-, β- and γ-subunits of enolase are consistent with the results of immunological titration of tissue extracts.

Preliminary work to assess the potential use of enolase isoenzymes as markers of pathological change has been carried out using the peroxidase/anti-peroxidase technique of immunohistochemistry on a wide range of tumours from tissues of neural-crest origin.

The results of the studies on cerebral tumours show that the enolase isoenzyme composition of a tumour is similar to that of the cell type from which it is derived. Thus astrocytomas, ependymomas and acoustic neuromas show strong staining for α-enolase, oligodendrogliomas only weak staining for this isoenzyme, whereas neuroblastomas and medulloblastomas showed no enolase staining. No cerebral tumour was found to contain γ-enolase. Ischaemic and necrotic tissues showed diminished enolase activity and this might be expected to result in a leakage of enolase into the cerebrospinal fluid, accounting for the raised concentrations found in the cerebrospinal fluid of many patients with cerebral tumours (Royds et al., 1981).

Melanomas are tumours derived from cells that originate from the neural crest, and immunohistolocalization studies of the α- and γ-enolases show that the malignant melanocytes of the dermis normally contain both of these isoenzymes. No enolase could be detected either in normal or malignant melanocytes that are found in the epidermis. This contrasts with the finding that benign intradermal naevi show strong staining for both α- and γ-isoenzymes with α-enolase usually present in excess. The α/γ ratio appears to increase with the degree of malignancy with some of the highly de-differentiated cells having no demonstrable γ-enolase.

Tumours of APUD cells have been studied using the same immunological staining technique. Most tumours stained for both α- and γ-enolase with the exception of the oat-cell carcinoma, where five out of 11 cases showed only α-enolase.

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Carbonic anhydrase III and other cytoplasmic enzymes in dystrophic mouse tissues

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Studies on dystrophic mice have shown clearcut changes in the levels of soluble muscle enzymes, notably a significant decrease in almost all glycolytic enzymes, only the pentose phosphate pathway activity was found to be significantly increased (Kitahara et al., 1977; Watts, 1978).

The discovery, in this laboratory, of very high levels of CAIII in normal rodent liver (an enzyme previously thought to be muscle specific, at high levels) (Carter et al., 1981) led to the assay of this enzyme and other soluble enzymes in dystrophic mouse liver and muscle. The objective was to define the expression of the dystrophic gene in muscle and liver and also to define other enzyme-regulating effects in liver related to sex differences.

Enzyme levels were measured with standard kinetic techniques and in addition CAIII was estimated by radial immuno-

* Abbreviation: CAIII, carbonic anhydrase III.

diffusion. The results are summarized in Table 1 and the following main conclusions can be drawn.

(a) There are highly significant (P = 0.001) differences in the normal male/female levels of liver and muscle CAIII. Thus there is a marked sex difference in tissue expression against which the dystrophic phenotype is now considered.

(b) In dystrophic (C57Bl/dy) muscle, enzyme levels are invariably lower (see Table 1), with the exception of glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase (both pentose phosphate pathway catalysts) and CAIII. These results are in agreement with the studies involving stimulation models of dystrophic muscle (Reichmann et al., 1981). The raised pentose phosphate pathway activity possibly represents increased lipid deposition in the sarcoplasm in the presence of degenerating fibres. The raised CAIII is a paradox but may be explained by increased content of red fibres, since the dark muscles in rat (of soleus) contain 10 times the CAIII level of fast muscle such as gastrocnemius (N. D. Carter & S. Jeffery, unpublished work). The enzyme has been shown to be totally cytoplasmic (R. Heath, S. Jeffery & N. D. Carter, unpublished work).

(c) In dystrophic liver there are marked reductions of CAIII
Table 1. Percentage difference in activities between enzymes in normal and dystrophic mice

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Dystrophic normal</th>
<th>Number of animals</th>
</tr>
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<tbody>
<tr>
<td>Muscle 6-phosphogluconate dehydrogenase</td>
<td>123%</td>
<td>Number of animals</td>
</tr>
<tr>
<td>Muscle glucose 6-phosphate dehydrogenase</td>
<td>207%</td>
<td>30d</td>
</tr>
<tr>
<td>Muscle pyruvate kinase</td>
<td>70%</td>
<td>13d</td>
</tr>
<tr>
<td>Muscle phosphoglucone isomerase</td>
<td>83%</td>
<td>28d</td>
</tr>
<tr>
<td>Muscle creatine kinase</td>
<td>35%</td>
<td>Small sample</td>
</tr>
<tr>
<td>Male liver** CO, hydrase CAIII</td>
<td>58%</td>
<td>22n</td>
</tr>
<tr>
<td>Female liver** CO, hydrase CAIII</td>
<td>93%</td>
<td>N.S.</td>
</tr>
<tr>
<td>Male Muscle** CO, hydrase CAIII</td>
<td>172%</td>
<td>13d</td>
</tr>
<tr>
<td>Female Muscle*** CO, hydrase CAIII</td>
<td>151%</td>
<td>9d</td>
</tr>
</tbody>
</table>

N.S., Not significant.
* Values from Mancini immunoassay (Mancini et al., 1964).
** CAIII valves are not combined for males and females since highly significant sex differences of CAIII are observed, with male-mouse liver homogenate having about 50% more activity than the female liver.

levels in both sexes though most strikingly in the male mouse (Table 1). Murine dystrophy is not sex-linked but from the male mouse data there appears to be a sex-influence on the gene expression. In human muscular dystrophy the disorder is thought to be expressed in brain cells and erythrocytes (Pennington, 1978). In the mouse the synthesis and turnover of CAIII in normal and dystrophic muscle and liver are different; however, the basic lesion of muscle degeneration and leakage of cytosol proteins from muscle to plasma is similar to the human phenotype.

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Radioimmunoassay of carbonic anhydrase III levels in plasma as an index of tissue damage

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Carbonic anhydrase III (CAIII) is a low-activity skeletal-muscle-specific enzyme in man, present at about 2% of soluble protein (Carter et al., 1979). Patients with Duchenne muscular dystrophy show markedly elevated plasma levels of CAIII (Carter et al., 1980). The present report describes the monitoring of CAIII in a range of clinical conditions where plasma enzyme levels are routinely determined. In particular creatine kinase (CPK) levels are compared with CAIII. The radioimmunoassay will be described in detail elsewhere (R. Heath, S. Jeffery & N. D. Carter, unpublished work). The other enzyme levels quoted were measured in the routine chemical pathology laboratory by standard procedures.

Fig. 1 shows the mean levels of CAIII and CPK in a range of clinical conditions and Fig. 2 shows a plot of CAIII against CPK. The basic findings are outlined below. (a) In all individuals, where there is skeletal-muscle breakdown or muscle trauma, CAIII is likely to be raised. (b) In conditions where there is cardiac-muscle damage, e.g. myocardial infarction, CAIII was not found to be significantly raised in spite of very high levels of myocardial CPK in plasma (Fig. 1). The tissue level of CAIII, in heart muscle, is only a fraction of skeletal muscle (approx. 1%) (Jeffery et al., 1980) and this provides the likely explanation. (c) In other diverse conditions plasma CAIII may, on occasions, be raised (e.g. pulmonary embolism hypothyroidism), but the consistency of these results awaits confirmation.

We conclude that CAIII measurement is a useful adjunct to CPK in determination of muscle disorders and, with the