Glycyl-leucine and leucyl-glycine could not be distinguished from each other. A small amount of glycyl-glycyl-glycine was also formed. In a two-dimensional separation in propanol, ammonia, and water (6:3:1 by volume) in one direction and butanol, formic acid, and water (77:10:13 by volume) in the other, the presence of glycyl-glycine, glycyl-leucine, leucyl-glycine, leucyl-leucine, and glycyl-glycyl-glycine was clearly established.

In futher experiments, the areas corresponding to the dipeptides were cut out from the chromatogram, eluted, and hydrolyzed with 6*N* HCl for approximately 24 hours in a sealed tube at 110°C. The hydrolysate was then chromatographed in one dimension with butanol, formic acid, and water. Figure 2 illustrates the result for the dipeptide glycyl-leucine. The two amino acids glycine and leucine were recovered almost quantitatively. Similar chromatograms for the hydrolysates of the dipeptides glycyl-glycine and leucyl-leucine showed the presence of only a single amino acid, glycine or leucine. The yields in these experiments were relatively small: about 1 percent for dipeptides and 0.1 percent for the tripeptide.

We have preliminary evidence to indicate that ultraviolet light alone can bring about the synthesis of peptides. Cyanamide, therefore, appears to enhance the yield. Dipeptides can also be formed by the action of heat on dilute solutions of amino acids in the presence of adenosine triphosphate and Mg^{++}. The formation of peptides under relatively simple abiological conditions lends support to the hypothesis of chemical evolution (*14*).

CYRIL PONNAMPERUMA
ETTA PETERSON

Exobiology Division, National Aeronautics and Space Administration, Ames Research Center, Moffett Field, California

References and Notes

1. S. W. Fox, Ed., *The Origins of Prebiological Systems and of Their Molecular Matrices* (Academic Press, New York, 1965).
2. S. L. Miller, *J. Amer. Chem. Soc.* **77**, 2351 (1955).
3. C. Palm and M. Calvin, *ibid.* **84**, 2115 (1962).
4. J. Oro, *Nature* **197**, 862 (1963).
5. K. A. Grossenbacher and C. A. Knight, "Amino acids, peptides, and spherules obtained from 'primitive earth' gases in a sparking system," in (*1*), pp. 173–186.
6. K. Harada and S. W. Fox, *Nature* **201**, 336 (1964).
7. S. W. Fox and S. Yuyama, *Ann. N.Y. Acad. Sci.* **108**, 487 (1963).
8. J. Oro and C. L. Guidry, *Arch. Biochem. Biophys.* **93**, 166 (1961).
9. J. Oro, *Ann. N.Y. Acad. Sci.* **108**, 464 (1963).
10. C. Ponnamperuma and P. Kirk, *Nature* **203**, 400 (1964).
11. A. Schimpl, R. M. Lemmon, M. Calvin, *Science* **147**, 149 (1965).
12. Westinghouse type 782H-10.
13. C. Ponnamperuma, P. Kirk, R. Mariner, B. Tyson, *Nature* **202**, 393 (1964).
14. A preliminary report was presented at the Pacific Slope Biochemical Conference, San Francisco, 27 and 28 August 1964.

16 February 1965

Dicyandiamide: Possible Role in Peptide Synthesis during Chemical Evolution

Abstract. *Dicyandiamide promotes certain dehydration condensations under conditions possible on primitive Earth. The effect of this compound in promoting peptide syntheses was studied.*

Dicyandiamide may have played a role in the first condensation reactions on primitive Earth. In aqueous solution, dicyandiamide promotes the appearance (*1*) of (i) pyrophosphate from orthophosphate, (ii) glucose-6-phosphate from glucose and phosphoric acid, and (iii) adenylic acid from adenosine and phosphoric acid. That dicyandiamide probably existed on primitive Earth was indicated by its appearance when dilute cyanide solutions were irradiated with ultraviolet light (*2*). In addition, the trimer of cyanamide, melamine, has been reported in the Orgueil meteorite (*3*), and it is known that dicyandiamide is converted to melamine at high temperatures (*4*).

We have further investigated the ability of dicyandiamide to promote condensations in aqueous media. We were particularly interested in the possible role of dicyandiamide in peptide formation.

