Preparation of *Entamoeba histolytica* Antigen for Column-Chromatographic Studies

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The main obstacle in obtaining a fairly pure antigenic extract in *Entamoeba histolytica* is the accompanying bacterial contamination (Ali Khan & Meerovitch, 1970). Laboratory cultures of parasitic amoebae are usually accompanied by a large number of a variety of living objects. This may include several types of bacteria, protozoa, moulds, yeasts, pollen grains and various other vegetative cells. In the present study an attempt has been made to get rid of a large number of these extraneous material from the amoeba-bacteria culture pools. The culture pools were sieved through a fine mesh and were subjected to several runs of centrifugation at various speeds. The amoeba pools were carefully washed with sterile diluents and were subsequently checked for the decrease in accompanying objects. From comparatively clarified amoeba pools the antigenic extracts were made according to the technique of Kessel *et al.* (1965). The above antigenic extract was prepared for column-chromatographic studies.

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Metabolism of (–)-Citramalate

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The metabolism of (–)-citramalate, unlike that of (+)-citramalate, has not been studied in detail. Earlier work from this laboratory has shown that citraconate is converted by a *Pseudomonas* sp. into (–)-citramalate (Subramanian & Rao, 1968). The present studies have shown that the *Pseudomonas* strain metabolizes (–)-citramalate by the following steps: synthesis of succinyl-CoA from succinate by succinyl-CoA synthetase, transfer of CoA from succinyl-CoA to (–)-citramalate to form citramalyl-CoA and cleavage of the latter to acetyl-CoA and pyruvate. This pathway resembles that described by Cooper & Kornberg (1964) and Nagai (1963). The three enzymes were separated from each other and partially purified. The reverse reaction, the synthesis of citramalate from acetyl-CoA and pyruvate, was also demonstrated, and the product was shown to be (–)-citramalate. There was no activity with (+)-citramalate. The properties of the three enzymes will

be described. The succinate-activating enzyme and the transferase were also active with itaconate. Findings on the metabolism of (–)-[Me-¹⁴C]citramalate by cell-free extracts also support the above pathway.

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Malate Metabolism in Mycobacteria

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In cell-free extracts obtained by sonication, malate dehydrogenase has been found to be NADP⁺-dependent in the two fast-growing strains *Mycobacterium* 607 and *Mycobacterium smegmatis*, whereas it has been found to be NAD⁺-dependent in the relatively slowgrowing species *Mycobacterium tuberculosis* H₃₇Rv, *M. tuberculosis* H₃₇Ra, *Mycobacterium avium* and *Mycobacterium phlei*. However, when the same species of *M. phlei* was grown in shake cultures to give faster growth, the enzyme was still NAD⁺-dependent. The differences in the cofactor requirements of this enzyme therefore do not appear to be related either to the rate of growth or to the virulence of the mycobacteria, but they appear to be species-specific. Both the NAD⁺- and NADP⁺-dependent malate dehydrogenase activities are localized in the 104000g supernatant fraction. The specific activity of the NADP⁺-dependent malate dehydrogenase (in *Mycobacterium* 607 and *M. smegmatis*) is very low when compared with those of the NAD⁺-dependent malate dehydrogenase in other strains. The malate dehydrogenase activity in *M. smegmatis* and *Mycobacterium* 607 increased with increase in the age of the culture, unlike the other enzymes of the tricarboxylic acid cycle and glycolysis, these showing a decline after a period.

'Malic' enzyme activity could be demonstrated in those strains that showed NADP⁺-dependent malate dehydrogenase activity but not in those strains that showed NAD⁺-dependent malate dehydrogenase activity, under our experimental conditions. This enzyme is also localized in the 104000g supernatant fraction.