Analysis of the trichloroacetic acid-soluble fraction from [^{14}C]glutamate showed that radioactivity had been incorporated into glutathione, ophthalmic acid, proline and pyrrolidone-5-carboxylic acid. Aspartate was not labelled. Most of the radioactivity in the trichloroacetic acid-soluble extract of the incubation with [^{14}C]aspartate was found in glutamate and glutathione. Alanine was also labelled. Most of the radioactivity from [^{14}C]alanine was recovered as unchanged alanine, but significant amounts of labelled lactate, glutamate, glutathione and aspartate were present.

These studies indicate that the role of the citric acid cycle in lens metabolism is greater than has been believed hitherto.

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Aromatization of Androst-4-ene-3,17-dione by Human Intestinal Bacteria

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As part of a wider study of cancer of the colon (Hill *et al.* 1971) we are investigating the nuclear dehydrogenation of steroids by intestinal bacteria. We report here the formation from androst-4-ene-3,17-dione of a novel steroid, tentatively identified as 17-methoxyoestratrien-1,3,5(10)-3-ol.

A culture of a strain of *Clostridium paraputrificum* (CC493) grown anaerobically at 37°C for 72h in Todd-Hewitt broth (Oxoid) containing 0.25mm-substrate was adjusted to pH 8 and shaken aerobically at 37°C for 96h after the addition of a further 0.25mm-substrate. The steroids were extracted with chloroform and t.l.c. analysis revealed the formation in moderate yield of a product that reacted with Folin reagent and with ferric chloride-ferricyanide reagent (both of which indicate the presence of a phenolic hydroxyl group). The product did not correspond to oestrone or oestradiol and behaved as a less-polar steroid.

The u.v.-absorption spectrum (maximum at 282nm, ϵ 2300) and the fluorescence spectrum (excitation maximum at 283nm with a corresponding emission maximum at 330nm) both indicated the presence of an aromatic ring A and a phenolic hydroxyl group, presumably still at C-3. The absence of a second absorption maximum at

about 300nm indicated that the product was not a seco-steroid produced by ring B fission.

Mass spectroscopy showed that the molecular weight of the product was 286, corresponding to methyloestradiol, the result of a migration of the 10-methyl group followed by aromatization of ring A. Such methyl migrations occur chemically to give 1-methyloestradiol or 1,17-dihydroxy-4-methyloestra-1,3,5(10)-triene (Dreiding, Plummer & Tomasewski, 1953). Both of these were synthesized and shown to differ from the bacterial product in t.l.c. behaviour and i.r. spectra. The trifluoroacetyl derivative was prepared and shown by mass spectroscopy to be the trifluoroacetate of oestradiol (molecular weight 464), indicating that the methyl group was lost during formation of the derivative. This suggested that the methyl group was not nuclear but at the 17-oxygen position.

Oestradiol was methylated with dimethyl sulphate, as was the bacterial product. Both yielded derivatives that were identical on t.l.c., and presumably corresponded to 3,17-dimethyloestradiol.

This result suggests that the bacterial product was the result of a migration of the methyl group from C-10 to the 17-oxygen function, 17-methoxyoestra-1,3,5(10)-trien-3-ol. Both androst-4-ene-3,17-dione and 17-hydroxyandrost-4-en-3-one were substrates for this novel migration. The reaction was carried out by a range of opalescent-negative clostridia, but no strains of the other genera tested (e.g. *Escherichia coli*, *Bacteroides* spp., *Bifidobacterium* spp.).

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Spectral Changes of a Cationic Dye after Interaction with Endotoxins

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The use of a carbocyanine dye for detection of undegraded endotoxins as defined by Nowotny (1969) was examined. Spectral shifts resulting from interactions of various compounds with the dye were observed by using a modified procedure of a method estimating polysaccharides (Edstrom, 1969).