Carbodiimides have been successfully used (*5*) in the preparation of amino acid polymers. Since it was initially postulated (*1*) that dicyandiamide operates by a mechanism similar to that of the carbodiimides, the following experiment was carried out: Chromatographically purified alanine-1-^{14}C (0.65 μc) was dissolved in 1 ml of $10^{-2}M$ HCl and $10^{-2}M$ dicyandiamide, and enough unlabeled alanine was added to bring its final concentration to $10^{-2}M$. After the solution had remained in the dark at room temperature for 20 hours, the product was chromatographed in parallel with true alanylalanine on Whatman No. 4 paper, a mixture of water-saturated phenol and concentrated NH_4OH (200:1) being the solvent. A radioactive spot (shown by x-ray film darkening) that had the same R_F value as alanylalanine (0.79) was eluted from the paper. Carrier alanylalanine was added, and the resultant mixture was chromatographed on paper with the organic phase of an *n*-butanol–acetic acid–water (4:1:5) solvent mixture in the first direction and isopropanol-water (4:1) in the second. Ninhydrin spray indicated that the position and shape of the carrier were in coincidence with the radioactive product. In the presence of phosphoric or phosphorous acid, no dipeptide appeared to be synthesized, but, when the *p*H was regulated with HCl, alanylalanine was obtained. The yield of alanylalanine from alanine was 1.2 percent; a trace of alanylalanylalanine also appeared.

It appears that a low *p*H is advantageous in the dicyandiamide-promoted condensations. When HCl was omitted from the reaction mixture, with all other conditions (time, temperature, concentrations) remaining the same, the yield of dipeptide was only about 0.1 percent. Furthermore, other work in this laboratory has indicated that dicyandiamide-induced phosphorylations also are most effective at around *p*H 2.

In order to prepare product solutions for paper chromatography, their volumes had to be reduced by evaporation under partial vacuum. To determine the effect of this, if any, on the reaction, the following experiment was performed. Synthesis of the dipeptide was carried out as described, but, before evaporation, the dicyandiamide was precipitated with silver acetate. Any residual silver was then removed with potassium chloride, and after filtration the volume of the resultant solution was reduced for chromatography. Another solution of alanine and HCl in the same concentrations as before was prepared and evaporated. However, this solution at no time contained dicyandiamide.

The first solution exhibited the same degree of synthesis of dipeptide as was noted earlier when dicyandiamide had not been removed before evaporation. The second solution appeared to indicate only a trace of dimer. The original appearance of alanylalanine may thus be attributed to dicyandiamide.

GARY STEINMAN
RICHARD M. LEMMON
MELVIN CALVIN

Lawrence Radiation Laboratory and *Department of Chemistry, University of California, Berkeley*

References and Note

1. G. Steinman, R. M. Lemmon, M. Calvin, *Proc. Natl. Acad. Sci. U.S.* **52**, 27 (1964).
2. A. Schimpl, R. M. Lemmon, M. Calvin, *Science* **147**, 149 (1965).
3. R. Hayatsu, *ibid.* **146**, 1291 (1964).
4. H. E. Williams, *Cyanogen Compounds* (Arnold, London, 1948), p. 28.
5. H. G. Khorana, *Some Recent Developments in the Chemistry of Phosphate Esters of Biological Interest* (Wiley, New York, 1961), p. 133.

6. Work sponsored in part by the AEC. G.S. is an NSF fellow, 1964–65.

11 January 1965

Protein Synthesis and the Mitotic Apparatus

Abstract. *Sea urchin eggs, exposed to a radioactive amino acid during the period from fertilization to metaphase of the first cleavage, incorporate the label into proteins. Some radioactivity is localized in the region of the mitotic apparatus (MA). The specific activity of proteins from isolated mitotic apparatus is more than three times that of the proteins from the rest of the cell, although under the conditions used for isolation only 11 percent of the total incorporated label is in the MA. The radioactivity is found in proteins that do not contribute significantly to the bulk of the MA protein, but this description might possibly apply to the fibrous skeleton. Electron microscope autoradiograms reveal a close association of radioactivity with the fibers.*

Some protein synthesis is required in order for a mitosis to be completed in the developing sea urchin embryo (*1*). When fertilized eggs are exposed to labeled amino acids during the first and subsequent cleavage cycles, the mitotic apparatus and its associated structures become radioactive. This was shown by Gross and Cousineau (*2*) with autoradiograms from sections of the intact embryo and by Stafford and Iverson (*3*) with preparations of isolated mitotic apparatus (MA). Their preparations were highly radioactive although made from eggs whose exposure to label had been terminated and followed by an excess of unlabeled amino acid before metaphase.

