converted to aromatic hydrocarbons and carbon oxides.

Another critical composition occurs as the amount of oxygen increases. For all C to H ratios, there is a sharp threshold of oxidation at which most of the biologically interesting compounds can no longer occur in the equilibrium mixture. For example, in a C—H—O composition in the ratio of 1 : 4 : 4 at 1 atmosphere and 500°K, the carbon and hydrogen are present as carbon dioxide and water. The free oxygen mole fraction is 10^{-4}. The only organic compound present is formic acid, at 0.2×10^{-29} mole fraction.

We believe that studies such as these are needed in order to separate problems of primordial chemical evolution into those which depend on thermodynamic equilibrium and those which depend on irreversible processes. Previous discussions of prebiotic evolution have included a number of special mechanisms, including heat to energize and catalyze various reactions. Even in the presence of a special mechanism which favors the production of particular compounds, however, there will also be a tendency to form all the products required by thermodynamic equilibrium. The concentration of these particular compounds must be maintained in spite of the possible presence of any broad specificity catalysts which might permit the establishment of equilibrium. In a detailed study of biological conditions on terrestrial planets, not only the presence of certain key compounds is important, but also the relative concentrations of other related compounds which could compete with them in the special reactions postulated for the origin of life. Information about equilibrium states is, in general, a necessary prerequisite for the study of nonequilibrium states and irreversible processes.

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D-Malate: Effects on Activity of L-Malate Dehydrogenase in Developing Sea Urchin Embryos

Abstract. *Sea urchin embryos grown in salt water containing* L-*malate showed a pattern of development of* L-*malate dehydrogenase which did not differ from that of embryos grown in salt water. However, embryos grown in* 10^{-4}M D-*malate for 6 or 12 hours had one additional band of* L-*malate dehydrogenase that was not present in control embryos of the same age.*

The regulation of cytodifferentiation in embryonic stages immediately after fertilization has received little attention because of the inherent experimental difficulties (*1*). The facts that eggs of sea urchins are available in quantity, can be fertilized in vitro under controlled conditions, can undergo synchronous development, and are relatively permeable to exogenous materials make them an ideal system for studying the regulation of macromolecule formation (*1*).

Moore and Villee (*2*) have shown that the unfertilized sea urchin egg contains five electrophoretically separable L-malate dehydrogenases (L-MDH). The number decreased to three in whole embryos early in development and then increased to four in the 12-hour embryo. Furthermore, when sea urchin embryos at the 64-cell stage (4-hour embryos) were dissociated and separated into large and small blastomeres, the large blastomere showed two and the small blastomere showed three bands of L-MDH activity by disc microelectrophoresis (*3*). It was of interest to determine whether this pattern of malate dehydrogenase (MDH) development could be altered by growing embryos in sea water containing D- or L-malate.

Embryos were collected (*2*) and homogenized in glass-distilled water with a Ten-Broeck hand homogenizer until microscopy showed that at least 95 percent of the embryos were disrupted. The homogenate was centrifuged, and the supernatant fluid was decanted and stored overnight at 4°C. Portions were analyzed spectrophotometrically for MDH activity (*4*), using NAD (*5*) and its analogues as hydrogen acceptors; other portions were subjected to disc-microelectrophoretic analysis on polyacrylamide gel as has been described (*3*). The electrophoresis was conducted in tris buffer at *p*H 8.3 instead of in glycylglycine buffer at *p*H 7.4, and cyanide was omitted from the reaction mixtures. Approximately the same total amount of activity was applied to each sample gel for microelectrophoresis in order to compare the MDH patterns of embryos at different stages of development and of embryos grown in sea water, L-malate, or D-malate.

Echinochrome content of the enzyme preparations inhibits polymerization of the sample gel; hence, the layer of the polyacrylamide that contained the sample was prepared by mixing 1 part each of enzyme preparation and water and 1.5 parts each of "upper gel" and 2.2*M* sucrose solution. The buffer layer for the cathode was applied slowly to prevent "wash-out" of the sample before the electrophoresis began.

