Some Aspects of Menaquinone Biosynthesis in *Escherichia coli* E 106

By J. R. S. ELLIS and J. GLOVER. (*Department of Biochemistry, University of Liverpool*)

The presence in enterobacteria of menaquinone-8 (MK-8) and demethylmenaquinone-8 (DMK-8) is now well established (Baum & Dolin, 1965; Whistance & Threlfall, 1968). Less certain is our knowledge of the biosynthetic relationship between these naphthoquinones.

The incorporation of an intact methyl group from L-[Me-²H]methionine into MK-8 was demonstrated by Jackman, O'Brien, Cox & Gibson (1967). We confirmed this, using L-[Me-¹⁴C,³H]methionine, but further showed that DMK-8 was devoid of radioactivity.

The role of shikimic acid in the biosynthesis of MK-8 in *Escherichia coli* was reported by Cox & Gibson (1966), and elaborated by Campbell, Coscia, Kelsey & Bentley (1967). We obtained incorporation of DL-[1,6-¹⁴C₂]shikimic acid into both MK-8 and DMK-8 in *E. coli*. The specific radioactivities of MK-8, purified in three chromatographic systems, were 110 700 and 114 000 disintegrations/min./μmole in two separate experiments. However, the specific radioactivities of DMK-8, purified in the same way, were respectively 36 800 and 40 700 disintegrations/min./μmole. This evidence suggests that only some of MK-8 is not on biosynthetic route to MK-8 in *E. coli*.

We failed to demonstrate incorporation of [1-¹⁴C]α-naphthol into either MK-8 or DMK-8 in *E. coli*; it was suggested by Leistner, Schmitt & Zenk (1967) that this compound is a precursor of MK-7 in *Bacillus megaterium*. However, we agree with these workers that [ring-³H]protocatechualdehyde is not a precursor of MK-8 in *E. coli*, as inferred by Cox & Gibson (1966). It is instead converted into an unknown compound(s) provisionally called 'compound L'. We shall discuss some evidence that 'compound L' may also be derived from DL-[1,6-¹⁴C₂]shikimic acid in *E. coli*, and from [ring-³H]-protocatechualdehyde and [U-¹⁴C]pyrocatechol in *Micrococcus lysodeikticus*, for which a dihydroxy aromatic compound is an essential growth factor (Salton, 1964).

J. R. S. E. acknowledges with thanks a Research Studentship from the Science Research Council.

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Sodium, Potassium, Calcium and Magnesium Contents in Nails of Children with Kwashiorkor

By P. J. LEONARD, W. P. MORRIS and R. BROWN. (*Department of Biochemistry, Trinity College, Dublin, Irish Republic, and Department of Preventive Medicine, Tufts University, Boston, Mass., U.S.A.*)

In children with kwashiorkor there is a loss of tissue potassium (Waterlow & Mendes, 1957) and a retention of sodium (Hansen & Brock, 1954). In addition osteoporosis and magnesium deficiency are frequently observed in this condition (Trowell, Davies & Dean, 1954; Caddell & Goddard, 1967). Normal plasma electrolyte contents are frequently observed in association with decreased tissue contents, rendering measurements of the former of little value in assessing the cellular electrolyte status. Direct measurement of tissue electrolytes involves either obtaining specimens of tissue for analysis or the use of radioactive tracer studies, neither of which is feasible, especially in large surveys. It was felt that analysis of nail electrolytes might be of value in conditions in which alterations in tissue electrolyte metabolism are expected.

In the present study sodium, potassium, calcium and magnesium contents were measured in nail clippings from 20 children with kwashiorkor whose ages ranged from 6 months to 6½ years (mean age 2.16 years); 25 children whose ages ranged from 1 to 6 years (mean age 3.58 years) and in whom alterations in tissue electrolyte metabolism would not be expected, acted as controls. The method of getting the nails into solution was essentially that described by Kopito, Mahoodia, Townley, Khaw & Schwachman (1965). Calcium and magnesium were determined fluorimetrically as described by Schachter (1954) and Phillips & Elevitch (1966) respectively, and sodium and potassium were measured by flame photometry.

