containing 5 g. of bovine plasma albumin/100 ml., 11 mM-glucose, amino acids as listed by Scholz & Bücher (1965), 1.5 mM-sodium lactate and 25 ml. of fresh heparinized whole rat blood/100 ml. Bile was analysed for bilirubin, β -glucuronidase and acid phosphatase by methods described by Slater & Griffiths (1965); total bile acids were measured by using 3α -hydroxy steroid dehydrogenase. Representative samples of the median lobe were fixed in 4% glutaraldehyde-0.1M-cacodylate buffer, pH 7.2, post-fixed in 1% osmic acid in Millonig's buffer, and examined in an AEI EM6B electron microscope.

The introduction of approx. 10μ moles of either sporidesmin or icterogenin into the perfusion medium (120 ml.) resulted in a rapid decrease in bile flow, which had virtually stopped within 30–45 min. of dosing. Electron-microscopic examination of sporidesmin-treated livers showed swelling of the microvilli in the biliary canaliculi and alterations in the endoplasmic reticulum. These results, together with changes found in bile acid excretion and in the acid hydrolase content of bile, will be discussed.

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Isoenzymes of β -Hydroxybutyrate Dehydrogenase in *Rhizobium* spp.

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Poly- β -hydroxybutyrate is one of the principal storage products in several bacterial species, including *Rhizobium* spp. (see Dawes & Ribbons, 1964). D(-)- β -Hydroxybutyrate, a key intermediate in the conversion of poly- β -hydroxybutyrate into cell material, is converted into acetoacetate by β -hydroxybutyrate dehydrogenase (Gavard, Combre & Tuffet, 1960; Shuster & Doudoroff, 1962).

In the present investigation, isoenzymic forms of β -hydroxybutyrate dehydrogenase were detected in several *Rhizobium* spp. The isoenzymic patterns of β -hydroxybutyrate dehydrogenase from fourteen strains of *Rhizobium*, representing six species, were compared. All the *Rhizobium* strains studied nodulated their respective host legumes. Some of the legume-*Rhizobium* combinations, however, were capable of fixing nitrogen whereas others were not.

Starch-gel (Smithies, 1955, 1959) and disc electrophoresis (Davis, 1964) were used to compare β -hydroxybutyrate dehydrogenase isoenzymes from different *Rhizobium* strains. Also, the rate of reaction of β -hydroxybutyrate dehydrogenase, from different rhizobial strains, with various coenzyme analogues (Kaplan, Ciotti, Hamolsky & Bieber, 1960) was compared with the rate of reaction of the enzyme with NAD. The following analogues of NAD were used: acetylpyridine-NAD, pyridine aldehyde-NAD, deamino-NAD, acetylpyridine deamino-NAD.

On both starch and polyacrylamide gels, β -hydroxybutyrate dehydrogenase from several rhizobial strains was separated into two or more isoenzymic forms. One strain of *R. leguminosarum*, for instance, had five isoenzymes when examined by starch-gel and disc electrophoresis and this pattern was reproduced on six different occasions. It was possible therefore to identify individual strains by their isoenzyme patterns. Likewise, the rate of reaction of β -hydroxybutyrate dehydrogenase with NAD, expressed as a ratio of the rate of reaction with one of the aforementioned coenzyme analogues, was characteristic of several strains. For example, the ratio of the reaction rates of β -hydroxybutyrate dehydrogenase with NAD and acetylpyridine deamino-NAD was twice as high for one strain of *R. leguminosarum* as that for another strain of the same species.

Zymograms of β -hydroxybutyrate dehydrogenase were also prepared from the bacteroid fraction of soya-bean root nodules. Zymograms obtained from the bacteroid fraction were very similar to zymograms prepared from the strain of *R. japonicum* used to inoculate the soya beans.

These investigations show that β -hydroxybutyrate dehydrogenase isoenzymes may have important applications in differentiating between *Rhizobium* strains and in studying the metabolism of *Rhizobium* bacteroids in legume root nodules.

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Studies on Purified Bovine Spleen Adenosine Deaminase

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Adenosine deaminase has been purified from bovine spleen. All the purified spleen preparations had a typical protein extinction spectrum with a maximum at 278 m μ . A study of the substrate specificity showed that the enzyme deaminates deoxyadenosine at approximately the same rate as adenosine, and the results are comparable with those obtained for adenosine deaminase from bovine duodenal mucosa. 6-Chloropurine riboside was dechlorinated by the spleen enzyme at a slower rate than by the duodenal enzyme. There was a marked difference between the heat stability of the two enzymes. The pH optimum and stability of the enzyme were similar to that reported by Brady & O'Sullivan (1967) for the duodenal enzyme.

Ag⁺, Hg²⁺ and Cu²⁺ ions as well as iodosobenzoic acid and iodine inhibited the enzyme. *p*-Chloromercuribenzoic acid and *N*-ethylmaleimide completely inhibited the enzyme, and these results point to the importance of a thiol group for the activity of the enzyme. In this respect it was similar to the bovine duodenal enzyme. The stability of the enzyme to freeze-drying decreased as enzyme purity increased, which again was similar to the duodenal enzyme. By electrophoresis the number of isoenzymes present in spleen extracts was found to vary from one to five, and the original pattern persisted throughout the purification. The electrophoretic pattern of the duodenal enzyme varied from intestine to intestine, giving a pattern of five or six isoenzymes when the intestines from various animals were mixed. The Michaelis constants of both enzymes were very similar.

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Disulphide Bonds in Relation to the Activity and Structure of Adenosine Deaminase

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Amperometric-mercurimetric titration of adenosine deaminase, with both inorganic and organic mercurials, revealed the presence of two disulphide bonds in the protein molecule. Results obtained by using two different procedures of the above titration technique (Leach, 1966), were compared and assessed in relation to the total sulphur content of this enzyme.

Reduction of the disulphide bonds of adenosine deaminase with sodium sulphite resulted in an instantaneous and marked fall in the enzyme activity. The residual activity of the reduced enzyme when assayed at pH 9.3 and 7.0 was similar, showing that reformation of disulphide bridges, which might be favoured by the lower pH, was not accompanied by reactivation of the enzyme. Iodoacetamide, which was shown to have no effect on the activity of the native enzyme, accelerated the rate of inactivation by sulphite.

Finally, the effect of reducing the disulphide bonds on the overall shape of the enzyme molecule was studied by the Sephadex-gel filtration technique. Comparable studies were carried out on other proteins of known disulphide bond content, and results were compared with those obtained for adenosine deaminase.

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The Induction of Glutamate Dehydrogenase and Aspartate Aminotransferase in *Rhizobium*

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Enzymes involved in the fixation of atmospheric nitrogen in legumes have been located in the bacteroid fraction of the root nodules (Kennedy, Parker & Kidby, 1966; Koch, Evans & Russell, 1967; Bergersen & Turner, 1967). Several enzymes active in the assimilation of ammonia, the primary product of nitrogen fixation, were also detected in the bacteroid fraction of legume root nodules and in *Rhizobium* grown *in vitro* (Fottrell & Montgomery,