globin. HRP bears a resemblance to the acute-phase proteins such as C-reactive protein (Hedlund, 1961) showing many of the characteristics of an antibody without being immunoglobulin.

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COMMUNICATIONS

Biosynthesis of *N*-Acetylanthranilic Acid by Aromatic Auxotrophs of *Aerobacter aerogenes* and *Escherichia coli*

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N-Acetylanthranilic acid has been isolated from culture filtrates of *Aerobacter aerogenes* N5-36 (a mutant lacking phosphoribose anthranilate transferase) utilizing either shikimic acid or quinic acid (Ratledge, 1967, 1968). The role of this compound in the biosynthesis and metabolism of aromatic compounds was not clear. Present work has shown that during growth of this mutant on quinic acid medium supplemented with 10 μ M-L-tryptophan anthranilate accumulation commences immediately whereas that of *N*-acetylanthranilate begins about 90 min later. Maximum concentrations are reached at the beginning of the stationary phase of growth and are 190 μ M and 100 μ M respectively. By increasing the tryptophan concentration to 30 μ M anthranilate accumulation is decreased and that of *N*-acetylanthranilate is completely arrested. At 50 μ M-tryptophan or above no anthranilate accumulates but if 70 to 360 μ M anthranilate is added into such a medium from *N*-acetylanthranilate can be detected once more in the culture filtrates.

Extracts of this mutant catalyse the synthesis of *N*-acetylanthranilate from anthranilate in the presence of either acetyl-CoA or acetate, CoA and ATP. No deacylation of the product could be demonstrated. The apparent K_m for anthranilate in crude extracts is about 10 mM. Similar acetylating enzymes are also present in extracts prepared from *A. aerogenes* NCW (parent wild-type), *A. aerogenes* 62/1 (a multiple auxotroph: phe⁻, tyr⁻, try⁻) and *Escherichia coli* B37 (a mutant lacking phosphoribose anthranilate transferase). These organisms also accumulate *N*-acetylanthranilate in the growth medium (but for *A. aerogenes* NCW 360 μ M-anthranilate had to be included in the medium;

and *Escherichia coli* B37 was grown on glucose with 10 μ M-tryptophan). *N*-Acetylanthranilate is not found, however, when *A. aerogenes* NC3 (a mutant lacking anthranilate synthetase) is grown on quinic acid medium but does accumulate upon the addition of 360 μ M-anthranilate into the medium.

All available evidence suggests that *N*-acetylanthranilate arises from, and is not a precursor of, anthranilate. The acetylase appears to be a constitutive enzyme as it is not repressed in *A. aerogenes* N5-36 by up to 150 μ M-tryptophan and is present in cells of *A. aerogenes* NCW and NC3 which do not, under normal conditions, accumulate anthranilate. The high K_m value suggests that this enzyme may well have a broad specificity although this has yet to be tested.

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The Precursors of Nicotinic Acid in Bacteria

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Tryptophan is the precursor of nicotinic acid in fungi (Bonner & Yanofsky, 1951) and mammalian tissues (Nishizuka & Hayaishi, 1963), but does not appear to be a precursor in most bacteria and plants.

In studies with *Escherichia coli*, the carbon skeleton of nicotinic acid was found to be derived from a C₄ dicarboxylic acid and a C₃ unit such as glycerol (Ortega & Brown, 1960). Similar findings were reported with *Mycobacterium tuberculosis*, when it was found that [1,3-¹⁴C]glycerol was

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 μmol of substrate in a buffered medium containing 5-phosphoribosyl 1-pyrophosphate (1 μmol), ATP (30 μmol), magnesium chloride (20 μmol), CoA (1 μmol) and pyridoxal phosphate (1 μmol). 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 5α bile acids in rats is the result of interconversion between bile acids of the 5α and 5β 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-5β-cholan-24-oic acid. The bacterial cultures were grown anaerobically in the presence of 0.01% 3-oxo-5β-cholan-24-oic acid and the washed cell suspensions were incubated with 0.1% 3-oxo-5β-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 5β-androstane-3,17-dione and 3,12-dioxo-5β-cholan-24-oic acid but not 17β-hydroxy-5α-androstan-3-one, 3,7-dioxo-5β-cholan-24-oic acid or 3,7,12-trioxo-5β-cholan-24-oic acid.

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