incorporated into nicotinic acid with an efficiency that suggested that the molecule remained intact (Gross, Schutte, Hubner & Mothes, 1963), and [2-¹⁴C]glycerol was found to be incorporated into the pyridine ring of nicotinic acid (Albertson & Moat, 1965).

Glycerol was again implicated as a precursor by Isquith & Moat (1965), who used *Clostridium butylicum*. However, purified extracts of *C. butylicum* did not incorporate [U-¹⁴C]glycerol; instead [U-¹⁴C]acetate and [1-¹⁴C]formate contributed to the nicotinic acid molecule (Isquith & Moat, 1966).

Scott & Matthey (1968) confirmed that aspartate and formate were incorporated and later showed the compound *N*-formyl-L-aspartate to be a precursor in *C. butylicum* (Scott, Bellion & Matthey, 1969).

In our experiments unpurified extracts of *E. coli* W (N.C.I.B. 8666), from cells grown under anaerobic conditions, were incubated with 10 μ mol of substrate in a buffered medium containing 5-phosphoribosyl 1-pyrophosphate (1 μ mol), ATP (30 μ mol), magnesium chloride (20 μ mol), CoA (1 μ mol) and pyridoxal phosphate (1 μ mol). Nicotinic acid was isolated and purified as previously reported (Scott *et al.* 1969).

The following results were obtained. (1) [1,3-¹⁴C]-Glycerol was incorporated into nicotinic acid with a 30-fold dilution of the molar specific radioactivity. (2) The incorporation of [1,3-¹⁴C]glycerol was 85% inhibited by the addition of aminopterin to the incubation mixture at a concentration of 0.1 mM. (3) Under identical conditions *N*[¹⁴C]-formyl-L-aspartate was incorporated without inhibition in the presence of aminopterin. This precursor showed a 1.5-fold dilution of the molar specific radioactivity.

These results show that glycerol does contribute to the nicotinic acid molecule in extracts of anaerobically grown *E. coli*, but indicate that the molecule is not incorporated intact. Aminopterin inhibits tetrahydrofolate dehydrogenase (EC 1.5.1.3), preventing formylation reactions by tetrahydrofolate coenzymes. The inhibition reported here suggests that part of the glycerol molecule is incorporated by a formylation reaction, and this suggestion is supported by the observed efficacy of *N*-formyl-L-aspartate as a precursor of nicotinic acid.

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The Formation of Unsaturated Bile Acids by Intestinal Bacteria

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Unsaturation of the steroid ring A of bile acids has been demonstrated with soil bacteria (Hayakawa, Fujii, Saburi & Eguchi, 1957; Halperin, Quastel & Scholefield, 1954) and with a strain of *Pseudomonas testosteroni* (Levy & Talalay, 1959). Kallner (1967) has shown that the occurrence of 5 α bile acids in rats is the result of interconversion between bile acids of the 5 α and 5 β series through the formation of the 3-oxochol-4-en-24-oic intermediate in the intestine. Norman & Sjoval (1958) have isolated metabolites of ¹⁴C-labelled cholic acid from rat faeces that could include 3-oxochol-4-en-24-oic acids. It seems likely therefore that the intestinal bacteria may be capable of introducing double bonds into the bile acid nucleus.

Initial screening of strains of bacteria isolated in this Department from human faeces revealed a strain of *Clostridium paraputrificum* CC510 that produced 3-oxochol-4-en-24-oic acid from 3-oxo-5 β -cholan-24-oic acid. The bacterial cultures were grown anaerobically in the presence of 0.01% 3-oxo-5 β -cholan-24-oic acid and the washed cell suspensions were incubated with 0.1% 3-oxo-5 β -cholan-24-oic acid in the presence of phenazine methosulphate (an artificial electron acceptor). The same unsaturation reaction was demonstrated when cultures were aerated by shaking for 2 days and the bile acids extracted from the broth.

Reaction products were identified by t.l.c. on silica gel G 254 (E. Merck A.-G., Darmstadt, Germany) and the identity of the 3-oxochol-4-en-24-oic acid was confirmed from the u.v.-absorption and the i.r. spectra.

