is almost precisely 1:1:1. However, this does not necessarily mean that the lamellar structure of the MLD granules consists of only one kind of abnormal membrane with equimolar amounts of cholesterol, galactolipids, and phosphatides, because the same analytical data could be obtained in various situations, for example, a mixture of equal amounts of two membrane structures, one with a ratio for cholesterol to galactolipids to phosphatides being 1:2:3, and the other 3 : 2 : 1. An extensive electron microscopic examination of biopsy specimens from three cases of MLD (*9*) has shown that the dimensions of these membranous structures appear to be constant, although the MLD granules vary in overall size or in the arrangements of the lamellae within the granules. This evidence supports the idea that the MLD granules contain only one kind of abnormal membrane with a cholesterol: galactolipids: phosphatides ratio of 1: 1: 1. The amount of gangliosides appears to be too small to be an integral part of the MLD granules and is probably derived from contaminating structures such as microsomes which are rich in gangliosides. Whether the considerable amount of protein in the MLD granules is an integral part of the abnormal membrane or whether it contains any enzyme activity is now unknown. The MLD granules appear to carry strong acid phosphatase activity as judged by light and electron microscopic histochemistry (*9*).

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Protein Synthesis in Micromeres of the Sea Urchin Egg

Abstract. *A method was developed for isolating large quantities of micromeres from the 16-cell stage of the sea urchin, and measurements were made of their ability to incorporate C^{14}-L-valine into protein as compared with that of a mixed suspension of micromeres, mesomeres, and macromeres. Per cell, the rate of incorporation was considerably less for the micromeres than for the suspension of mixed cells. Per unit volume, however, the two types of suspensions showed no significant differences.*

In sea urchins the first three cleavages divide the fertilized egg into eight equal-sized, and similar-appearing, cells. At the fourth cleavage the "lower" (vegetal) four cells divide unequally and across the polar axis, forming four micromeres (vegetally) and four macromeres, while the upper four cells divide equally and meridionally to form a layer of eight mesomeres. Experiments (*1*) in which these and derivative cell types have been removed and regrouped have shown that they possess special properties and that they interact in a gradient system. Previous investigators (*2*), using cytochemical and autoradiographic methods, have reported that protein content and rate of incorporation of labeled amino acid into protein are approximately the same in the different cell types. In order to obtain more quantitative information on this subject we have developed a method for isolating micromeres in large quantity and have measured their ability to incorporate labeled amino acid into protein.

Gametes of the sea urchin *Lytechinus pictus* were obtained by injection of the animals with isotonic potassium chloride. Artificial sea water (*3*) was used throughout. Typically, 25 ml of a suspension of washed eggs were inseminated with 25 ml of a dilute sperm suspension containing $10^{-4}M$ ethylenediaminetetraacetate (EDTA) at *p*H 8.1.

The fertilization membrane was removed 4 minutes (20°C) later by a single rapid squirting of the suspension against the side of a beaker through a 50-ml pipette. The eggs were then washed, suspended in 250 ml of sea water, cultured to the 16-cell stage in a large (500 cm^2 area) pyrex dish, and transferred, with one washing, to $0.55M$ KCl containing EDTA at $2 \times 10^{-3}M$, *p*H 8.1. After 10 minutes of gentle agitation in this medium, the component cells of the eggs were generally completely separated from each other. The suspension was then centrifuged at about 1000*g* for 2 minutes in order to reduce its volume to 40 ml, and 10-ml portions were layered, in test tubes, over 10 ml of a mixture of isotonic sucrose and EDTA-KCl (3:7). The cells were allowed to settle for 10 to 15 minutes, and the upper 10-ml of the suspension were removed. This was composed predominantly (99 percent) of micromeres. This suspension was further concentrated by centrifugation at 1000*g* for 2 minutes. The entire procedure was carried out at 20°C. When returned to sea water, the micromeres exhibit normal reaggregation and continue with normal cell division. After such treatment, mixtures of micromeres, mesomeres, and macromeres will reaggregate and frequently gastrulate. Only a few of the aggregates develop into normal embryos, as expected in view of the random nature of the kinds and positions of the different cell types, which evidently retain their distinctive properties during the period of isolation (*1*).

For the measurements of incorporation of labeled amino acid into the proteins of micromeres, 0.05 ml of the suspension in sea water (containing 8 to 20 $\times$ 10^3 cells, depending on the experiment) were added to 0.25 ml of C^{14}-L-valine (specific activity 200 c/mole, 0.5 μc/ml) in sea water and incubated for 1 hour at 20°C with

Table 1. Incorporation of uniformly labeled C^{14}-L-valine into micromere and mixed-cell suspensions.

