Colleran & Ó Carra, 1969). As specific cleavage of haem at the α -bridge to yield the IX α -isomer of biliverdin is the most outstanding characteristic of the process *in vivo*, we have analysed the isomer composition of the products obtained *in vitro* as follows.

The biliverdin product is treated with methanolic 5% H_2SO_4 at 2° for 16 hr. The resulting biliverdin dimethyl ester is extracted into chloroform after addition of 4 vol. of water and subjected to t.l.c. on silica gel G in *n*-heptane-ethyl methyl ketone-acetic acid (10:5:1, by vol.). After double development in one dimension, the dimethyl esters of the four biliverdin isomers separate cleanly in the order (bottom to top): α ; β or δ ; δ or β ; γ . They are scraped off, eluted into methanol and estimated spectrophotometrically at 670 nm.

Myoglobin yielded the most clear-cut results, owing apparently to the absence of cysteine residues from its structure. After aerobic incubation with ascorbate at pH 7.4 and 37° for 2 hr. the biliverdin formed (which was extracted by the method of Lemberg, Legge & Lockwood, 1938) was found to consist entirely of the IX α -isomer. When the incubation was carried out under denaturing conditions (e.g. in the presence of 8 M-urea) the specificity of the cleavage was destroyed and a mixture of the four isomers was obtained.

This 'suicide reaction' of myoglobin thus has the specificity characteristics of an enzyme reaction, the haem-binding site being analogous to an active site with three co-substrates: haem, ascorbate and oxygen. It is interesting that it is the α -carbon bridge of the haem group that is specifically labilized, since the X-ray-diffraction data (cf. Kendrew, 1963) show this bridge to be the most buried and least accessible of the four.

These results suggest that the coupled oxidation of myoglobin with ascorbate is a model that may be of significance *in vivo*.

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The Function of the Non-Specific Adenylyl Aminohydrolase of *Aspergillus oryzae*

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The adenylyl aminohydrolase of *Aspergillus oryzae* has been purified from Taka-diastrase powder and shown to deaminate all naturally occurring

adenine nucleotides and coenzymes with the exception of those having a phosphate group at the 2'-position (Minato, Tagawa & Nakanishi, 1965; Minato, Tagawa, Miyaki, Shimizu & Nakanishi, 1966; Wolfenden, Sharpless & Allan, 1967). However, little interest has been shown in the function of this enzyme.

We have found that the enzyme was induced when the organism was transferred to a nitrogen-deficient medium (6% glucose in 0.1 M-phosphate buffer, pH 5.9) after 42 hr. growth in complex medium. Cycloheximide prevented the induction of the enzyme and ammonium chloride repressed the enzyme synthesis. Acid phosphatase and ribonuclease activities were similarly induced and repressed, suggesting that the three enzymes are involved in the catabolism of RNA in nitrogen deficiency to provide a source of nitrogen for growth.

Measurement of the concentrations of RNA in mycelial extracts showed that the catabolism of RNA proceeded to a greater extent when the organism was suspended in nitrogen-deficient medium than when it was in medium containing ammonium chloride. Later investigation showed that most of the ribonuclease activity was extracellular whereas the adenylyl aminohydrolase activity was found mainly in the mycelial extracts. It seems likely that the hydrolysis of RNA took place in the medium into which it was released by autolysis. AMP produced in this way could be degraded first to IMP by adenylyl aminohydrolase and then to inosine by acid phosphatase. The low rate of extraction of these two enzymes by ultrasonic disintegration suggested that they might be bound to the cell wall or membrane.

The adenylyl aminohydrolase thus appears to be part of a digestive system, and its function may be compared with that of the specific adenosine aminohydrolase that is found in similarly high activities in mammalian intestinal mucosa.

It is unlikely that the increase in the activity of the enzyme which occurred when the organism was suspended in nitrogen-deficient medium was due to substrate induction because the addition of adenosine to the medium prevented the increase. The apparent repression of the enzyme by adenosine was probably due to its conversion of inosine and ammonia. Both of these substances repressed the enzyme synthesis when added to the medium but, of the two, inosine (or IMP) seemed more likely to be the repressor.

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Purification of Adenosine Deaminase from Bovine Lung and Spleen

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Adenosine deaminase has been purified from a number of bovine tissues: intestinal mucosa (Brady & O'Connell, 1962), ox heart (Rockwell & Maguire, 1966), spleen (Pfrogner, 1967; O'Brien & Tully, 1968) and serum (Cory, Weinbraun & Suhadolnik, 1967). Although considerable similarities are apparent in some of their properties, the reported specific activities of these enzymes show wide variation from a value of 1 for the serum enzyme to 450 for the mucosal enzyme.

It was therefore decided to purify the enzyme from a number of tissues with a view to comparing their properties. In the present communication purifications from bovine lung and spleen have been made yielding preparations with specific activities of 400.

The deaminase from lung tissue was extracted from homogenized tissue with 0.02M-phosphate buffer, pH 7.0, followed by heating at 70° for 10 min. and acidification to pH 4. The neutralized supernatant was fractionated with acetone. Adenosine deaminase was precipitated between 100–150% (v/v) acetone. The dissolved enzyme was chromatographed on DE-52 DEAE-cellulose (Whatman), pH 5.8, with a 2–33 mm gradient of citrate buffer, and finally on Sephadex G-75 (superfine grade). The preparation had a specific activity of 420.

An improved method that introduced two non-consecutive ammonium sulphate fractionations in the purification of adenosine deaminase from spleen gave a preparation with a specific activity of 400.

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The Relationship between Plasma and Tissue Vitamin E Concentrations in Man

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Vitamin E is widely distributed in human tissues (Pappenheimer & Victor, 1946). Deficiency of the vitamin in man is thought to occur at plasma concentrations less than 0.5 mg./100 ml. (Leonard, Losowsky & Pulvertaft, 1966). Tissue concentrations, although not necessarily reflected by plasma concentrations, are a better index in assessing deficiency. Apart from information obtained by prior loading with small doses of labelled or large doses of unlabelled vitamin E, little is known of the relationship between tissue and plasma vitamin E and the significance of the latter as a guide to tissue vitamin E depletion in man.

In the present study vitamin E concentrations were examined in tissues by using biopsy and post-mortem specimens and were compared with the concentrations found in the corresponding plasmas. Thirty specimens of rectus abdominis muscle were obtained at operation and 22 liver and 11 heart specimens were obtained *post mortem*. Tissue vitamin E was determined by the method of Bieri (1961) and plasma vitamin E by the method of Martinek (1964). A similar pattern of results was obtained for all tissues. There was an almost linear increase in the tissue concentration as the plasma concentration rose to 0.5 mg./100 ml., but the curve flattened off at higher plasma concentrations. Tissue concentrations in excess of 4 µg./g. were always associated with plasma concentrations greater than 0.5 mg./100 ml. It is concluded that plasma vitamin E concentrations less than 0.5 mg./100 ml. reflect low vitamin E concentrations in the tissues studied.

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