Unfertilized eggs have six bands of L-MDH activity and perhaps a seventh which appeared as a thin band (Fig. 1); the number of bands decreased to three in 4- and 6-hour embryos before increasing to four in 12-hour embryos. The major bands of MDH activity in unfertilized eggs appeared to be Nos. 3, 5, and 6, but activities were not determined quantitatively. The ratio of L-MDH activity with APAD to activity with NAD (as hydrogen acceptors) was less than 1 for preparations from both unfertilized eggs and embryos, a figure which agrees with the observations of Moore and Villee (*3*).

The effect of varied concentrations of L- and D-malate on fertilization and development of the eggs was investigated. D-Malate at $10^{-1}M$ or $10^{-2}M$ inhibits fertilization; $10^{-3}M$ D-malate inhibits neither fertilization nor development. In $10^{-1}M$ L-malate, the percentage of eggs fertilized ranged from 50 to 100 percent, but in $10^{-2}M$ L-malate both fertilization and development were normal.

Embryos grown for 4 hours in sea water, $10^{-2}M$ L-malate in sea water, or $10^{-3}M$ D-malate in sea water had iden-

tical electrophoretic patterns of L-MDH. However, embryos grown for 6 hours in D-malate had one more band of L-MDH activity than embryos grown in sea water or L-malate (Fig. 2); this additional band corresponded to band 4 of the preparation from unfertilized eggs (Fig. 1). Kinetic studies of the MDH activity with L- and D-malates as substrates and NAD and APAD as cofactors revealed no differences between the properties of the enzyme preparations from embryos grown in sea water, L-malate, or D-malate.

Embryos grown for 12 hours in D-malate also had one more band of L-MDH activity than embryos grown in sea water for 12 hours (Fig. 3). Again no kinetic differences were found in enzyme preparations from embryos grown for 12 hours in sea water, L-malate, or D-malate. The additional band in embryos grown in D-malate corresponded in electrophoretic mobility to band 2 of the unfertilized eggs (Fig. 1).

Unfertilized eggs and embryos grown for 5.5 hours in sea water each comprised two groups, one group being homogenized in water and the other in $10^{-2}M$ D-malate. The homogenates were centrifuged, and the supernatants were stored at 4°C for 18 hours. When subjected to disc electrophoresis, both preparations of unfertilized eggs (homogenized in water or in D-malate) had seven bands with L-malate and NAD in the staining mixture. The addition of $10^{-3}M$ D-malate to the L-malate and NAD in the staining mixture did not change the pattern of seven bands observed in preparations from unfertilized eggs homogenized in water. Six-hour embryos had three L-MDH bands, whether homogenized and stored in water or in D-malate. Thus, the effect on the L-MDH pattern of growing embryos for 6 or 12 hours in D-malate is not apparently due to any "protective" influence of unmetabolized D-malate during homogenization and storage.

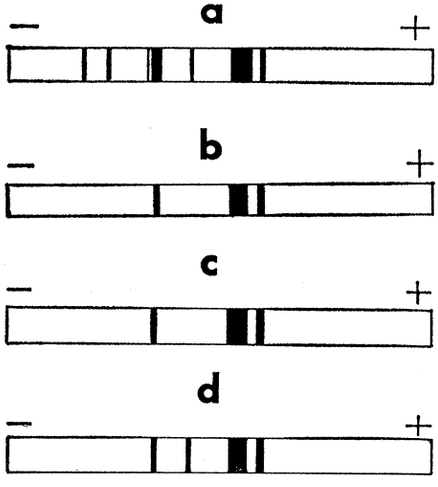

Fig. 1. Bands of L-MDH separable by disc microelectrophoresis on polyacrylamide gels. The staining reaction mixture contained L-malate, NAD, phenazine methosulfate, nitro blue tetrazolium, and magnesium chloride (*3*). *a*, Unfertilized eggs. Six distinct bands were present, plus a very thin one to the left of band 3. *b*, Four-hour embryos. Three bands were present, corresponding in electrophoretic mobilities to bands 3, 5, and 6 of unfertilized eggs. *c*, Six-hour embryos. *d*, Twelve-hour embryos. Four bands were present, corresponding in electrophoretic mobilities to bands 3, 4, 5, and 6 of unfertilized eggs.

