was covered with a sheet of plywood, covered with plastic, 1 m by 2 m, to prevent evaporation of tritium into the immediate environment.

Six small trees (*Quercus laevis* Walt., *Q. incana* Bartr., and *Q. stellata* Wang., 1 to 9 m tall) in each plot were selected for sampling; their average distance from points of application of tritium was 2.1 to 2.4 m. Natural diversity of the vegetation precluded exact replication of species and distances.

Transpired water was collected from leaves by enclosing the ends of branches (5 to 20 leaves) in plastic bags (35 by 50 cm) which were sealed to the branch with plastic tape. Although temperatures inside the bags reached 51.5°C the leaves were not killed if the bags were removed each evening; leaves in contact with the bags were killed, however. Water was extracted from the bags with disposable plastic hypodermic syringes; bags and syringes were discarded after one use. We used an estimated 1 percent of the total leaf-surface area available on the trees studied.

Transpired water thus collected was filtered, decolorized with charcoal, and added to a scintillation mixture for liquid scintillation counting. When the liquid scintillation mixtures were "spiked" with a tritium standard and then recounted, a correction factor was obtained which showed that distillation of the water samples prior to counting was not necessary to obtain acceptable counting efficiency.

Polyethylene bags were placed on branches immediately after tritium was applied to the soil (sampling day "0") and were allowed to remain for 4 hours. In each plot, significant quantities of water moved from the soil to the leaves of at least one tree, even in this short period (Table 1), indicating rapid movement of water through the transpiration stream.

The amounts of tritiated water collected in new bags 2 days after application indicated that the rate of uptake of water was relatively much greater in surface roots than in roots in deeper soil. While this was expected, the 38 : 1 ratio of uptake between roots in the 0- to 30.5-cm zone and those in the 61- to 91.5-cm zone was somewhat greater than was expected; a greater root surface area in the shallow zone probably explains the ratio, which would undoubtedly be smaller during periods of drought.

The greatest amount of tritium recovered from one tree on one day was 4 μc per milliliter, which was obtained from a tree in the plot where the tritium had been applied in the shallowest zone. The greatest amount of water transpired by a single tree during a 4-hour period was about 25 ml. At no time was tritium present as atmospheric water vapor in quantities detectable by an extremely sensitive portable "sniffer," an ionization chamber designed specifically for tritium. Once transpired from leaves, water was blown into the surrounding environment with great speed. The large dilution factor also provided a measure of safety for the investigator; no special protective masks and clothing were necessary.

Table 1. Relative amounts of tritium recovered from leaves after tritium-water (5 c/500 ml) was applied to the soil at three different depths. The results are expressed as 10^{-6} μc/ml; each is the average for five trees.

Day of sample collection	Depth of application (cm)		
	0 to 30.5	30.5 to 61	61 to 91.5
−1 (check)	40	47	46
0*	15,700	291	186
1	299,000	10,500	138
2	363,000	19,000	9,500

* Day of application.

Rates of water movement through plants were highly variable and depended on incident solar radiation, which determined temperatures within the plastic bags. Repeated instrument failures prevented collection of radiation data. Only leaves which could be reached without climbing were sampled; in more intensive work it would be desirable to sample all parts of tree crowns.

For useful results the same leaves must be sampled on successive days. This is possible only if the plastic bag collection technique is used, but destructive sampling of leaves can provide precise measurements over short intervals. Although certain atmospheric gases diffuse through polyethylene bags, mass air movement within the bags was slight. The polyethylene-bag technique may therefore be a useful tool for isolating the various components which affect transpiration of large trees, such as air movement and temperature.

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Note

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Cyanamide Formation under Primitive Earth Conditions

Abstract. *The dimer of cyanamide, dicyandiamide, is formed upon ultraviolet irradiation of dilute cyanide solutions, and by the electron irradiation of a mixture of methane, ammonia, and water. Thus cyanamide may have had an important role in chemical evolution.*

Steinman *et al.* have pointed to cyanamide and its dimer, dicyandiamide, as possible key compounds in chemical evolution (*1*). These compounds cause the formation of pyrophosphate from orthophosphate, glucose-6-phosphate from glucose and orthophosphoric acid (H_3PO_4), and adenosine-5′-phosphate from adenosine and H_3PO_4. In all these reactions appreciable yields (1 to 3 percent) of products were obtained in a few hours from dilute (about 1m*M*) aqueous solutions at room temperature.

If cynamide played a major role in chemical evolution, it must have been formed steadily on primitive Earth. Consequently, we looked for cyanamide formation under "primitive Earth conditions" (such as, ultraviolet irradiation of hydrogen cyanide solutions, ionizing irradiations of mixtures of methane, ammonia, and water) that are known to form such biologically important compounds as the amino acids (*2*), sugars (*3*), and adenine (*4, 5*).

The ^{14}C-labeled cyanide, K ^{14}CN (15.4

Table 1. Formation of dicyandiamide in "primitive Earth" experiments. UV, ultraviolet; e$^-$, electron irradiation.

