after infection and by the 15th minute reaches a maximum level comparable to that of the most active enzymes observed in *E. coli* infected with T-even phage. It seems likely that this phage-specific enzyme is responsible for supplying the deoxyuridylic acid that ultimately enters the 5-hydroxymethyluridylate in DNA from bacteriophage SP8. Thus it is apparent that host-specific mRNA and phage-specific mRNA can be synthesized in the same cell. Whether both or only one of the DNA strands of *B. subtilis* acts as a template for mRNA synthesis has not been determined in this study.

Other evidence, suggesting the transcription in vivo of only one of two DNA strands, comes from studies of other bacteriophage systems. Bautz and Hall (*17*) observed that the RNA formed after infection with T4 bacteriophage has a base composition such that adenine is not equal to uracil and cytosine is not equal to guanine. In an experiment similar to that reported here, Hayashi *et al.* (*18*) noted that the messenger RNA induced by infection with bacteriophage ΦX174 does not hybridize with the DNA from the mature bacteriophage particle but does hybridize with the double-stranded replicating form of ΦX174 DNA (*19*).

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Malate Dehydrogenase: Multiple Forms in Separated Blastomeres of Sea Urchin Embryos

Abstract. *Sea urchin embryos at the 64-cell stage were dissociated by treatment with trypsin and separated by centrifugation on a sucrose gradient. The large blastomeres have two and the small blastomeres have three bands of* L-*malate dehydrogenase activity, which are separated by disk microelectrophoresis on polyacrylamide gel, whereas unfertilized eggs have five.*

Changes in the number and amounts of the multiple forms of enzymes in a given tissue, during the latter part of embryonic development, have been demonstrated for lactate dehydrogenases (*1*, *2*) and for malate dehydrogenases (*2*). The relationship of this phenomenon of multiple molecular forms of an enzyme to the "one gene-one enzyme" theory and to the problem of cellular differentiation is of interest. All the tissues of adult echinoderms that have been tested contain malate dehydrogenases which have characteristic ratios of activity with nicotinamide-adenine dinucleotide (NAD) and its analogs (*3*). Electrophoresis on either starch granules or gel, or adsorption to and elution from diethylaminoethanol (DEAE) cellulose columns, revealed three to five molecular forms of malate dehydrogenase in these tissues (*4*, *5*). Unfertilized eggs of the sea urchin, *Arbacia*, have five NAD-malate dehydrogenases, 6-hour embryos have three, and 12- to 48-hour embryos have four. We have now investigated the multiple forms of malate dehydrogenase in separated blastomeres of early embryos.

Eggs and sperm, collected by electric shocks (10 v), were washed and diluted for fertilization. Embryos were grown at 25°C to the 64-cell stage, collected by gentle centrifugation and frozen briefly. They were suspended in a small volume of 0.53*M* NaCl containing $2 \times 10^{-3}M$ ethylenediamine tetraacetate, *p*H 5.0, for 3 minutes at 37°C to remove the egg membranes and jelly. Two volumes of 0.265*M* Tris buffer, *p*H 7.9, containing 0.01 percent recrystallized trypsin were added and the mixture was placed in a glass homogenizer in a water bath maintained at 37°C. Gentle movements of the pestle were continued until microscopic examination revealed that the blastomeres were completely dissociated. The dissociated blastomeres were layered on a 0.29 to 0.87*M* sucrose gradient and centrifuged at 750*g* for 25 minutes. The large blastomeres formed a pellet at the bottom of the tube and the small blastomeres formed a layer between the 0.29 and 0.87*M* sucrose. The layers were separated and examined microscopically, then the cells were distintegrated by ultrasonic techniques to make the enzymes soluble.

The resulting preparations were assayed spectrophotometrically for the rate of reaction of malate dehydrogenase with NAD and its analog, by means of a Beckman DB spectrophotometer and a recording potentiometer (*5*). Aliquots of each preparation were subjected to disk microelectrophoresis on polyacrylamide gels for 70 minutes at 5 mA in tris-glycine buffer, *p*H 8.6. Positions of malate dehydrogenase activity were located by staining the gels in 10 ml of a solution containing: 0.05*M* L-malate; 0.001*M* NAD; 0.002*M* KCN; 0.0005*M* $MgCl_2$; 0.05*M* glycyl glycine; 0.05 mg/ml phenazine methosulfate; and 0.3 mg/ml nitro blue tetrazolium. The *p*H of the solution was 7.4.

Table 1. Malate dehydrogenase (MDH) activity in eggs and embryos of the sea urchin, *Arbacia punctulata*, as determined by spectrophotometry (column 2) and electrophoresis (column 3).

Stage of development	Ratio of activity with APAD/NAD	Bands of MDH activity	
		With NAD	With APAD
Unfertilized eggs	0.68	5	8
Whole embryos (6 hours)	0.63	3	
Whole embryos (48 hours)	2.2	4	
Small blastomeres	1.4 ± 0.13*	3	2
Large blastomeres	2.3 ± 0.20*	2	1

*The mean value of eight experiments ± standard error.