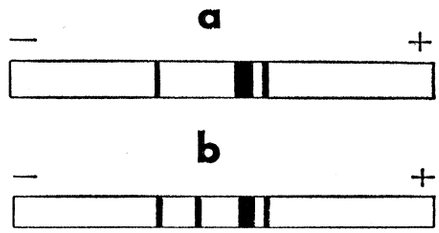

Fig. 2. Bands of L-MDH from embryos grown 6 hours in sea water, L-malate, or D-malate. The staining mixture is described in legend to Fig. 1. *a*, Embryos grown for 6 hours in sea water or $10^{-2}M$ L-malate in sea water. *b*, Embryos grown in $10^{-3}M$ D-malate in sea water for 6 hours.

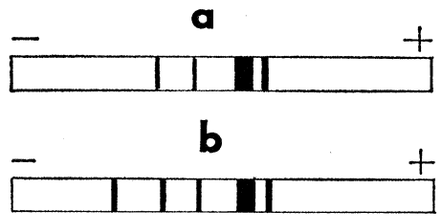

Fig. 3. Bands of L-MDH from embryos grown for 12 hours in sea water, L-malate, or D-malate. The staining mixture is described in legend to Fig. 1. *a*, Embryos grown for 12 hours in sea water or L-malate in sea water. *b*, Embryos grown in $10^{-3}M$ D-malate in sea water for 12 hours.

Kinetic analysis of the supernatant fraction from homogenized unfertilized eggs revealed a complex enzymic pattern. High concentrations ($1.67 \times 10^{-2}M$) of L-malate itself inhibited L-MDH activity which was greater at concentrations of approximately $2 \times 10^{-3}M$ L-malate than it was at concentrations of $3 \times 10^{-4}M$ or less. This result is reminiscent of the observation of Wolfe and Neilands (*4*) of a marked increase of activity of MDH from heart muscle when substrate concentrations exceeded $10^{-4}M$. In our experiments, addition to the reaction mixture of D-malate at concentrations ranging from $1.67 \times 10^{-3}M$ to $1.67 \times 10^{-2}M$ inhibited L-MDH activity; the nature of this inhibition is unknown. Thus, kinetic data from experiments with unpurified fractions from the supernatant fluid from homogenized tissues must be interpreted with caution.

The aforementioned effect of D-malate on the L-MDH patterns of the 6- or 12-hour embryos may be attributable to: (i) a quantitative effect on gene transcription; (ii) an effect on protein synthesis (or the cellular site of protein synthesis); (iii) a protective effect on L-MDH from either inhibitors or other inactivating agents, for example, proteolytic enzymes whose concentrations themselves may vary with development (*6*); or (iv) activation of "inactive" L-MDH. At present there is no reason for preferring any one of these alternatives. Interaction of D-malate with L-MDH, such as inhibition by D-malate of L-MDH activity, favors either (iii) or (iv).

The apparent lack of effect of L-malate on the pattern of L-MDH in developing embryos may reflect either its rapid metabolism after it enters the cells, a lower rate of uptake into the cells than for D-malate, or a true lack of effect on the enzyme-synthesizing system.

These and earlier observations (*1*, *3*) suggest a system for studying factors which influence the formation of macromolecules in a uniformly dividing cell population which has not yet undergone extensive differentiation.

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7. Aided by grants from the Charles and Marjorie King Fund, the Lalor Foundation, and the National Institute of Child Health and Human Development (grant HD6).

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