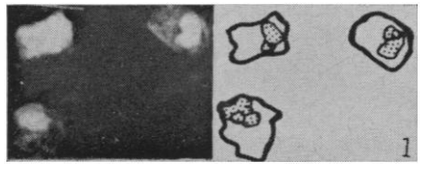
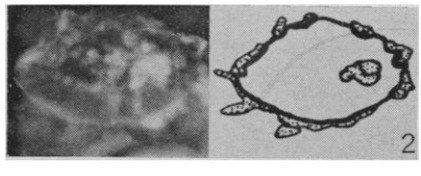
other than RNA. Other specificity studies were done: Treatment with deoxyribonuclease left RNA intact, but DNA fluorescence of nuclei disappeared; trypsin digestion did not alter DNA or RNA fluorescence; pepsin digestion was not completely satisfactory because of the low tissue *p*H necessary, but under these conditions no change in RNA or DNA occurred; hot trichloracetic acid extracted all detectable RNA and DNA; a methylene blue extinction series corresponded to RNA fluorescence. The possibility of diffusion artifacts during fixation was eliminated by comparing the appearance and distribution of the polychromatic fluorescence with embryonic tissues which had been prepared by the freeze-dry technique (*7*).

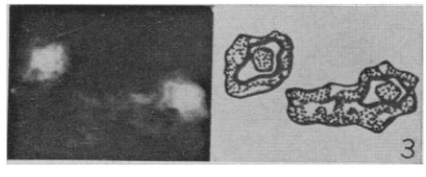
The following six figures show the successive steps in differentiation of primordial embryonic cells into epithelia. These are halftone reproductions of fluorescent color photomicrographs and do not show the marked polychromatic differentiation which is observed directly through the microscope or which is seen in great contrast on color film. The line tracings adjoining the prints are camera lucida tracings from the color slides. RNA fluoresence is stippled, and the green fluorescing nuclei are drawn in heavy line.



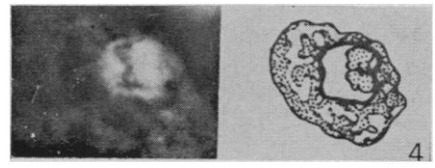
The first change seen is the production of nucleolar RNA in the nucleus of the highly condensed primordial embryonic cell (about × 1000).



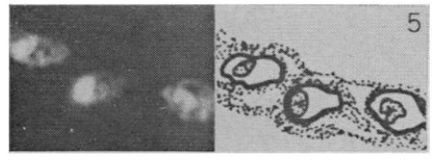
RNA is promptly seen at various areas along the periphery of the nucleus (about × 2000).



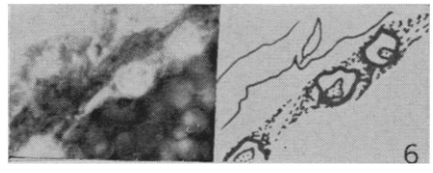
RNA is then seen in the cytoplasm (× 1000).



RNA in the cytoplasm increases as the cell takes shape (× 1000).



Finally cells coalesce as an epithelium emerges (× 1000).



In Fig. 6 is seen a deeper epithelial layer, approximately 24 hours after fertilization and after the blastoderm has been established. The coalescence is similar to Fig. 5. The thin lines in the upper left are outlines of epithelia, purposely left unstippled (× 1000).

The RNA in the unfertilized ovum is currently under investigation as a possible source of early "structural information" (*8*). Most RNA is found toward the animal pole of the egg, the bulk nearest the blastodisc, but fanning out from here in a diminishing arc well beyond the blastodisc. It has been observed repeatedly that early embryonic cells seem to "arrange" themselves in this RNA which is no longer found by about 24 hours after fertilization when the blastoderm has formed. In local areas, RNA seems to disappear earlier, perhaps depending upon the thickness of the forming cell layers.