In the control group the mean concentrations (μg./mg. ± S.D.) were: sodium, 3.01 ± 0.97; potassium, 2.40 ± 1.15; calcium, 3.07 ± 2.01; magnesium, 2.48 ± 1.66. In the kwashiorkor children the mean values obtained were: sodium, 5.32 ± 2.55; potassium, 3.43 ± 2.08; calcium, 5.35 ± 3.21; magnesium, 1.38 ± 1.08. The increased sodium and calcium concentration and the decreased magnesium concentration in the kwashiorkor group were statistically significant ($P < 0.01$, $P < 0.01$ and $P < 0.05$ respectively). These results suggest that the analysis of nail electrolytes may be of considerable value as a guide to alterations in tissue electrolyte

metabolism. Because of the ease with which specimens can be collected, stored and transported nail analyses may prove to be a valuable tool in large surveys, particularly in areas remote from laboratory facilities.

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Some Factors Influencing Mycobactin and Salicylic Acid Production in *Mycobacterium smegmatis*

By C. RATLEDGE. (*Department of Biochemistry, The University of Hull*)

In response to an environment deficient in iron, *Mycobacterium smegmatis*, in common with other mycobacteria, produces salicylic acid extracellularly and a mycobactin intracellularly (Snow, 1965; Ratledge & Winder, 1966; White & Snow, 1968; Antonie & Morrison, 1968). Both compounds are iron-chelators and their productions are possibly related, as salicylic acid forms part of the larger mycobactin molecule (Snow, 1965). Before beginning a detailed examination of their biosyntheses, it seemed prudent to examine the effects of various factors on the production of these two compounds so that optimum conditions for their accumulation could be determined, and also to see if both have similar patterns of control.

M. smegmatis was grown for 6 days on glycerol (20 ml.), L-asparagine (5 g.), KH_2PO_4 (5 g.) and

MgSO_4 (0.4 g.), with NaOH added to pH 6.8. Precautions were taken to exclude trace elements from the glassware and water. Mycobactin was extracted and assayed by the methods of White & Snow (1968). Without supplementation the basal medium yielded 1.04–1.77 g. dry wt. of cells/l. with different batches of medium on separate occasions.

The effects of varying the concentrations of Fe^{2+} , Zn^{2+} , EDTA, citrate and salicylate on concentrations of mycobactin and salicylic acid have so far been examined. Preliminary results show that addition of Zn^{2+} (0.46 $\mu\text{g./ml.}$) stimulates mycobactin production from about 2 $\mu\text{g./mg.}$ dry cells to 20–30 $\mu\text{g./mg.}$ but has little effect on salicylic acid. This is an unexpected finding in contrast with *Mycobacterium phlei* (Antonie & Morrison, 1968). The effect produced by Zn^{2+} was not altered by the addition of EDTA (10 $\mu\text{g./ml.}$) or citrate (up to 2 mg./ml.).

Addition of Fe^{2+} (up to 6.25 $\mu\text{g./ml.}$) together with Zn^{2+} (0.46 $\mu\text{g./ml.}$) to the basal medium suppressed only salicylic acid production. However, if citrate (1 mg./ml.) or salicylate (20 $\mu\text{g./ml.}$) was also added then mycobactin production was simultaneously suppressed. This effect was more pronounced with citrate than with salicylate, though neither compound when added alone was an effective inhibitor of mycobactin synthesis. The formation of mycobactin could be entirely eliminated by the dual addition of citrate and Fe^{2+} at 1 mg./ml. and 2.5 $\mu\text{g./ml.}$ respectively.

The results so far suggest that an extracellular iron-chelator is needed before adequate transport of iron to the cell interior can occur. The cell presumably attempts to meet this need by forming and excreting salicylic acid.

I am indebted to Dr G. A. Snow for an advance copy of his paper with A. J. White.

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