Cell suspension	Radioactivity (count/min)	
	Per 10^3 cells	Per unit volume
	Experiment 1	
Micromere	32	131
Mixed	183	183
	Experiment 2	
Micromere	99	406
Mixed	480	480
	Experiment 3	
Micromere	120	492
Mixed	370	370

gentle agitation. This was followed by washing with a large excess of C^{12}-L-valine and three washings with ice-cold sea water. The eggs were processed by a modification (*4*) of a filter-paper technique (*5*) for determination of the radioactivity of the proteins insoluble in trichloroacetic acid. A liquid scintillation spectrometer with a counting efficiency of 50 percent was used. A cell suspension consisting of a mixture of micromeres, mesomeres, and macromeres, in the normal ratio of 4:8:4, was prepared, incubated, and processed in similar fashion to the micromere suspension to serve as control. For cell counts, three 0.05-ml portions were taken from each suspension, appropriately diluted, and all the cells enumerated under the microscope at × 100 (Table 1).

On a "per-cell" basis, micromeres incorporate C^{14}-L-valine into protein at a rate about 17 to 32 percent of that exhibited by the mixed cell suspension. The diameters of micromeres, mesomeres, and macromeres are approximately 22 μ, 34 μ, and 44 μ, respectively, in *Lytechinus pictus*. Calculated as a sphere, the average volume of a cell in the mixed suspension (with the normal 4:8:4 ratio of numbers of micromeres to mesomeres to macromeres) is approximately 4.1 times that of a micromere. When the radioactivity of the micromeres is converted to radioactivity per unit volume (multiplication of column 2, Table 1, by 4.1) the results show no significant difference between micromeres and mixed-cell suspensions in ability to incorporate C^{14}-L-valine into protein.

It appears, then, that there are no marked differences in the rates of protein synthesis among these cell types. This conclusion is subject to the general or implied assumption in experiments in incorporation of labeled precursors into macromolecular substances in vivo, and sometimes in vitro, that there are no appreciable differences in the precursor pool. In the present instance there are no a priori reasons for expecting appreciable differences. Also, the relatively small changes in amino acid pool that have been noted (*6*) during these early stages of development in sea urchins tend to argue against there being appreciable differences in the different cell types.

At present, then, the results are consistent with the view (*4*) that the bulk of the protein synthesis during early development employs messenger RNA that was present (in inactive form) in the unfertilized egg. This maternal messenger RNA does not appear to be distributed differentially to any great extent among the three cell types of the 16-cell stage. While there is evidence (*7*) that new messenger is also being formed during this period, it, in turn, appears to remain inactive, or relatively so, until later stages of development.

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Rotifer Ecology and Embryological Induction

Abstract. *The rotifer* Asplanchna *releases into its environment a. water-soluble, nondialyzable, pronase-sensitive factor which causes uncleaved eggs of another rotifer,* Brachionus calyciflorus, *to develop into individuals with a pair of long, movable spines which neither their mothers nor the unaffected controls have. These appendages protect the* Brachionus *from* Asplanchna *predation.*

Beauchamp (*1*) briefly described a remarkable predator-prey relationship between the rotifers *Asplanchna* and *Brachionus calyciflorus* (*2*). When *B. calyciflorus* were introduced and maintained as food organisms in cultures of the considerably larger and predominantly carnivorous *A. brightwelli*, the female, parthenogenetic offspring of these prey animals had, in addition to the normal complement of three relatively short pairs of spines, a pair of long posterolateral spines—structures that were completely lacking in the previous generation. The production of these extra spines was mediated by a factor released into the medium by *Asplanchna* and represents a phenotypic response of undoubted adaptive significance for the *B. calyciflorus*, long-spined forms being more difficult for *Asplanchna* to eat.

This interaction between *Asplanchna* and *B. calyciflorus* is of such unusual biological interest with respect to exogenous substances, population dynamics, evolutionary biology, and embryological induction that a more detailed analysis of the phenomenon was undertaken (*3*).

Clones of *Asplanchna sieboldi* Leydig (*4*), collected by N. D. Meadow in the Philadelphia area, and a Lake Washington strain of *A. girodi* de Guerne were reared in an inorganic medium and fed *Paramecium bursaria* that had been harvested by centrifugation from dilute, xenic, wheat-grain media kept under constant illumination. A clone of *B. calyciflorus* from Lake Washington was cultured in inorganic medium and fed *Euglena gracilis* Klebs, strain Z, obtained from the Culture Collection of Algae at Indiana University (*5*). Stock cultures of all three rotifers were maintained in Syracuse watch glasses and incubated at 25°C in the dark. The *B. calyciflorus* clone never produced individuals with posterolateral spines unless the medium was affected by *Asplanchna*. Hereinafter, all references to spines are to the posterolateral spines, unless otherwise stated.

Newly hatched, spineless *B. calyciflorus* cultured in media extensively conditioned by *A. sieboldi* remain visibly unaffected throughout their lifetime, but their first offspring invariably have long posterolateral spines. Rotifers with these *Asplanchna*-induced evaginations of the body wall also have significantly longer anterior and posteromedian spines (Figs. 1 and 2, Table 1), the amount of elongation being roughly directly proportional to the length of the posterolateral spines. There are no associated changes in body length.

The altered morphology of these individuals is determined before the eggs from which they hatch are extruded from the maternal body cavity and begin to cleave. Lacto-orcein squash preparations and direct observations with a Zeiss-Nomarski differential interference-contrast microscope (*6*) showed that