The significance of these observations is, of course, yet to be established, but current investigation is proceeding along temptingly speculative lines as if this "free RNA" in the ovum carries information for primordial structure and perhaps is a contribution solely from the maternal organism.

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7. The techniques for ribonuclease studies, freeze-dry method, and others are described in A. G. E. Pearse, *Histochemistry Theoretical and Applied* (Little, Brown, Boston, 1960).
8. With this modified method studies have been in progress of both qualitative and correlative quantitative aspects of organ development and embryo development. Of special interest has been the "life cycle" of RNA in particular cell lines, the manuscript of which is now in preparation.
9. Supported by grants from the Association for the Aid of Crippled Children and the James Hudson Brown Memorial Fund of the Yale University School of Medicine. Presented in part before the Society for Pediatric Research, Atlantic City, N.J., 8–10 May 1962. We are grateful to Dr. Russell J. Barnett, Department of Anatomy, Yale University School of Medicine, for advice and criticism.

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Amino Acids in Deep-Sea Water

Abstract. Approximately 17 different amino acids in acid hydrolyzates of dissolved organic material in sea water to depths of 3500 meters from the central Gulf of Mexico have been identified by ion-exchange resin chromatography. They are glutamic acid, lysine, glycine, aspartic acid, serine, alanine, leucine, valine plus cystine, isoleucine, ornithine, methionine sulfoxide, threonine, tyrosine and phenylalanine, histidine, arginine, proline, and methionine. The results indicate that the dissolved organic material in deep-sea water may make an important contribution to the organic budget of the sea.

Parsons and Strickland have determined the concentration of organic material in oceanic detritus in the northeastern Pacific Ocean (*1*). They found that the amount of particulate carbon at 3000 m was not appreciably different from that at 300 to 500 m. They detected glycine, alanine, glutamic acid, aspartic acid, lysine, arginine, and proline in the oceanic detritus by paper chromatography. Erdman *et al* have reported the presence of ten amino acids in sediments from the Gulf of Mexico (*2*).

To identify dissolved organic material in sea water one must deal with the presence of about 10^4 times by weight more inorganic salts than organic material. Various techniques which have been evaluated for the isolation of dissolved organic material include dialysis, adsorption, ion exchange, sol-

vent extraction, and coprecipitation (*3*). None of these techniques has resulted in convenient and complete isolation of the organic material from sea water.

Tatsumoto *et al.* investigated amino acids in surface waters by an iron coprecipitation method (*4*). In our work, we extended a similar study to deep-sea waters to a depth of 3500 m.

During July 1960, at latitude 25° 45′N, longitude 94°10′W, near the center of the Gulf of Mexico, aboard the research vessel *Hidalgo,* we collected six 200-liter samples from depths of between 10 and 3500 m with Fiberglas and polyethylene water samplers. The water was immediately filtered through Millipore HA filters, and 3 g of mercuric chloride were added to each sample. The dissolved organic material was coprecipitated with ferric hydroxide which was made from 2*N* sodium hydroxide and 2*M* ferric chloride solutions. The precipitates had a *p*H of 9.0 to 9.7, as measured during the return to a shore laboratory, where they were then refrigerated.

At the shore laboratory, the iron precipitates, which contained organic material, were centrifuged to remove most of the remaining sea water, and were then dissolved in hydrochloric acid. Further desalting was accomplished by passing this solution through columns of Dowex-50 cation-exchange resin and Amberlite IRA-400 anion-exchange resin (*5*). Acid hydrolysis in 6*N* hydrochloric acid followed, and the amino acids in the salt-free concentrates were resolved by Amberlite CG-120 ion-exchange resin chromatography (*6*).

Table 1. Distribution of amino acids obtainable by iron coprecipitation in order of abundance: +++ high, > 1 mg/m³; ++ medium, 1 to 0.5 mg/m³; + low, <0.5 mg/m³.