The nuclear dehydrogenase from CC510, which was substrate-induced, was inhibited by 10 mM-Cu²⁺, 10 mM-Ca²⁺, 0.1% formaldehyde and 4 mM-periodate, but not by 0.01% merthiolate, 15 mM-Mg²⁺, 10 mM-EDTA, 50 mM-azide, 10 mM-fluoride or 10 mM-iodoacetate. The reaction had a broad pH optimum at about pH 7.0. Steroid substrates included 5 β -androstan-3,17-dione and 3,12-dioxo-5 β -cholan-24-oic acid but not 17 β -hydroxy-5 α -androstan-3-one, 3,7-dioxo-5 β -cholan-24-oic acid or 3,7,12-trioxo-5 β -cholan-24-oic acid.

Further intestinal bacteria were screened for C₄-C₅ nuclear dehydrogenation activity, by using

the assay system of shaking cultures aerobically for 2 days, and extracting the steroid substrate from the broth. Activity was demonstrated with five out of 12 strains of lecithinase-negative clostridia and relatively weakly with five out of 11 strains of lecithinase-positive clostridia but not with nine strains of enterobacteria, nine strains of enterococci or 16 strains of the non-sporing strictly anaerobic bacteria.

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The Glyceraldehyde 3-Phosphate Dehydrogenase of *Azotobacter beijerinckii* and its Possible Significance in Poly- β -hydroxybutyrate Biosynthesis

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Azotobacter beijerinckii can accumulate the storage polymer poly- β -hydroxybutyrate up to 70% of its dry weight when grown on glucose as the sole source of carbon and energy in the absence of a fixed nitrogen source. The enzymes β -ketothiolase, acetoacetyl-CoA reductase and polyhydroxybutyrate polymerase effect the synthesis of one hydroxybutyrate monomer from 2 molecules of acetyl-CoA, with the concomitant release of 1 CoA molecule and the reoxidation of 1 molecule of either NADPH or NADH. An acyl-carrier protein is not involved in polyhydroxybutyrate biosynthesis (Ritchie & Dawes, 1969). The reductive step is catalysed by acetoacetyl-CoA reductase, which reacts with NADPH at five times the rate with NADH (Ritchie, 1968). Our interest in the glyceraldehyde 3-phosphate dehydrogenase of the organism stems from a survey of reactions generating the large amounts of NADPH necessary for the massive synthesis of polyhydroxybutyrate which occurs when the organism is fixing nitrogen, a process that itself makes considerable demands on the production of reducing equivalents.

A glyceraldehyde 3-phosphate dehydrogenase has been isolated and partially purified by ammonium sulphate fractionation and ion-exchange cellulose chromatography. The enzyme reacts preferentially with NADPH ($V_{\max}$ 63.1 and 11.1 nmol/min with NADPH and NADH respectively) and thus resembles that more commonly encountered in plants,

which, however, operates in the direction of reduction. Johnson & Johnson (1961) recorded a similar coenzyme preference by the enzyme from *Azotobacter agilis*. The *A. beijerinckii* enzyme requires a thiol group for activity, as indicated by the inhibitory action of *p*-chloromercuribenzoate, *N*-ethylmaleimide, iodoacetate and mersalyl. The pH optimum is 7.5 and with phosphate concentrations higher than 100 mM there is an inhibitory effect at pH values above 6.5. The equilibrium of the reaction at pH 7.5 and 30°C greatly favours oxidation (K_{eq} approx. 10^7) and we believe that this reaction may play an important role in furnishing reducing equivalents for polyhydroxybutyrate biosynthesis.

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Conversion of Pyruvate into Citrate by Mitochondria Isolated from Rat Epididymal Fat-Cells

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From a previous study of the total activities and location of enzymes in isolated fat-cells and the permeability properties of intact fat-cell mitochondria (Martin & Denton, 1970) we have concluded that acetyl units for cytoplasmic fatty acid synthesis are transferred from mitochondria as citrate (as postulated by other workers largely on the basis of the parallelism between ATP citrate lyase activity and fatty acid synthesis rates), that transfer of citrate requires malate, presumably for counter-transport, and that the intramitochondrial metabolism of citrate and malate is restricted to allow transfer of acetyl units to the cytoplasm and to limit the loss of cytoplasmic reducing power into mitochondria.

Support for these conclusions has come from an investigation of the metabolism of pyruvate by isolated fat-cell mitochondria. The mitochondria were incubated with pyruvate, ADP and P_i and the effects of additions of malate, hydroxymalate (a non-oxidized malate analogue) and bicarbonate on the time-courses of pyruvate uptake, citrate output, malate uptake (or output) and oxygen consumption were determined. On addition of bicarbonate alone there was an appreciable lag in citrate production presumably because in the absence of