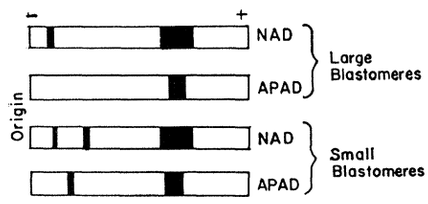

Fig. 1. Bands of activity of L-malate dehydrogenase with NAD and APAD, separated by disk electrophoresis on polyacrylamide gel and stained with nitro blue tetrazolium.

The ratio of the rates of reaction of malate dehydrogenase with acetylpyridine-adenine nucleotide (APAD) compared with NAD is 0.68 in unfertilized eggs, but rises during the early embryonic period and reaches 2.2 in 48-hour embryos (*5*). In the preparations from blastomeres separated at the 64-cell stage, the APAD/NAD ratio for malate dehydrogenase activity was 1.4 in preparations from small blastomeres and 2.3 in preparations from large blastomeres (Table 1).

Staining of the acrylamide gels revealed a slowly migrating band of NAD-malate dehydrogenase activity in small blastomeres which is not present in preparations from large blastomeres. The small cells have a total of three NAD-malate dehydrogenases which are separable by electrophoresis and the large cells have two (Fig. 1). Two of the bands in the small cells appear to be identical to the two bands seen in preparations from large blastomeres. Preparations from small blastomeres subjected to electrophoresis show two bands of APAD-malate dehydrogenase activity whereas preparations from large blastomeres show only one. The additional band of APAD-malate dehydrogenase in the small blastomeres moves slowly and remains near the anode, but it is not identical with the additional band of NAD-malate dehydrogenase. Material obtained from unfertilized eggs by homogenization or ultrasonication and subjected to disk electrophoresis on polyacrylamide gels showed five bands of malate dehydrogenase activity with NAD, and eight with APAD. Thus, as development proceeds from the egg to the 64-cell embryo, the number of malate dehydrogenases which are separable by electrophoresis decreases from five to three in small blastomeres, and to two in large blastomeres.

The method of separating small and large blastomeres in quantities large enough for chemical analysis makes possible studies of the development of biochemical differences in cells during early embryonic development. Later in development the differences in the size and density of the cells are too small to permit separation. The present experiments with the multiple forms of malate dehydrogenase provide an example of the differentiation of enzymes early in development, and indicate the feasibility of a study of the genetic and biochemical mechanisms controlling the formation of enzymes during development, analogous to the classic experiments in microorganisms (*6*).

The finding that there are as many as five forms of NAD-L-malate dehydrogenase might suggest that these are tetramers of two types of subunits, and that the five forms represent the five possible combinations of the two subunits, as suggested for mammalian lactate dehydrogenases (*7*). Vertebrate tissues have only two malate dehydrogenases which are separable by electrophoresis (*2*). The fundamental genetic control of malate dehydrogenase may be similar to that postulated for lactate dehydrogenase—that is, two genes, one for each type of subunit; but the two types of subunits may be prevented from forming hybrids in vertebrates although this is possible in the echinoderm system (*8*).

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Actinomycin: Inhibition of Cortisone-Induced Synthesis of Hepatic Gluconeogenic Enzymes

Abstract. *The cortisone-induced de novo synthesis of liver glucose-6-phosphatase, fructose-1,6-diphosphatase, aldolase, and lactic dehydrogenase was prevented by injections of Actinomycin D in the rat. In the in vitro assay systems, addition of actinomycin exerted no effect on the enzyme activities examined. The evidence favors the concept that the increased gluconeogenesis induced by corticoids entails at a certain stage an increased rate in the synthesis of gluconeogenic enzymes.*

Hepatic glucose-6-phosphatase activity which represents the final common path of gluconeogenesis and glycogenolysis increases after administration of corticoid (*1*), and the findings have been confirmed (*2*). Subsequently it was demonstrated that the activity of other enzymes involved in gluconeogenesis, such as fructose-1,6-diphosphatase, phosphohexose isomerase, and lactic dehydrogenase, also increases after cortisone is injected into normal, adrenalectomized, and hypophysectomized rats (*3*). Injection of ethionine (*4*) or puromycin (*5*) also inhibits these increases, suggesting that corticoid injection induces *de novo* synthesis of the gluconeogenic enzymes. Recently it was demonstrated that injection of puromycin and actinomycin prevents deposition of liver glycogen in starved rats injected with cortisone (*6*). Since actinomycin inhibits selectively deoxyribonucleic acid-directed synthesis of RNA in both microorganisms and mammalian cells (*7*), we tested the effect of this compound on cortisone-induced increases in liver gluconeogenic enzymes. Actinomycin completely inhibits cortisone-induced enzyme synthesis, nitrogen increase, and glycogen deposition in liver.

Male Wistar rats, weighing 90 to 100 g and maintained on Purina laboratory chow and water ad libitum, were divided into three experimental groups: (i) normal rats, (ii) rats treated with cortisone, and (iii) rats treated with cortisone and actinomycin. Normal rats were used for controls because preliminary experiments showed that physiological saline injections in volumes and regimens identical with those used for the treated groups had no effect on the enzymes examined or on the nitrogen or glycogen content. In the second