Distribution at depths indicated					
10 m	500 m	900 m	1500 m	3000 m	3500 m
Glutamic acid					
+++	+++	+++	+++	+++	+++
Lysine					
+++	+++	+++	+++	+++	+++
Glycine					
+++	+++	+++	+++	++	+++
Aspartic acid					
++	+++	+++	+++	++	+++
Serine					
++	+++	+++	++	+	++
Alanine					
++	++	++	++	++	++
Leucine					
++	++	++	++	++	++
**Valine plus cystine*					
++	++	++	++	+	++
Isoleucine					
++	++	+	++	+	+
Ornithine					
+	++	+	+	+	+
Methionine sulfoxide					
+	+	+	+	+	+
Threonine					
+	+	+	+	+	+
Tyrosine and phenylalanine					
+	+	+	+	+	+
Histidine					
+	+	+	+	+	+
Arginine					
+	+	+	+	+	+
Proline					
+		+	+	+	+
Methionine					
+	+		+	+	+

* The peaks corresponding to these amino acids were poorly resolved on the ion-exchange chromatograms.

Table 1 shows that identifiable amino acids were rather uniformly distributed throughout the wide range of depths sampled. Amino acid concentrations in the acid hydrolyzate were about three times greater than in an unhydrolyzed portion of an identical sample which had been taken at 3000 m. However, unidentifiable ninhydrin-positive resolution peaks in the unhydrolyzed sample indicated the existence of substances similar to peptides in deep-sea water.

Although the analyses were not strictly quantitative (*7*), our findings indicate that there are substantial amounts of amino acids in deep-sea water (*8*). Along with other dissolved organic material, these may eventually be adsorbed on and incorporated in the deep-sea sediments or used by deep-sea microorganisms (*9*).

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5. Detailed laboratory procedure on the isolation of amino acids from sea water is given in a Ph.D. thesis by Kilho Park, Texas A. & M. College, 1961.
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7. Measurements by carbon-14 tracer techniques indicated that the percentage yield of free amino acids by the iron coprecipitation method ranges from a minimum of 30 percent for alanine to a maximum of 85 percent for lysine. A study of the recovery of peptides and proteins is current at Texas A. & M. College. Preliminary results indicate nearly quantitative yields. The data in Table 1 are not corrected for any losses during fractionation.
8. Although virtually all kinds of organic matter in the sea can be mineralized by marine bacteria [see C. E. ZoBell, *Marine Microbiology* (Chronica Botanica, Waltham, Mass., 1946)], dissolved organic matter is continuously replenished by marine life. A list of various dissolved organic substances found in sea water has been compiled by E. K. Duursma [*Neitherlands J. Sea Res.* **1**, 1 (1960)].
9. This work was supported by grants A–003 and A–022 from the Robert A. Welch Foundation, Houston, Texas. The assistance of Mrs. A. F. Isbell is appreciated.
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Water Relations of Plant Communities as a Management Factor for Western Watersheds

Abstract. Altering natural succession may become a major way of modifying plant cover thereby increasing water yield.

Scarcity of water for human use has influenced the history of mankind profoundly (*1*) and is currently a source of national concern. Much research effort has been focused recently on watersheds as our primary source of water (*2*).

Yield from many watersheds may be considered simply as the excess of precipitation over direct evapotranspirational loss from soil and plants. Hoover (*2*) indicated that, although altering plant cover to reduce this evapotranspirational loss is probably the major practical means of increasing yield from most watersheds, very little information is available as a basis for the practice. He summarized as follows: "The real need is to spell out what kind, size, age, and arrangement of plant cover is most effective for specific situations. This is the sort of information that land managers can use."

The current need is slightly different for Western watersheds, for these are mostly extensive wildlands on remote mountain slopes where intensive manipulation to maintain ideal cover is still impractical. Here a watershed manager can probably aspire to little more than altering the speed or direction of natural succession (the gradual replacement of one species by another). His basic decision will probably involve only desirable trends of vegetational change rather than the most effective