New absorption maxima were found in the range 430–485nm. They occurred with certain endotoxin preparations and long-chain aliphatic sulphates. Under various conditions the shifts could serve as

an accurate and sensitive measure of sodium dodecyl sulphate and some endotoxins. When direct sunlight was avoided and measurements were made within certain standardized times, 1 min for sodium dodecyl sulphate and 5 min for endotoxins, the procedures were usable for the quantitative determination of sodium dodecyl sulphate in the range 0.4–4.0 μg and of a lipopolysaccharide preparation derived from *Escherichia coli* A.T.C.C. 12408 in the range 2.0–20 μg .

Apparently certain structural features of the reacting molecules determined the spectral changes that took place. Spectra from model compounds indicated that the reactive groupings possess strong anionic sites with long aliphatic chains attached to them in a certain spatial configuration. The spectral responses of endotoxins were diminished or altered by different treatments of lipopolysaccharide solutions such as mild acid hydrolysis (pH 1, 100°C, 30 min), refluxing in dioxan [aq. 99% (v/v) dioxan, 120 min], cleavage of fatty acids leading to loss of toxic properties (Nowotny, 1964), mild alkaline hydrolysis (1M-sodium hydroxide, room temp., 120 min), urea treatment (4M, 8M), reactions with sodium deoxycholate and with proteins, e.g. with specific antisera.

These effects and additional evidence based on i.r. spectra, sulphate and total sulphur determinations (S was 0.2–0.4%) suggest that the endotoxin anionic sites in question are attached to fatty acids and the sites might contain sulphur atoms. Details of the nature of the reactive endotoxin groupings remain to be established; nevertheless the spectral shifts are useful evidence for the existence of groupings in endotoxins that have not been detected by other means. The spectral shifts could be also used for determination of these endotoxin groupings in fermentation fluids.

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Mutants of *Pseudomonas* AM1 that Require Glycollate or Glyoxylate for Growth on Methanol or Ethanol

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The pathway leading to glycolytic intermediates during the growth of *Pseudomonas* AM1 on C_1 compounds involves condensation of a C_1 unit with

glycine to form serine, which is converted via hydroxypyruvate and D-glycerate into 3-phosphoglycerate (Large & Quayle, 1963; Heptinstall & Quayle, 1970). The net assimilation of C_1 compounds into cell constituents by this 'serine pathway' requires the regeneration of 1 molecule of glycine for each molecule of 3-phosphoglycerate formed. The mechanism of synthesis of the glycine skeleton from C_1 compounds in this organism is unknown. On the basis of this assimilatory scheme, mutants might be isolated containing a lesion in the pathway leading to the regeneration of glycine, leaving the oxidation of C_1 compounds unimpaired. Such mutants would require substrate amounts of glycine for growth with a C_1 compound as energy source, but the toxicity of glycine to *Pseudomonas* AM1 even at low concentrations precludes use of this phenotype for screening purposes. However, Harder & Quayle (1971) showed that glyoxylate (which can be tolerated at substrate concentrations in the growth medium) is a precursor of glycine during growth on C_1 compounds. This fact has permitted preparation of mutants that require substrate quantities of glyoxylate for growth with methanol as energy source. A number of such mutants have been obtained, none of which grows in the absence of either of these substrates. A typical mutant, G5, was used for detailed study. This mutant can use glycollate in place of glyoxylate as supplement in the methanol growth medium. During growth tests with other compounds it was found that mutant G5 also requires substrate amounts of glyoxylate or glycollate for growth with ethanol as energy source. This implies a connexion between the pathways of assimilation of methanol and of ethanol in the wild-type organism, perhaps by way of glyoxylate or a common C_2 precursor of glyoxylate.

Although only a weak enzymic oxidation of glycollate to glyoxylate has been observed in cell-free extracts of *Pseudomonas* AM1, double mutants have been obtained from mutant G5 for which glycollate no longer replaces glyoxylate as a growth substrate with methanol or ethanol as energy source. Further work is needed to establish whether glycollate is an obligatory intermediate between methanol or ethanol and glyoxylate.

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