These observations suggest that components of the MA are among the first products of the protein synthesis that are activated at fertilization (*4*), and the suggestion is strengthened by the finding in whole-egg autoradiograms that the spindle region is preferentially labeled (*2*). Thus the new proteins that associate with the MA might constitute an important fraction of those made in the period of early cleavage. Preferential localization is lost after the first few divisions; this implies that the "mitotic" proteins, if such indeed are being observed, become a less prominent class among those synthesized as development proceeds.

This interpretation has a number of important consequences for the analysis of mitosis, and a careful study of possible alternatives is therefore justified. Some of the more obvious ones were considered in the earlier papers (*2*, *3*), and have been discarded. Examination of one of the important remaining alternatives is the objective of experiments to be described.

Let us suppose that most of the protein in the region of the MA, *in situ* and as isolated, is associated specifically with the structure or function of that organelle. Contaminant proteins would make, by definition, a minor contribution to the mass. In consequence, the observed patterns of localization of radioactive protein would imply that some of the proteins of the mitotic apparatus must be newly synthesized during the cell cycle. But there is reason to believe that at least a large fraction of the protein in the MA is not specifically associated with its structure or function. Evidence from electron microscopy provides the main support for this statement (*5–7*), and more is provided by the experiment presented here. The region in which the MA is forming extrudes large cytoplasmic particulates—pigment granules, yolk particles, and mitochondria—but seems to retain, at least in the electron-microscope image, a normal sample of the ground cytoplasm, including ribosomes and smooth-surfaced vesicles. Thus, the concentration of large particulates (not likely to contain much newly synthesized protein) is perforce increased in the peripheral cytoplasm, while that of the ground cytoplasm, in which complete new proteins presumably first appear, is increased in the region of the MA. The earlier autoradiographic observations might therefore be explained on the basis of a passive concentration of new proteins having no structural or functional association with the MA itself.

The experiments which aid in evaluating this explanation are concerned with (i) the specific radioactivity of the proteins in the isolated MA as compared with the specific activity of the rest of the cell, (ii) with the chromatographic behavior of the proteins isolated from the MA, and (iii) with the localization of radioactivity in the isolated MA as indicated by the electron microscope. The results fit the original interpretation well, and can be reconciled with the alternative given only with some difficulty.

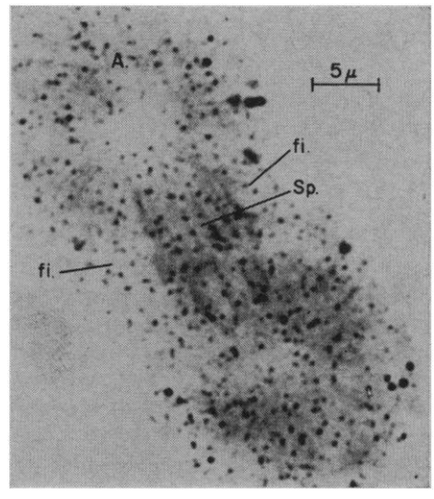

Fig. 1. Optical autoradiogram of a 1-μ section from the MA pellet. Stained with toluidine blue. Ilford L-4 emulsion exposed for 7 days. *A.*, aster; *Sp.*, spindle. The MA shown is at very early anaphase. Fibers (*fi.*) stain more darkly than the matrix, and silver grains tend to be distributed along their length.

The MA was isolated by a modification of the method of Kane (*8*). *Arbacia punctulata* were induced to shed their gametes (*9*). The eggs were fertilized, and their fertilization membranes were removed by a brief exposure to 1*M* urea, after which the zygotes were washed and resuspended

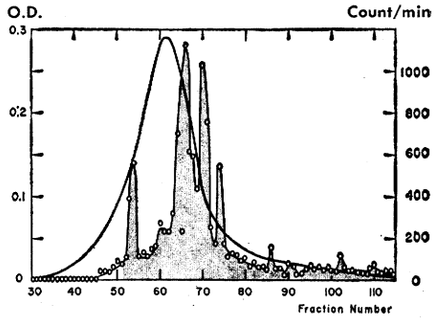

Fig. 2. Elution pattern of MA proteins dissolved in urea, from a column of Sephadex G-200. A part of the elution profile (85 of 130 fractions) containing more than 95 percent of optical density (O.D.) and counts, is represented. The continuous line represents optical density in the effluent. Circles and shaded region represent radioactive protein. Counts and O.D. have been adjusted to correspond, by correction for the dead space in tubing (0.75 ml). Fractions were collected in drops to 0.34 ml each.