Energy source	Radioactivity		
	Total (μc)	Non-volatile (%)	Dicyandiamide* (%)
1 ml of 7.5 × 10^{-5}M H ^{14}CN			
UV	10	7.3	1.9
1 ml of 7.5 × 10^{-5}M NH_4 ^{14}CN and 1.8 × 10^{-3}M NH_3			
UV	10	3.2	3.5
$^{14}CH_4$, NH_3, H_2O			
e$^-$ beam	500	2.4	0.02
e$^-$ beam	500	1.2	0.002

* There was no detectable cyanamide monomer produced in these experiments. However, the monomer is known to dimerize readily in both acidic and basic solutions (*9*).

μc/mg) (*6*) was treated with concentrated sulfuric acid to give $H^{14}CN$ which was collected by a vacuum line and trapped at 77°K. Ammonium cyanide solutions were prepared by adding NH_4OH solutions directly to the labeled HCN. Chromatography of the unirradiated solutions indicated the absence of any detectable cyanamide or dicyandiamide.

For the ultraviolet irradiations the solutions (Table 1) were placed in quartz tubes and irradiated for 20 hours with a high-pressure mercury arc (*7*) at a distance of 7.5 cm. During the irradiations the samples were kept at 25° to 35°C by an air stream. After irradiation, the reaction mixtures were evaporated to dryness at room temperature at reduced pressure, and the total (nonvolatile) radioactivity was determined. Portions were subjected to paper chromatography on Whatman No. 4 paper (washed with oxalic acid) or on "Ederol" chromatography paper (*8*). The initial solvent systems used were *n*-butanol, propionic acid, and water (75:36:49 by volume) and propanol, 16*N* NH_4OH, and water (6:3:1). Radioactive spots (shown by autoradiographs) that had the same R_F values as those for cyanamide and dicyandiamide were cut out, eluted, and co-chromatographed with the authentic compounds in (i) *n*-butanol, ethanol, and water (4:1:1) and (ii) isopropanol, methanol, and water (18:1:1). The cyanamide and dicyandiamide were made visible by spraying the paper with a solution of 5 percent potassium nitroprusside, 10 percent NaOH, 3 percent H_2O_2, and water (2:1:5:15).

The electron irradiations of the $^{14}CH_4$-NH_3-H_2O mixture were carried out as described (*4*), except that no hydrogen was used in the present experiment. After the irradiation, the chromatographic search for cyanamide and dicyandiamide was done in the same way as in the case of the cyanide solution that had been irradiated (Table 1).

The foregoing experiments lend support to the idea that the cyanamide dimer (dicyandiamide) was formed on prebiotic Earth—before the era of life as we know it—and that this compound could have played a role in chemical evolution.

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Ribonucleic Acids of the Ilyanassa Embryo

Abstract. *From the* Ilyanassa *embryo an RNA component having a base composition similar to that of DNA has been separated by elution from a methylated albumin column. This material is judged to be a messenger RNA.*

The descriptive, experimental, and chemical embryology of the *Ilyanassa* egg has been reviewed (*1*). I now report the separation, and characterization by base composition, of the RNA of the embryo of the marine mud snail *I. obsoleta*. The results support the conclusion that a messenger RNA (mRNA) is synthesized by the embryo before and during formation of organ primordia.

Forty thousand 5-day *Ilyanassa* embryos reared at 19°C were incubated in sea water containing phosphoric acid–P^{32} (1 μc/ml) for 4 hours at 20°C. The embryos were washed and homogenized, and the RNA was separated according to Scherrer and Darnell's modification (*2*) of the phenol procedure. The RNA was precipitated with a mixture of sodium chloride and ethanol, dissolved in tris buffer and 0.01*M* $MgCl_2$, *p*H 6.7, and treated with crystalline deoxyribonuclease (10 μg/ml) for 30 minutes at room temperature. After dialysis against 0.01*M* tris buffer containing 0.001*M* $MgCl_2$, the dialyzate was made 0.05*M* with NaCl and sorbed onto a methylated albumin column (*3*). The RNA was eluted from the column with a linear gradient, 0.05 to 1.0*M*, of NaCl in 0.05*M* phosphate buffer, *p*H 6.7; 3-ml fractions were collected. That the eluted RNA was protein-free was shown by its minimal absorption at 230 mμ, which reflects the absence of peptide bonds; the ratio of optical density at 260 mμ was 2.27.

The RNA eluted from the methylated albumin column was hydrolyzed, after the addition of carrier RNA, with 0.3*N* KOH for 20 hours at 37°C. The hydrolyzate was brought to *p*H 7.0, sorbed onto charcoal, and eluted with 50 percent ethanol containing 1 percent NH_4OH; the eluate was lyophilized to remove the eluant, and the residue was dissolved in tris buffer and applied to a Dowex-1 formate column. The nucleotides were separated by elution with formic acid and ammonium formate, and the base composition of the labeled sample was calculated from the specific activity of each nucleotide (*4*).

The base composition of bulk RNA was determined from RNA obtained from embryos that had been reared throughout in the presence of phosphoric acid–P^{32} (1 μc/ml). Thus, all RNA synthesized during embryogenesis was uniformly labeled with phosphorus-32. The base composition was determined by isotopic dilution as described.

The DNA was obtained from the digestive gland of the adult snail (*5*) and hydrolyzed with 72 percent perchloric acid for 1 hour at 100°C; the bases were separated by paper chromatography (*6*) and quantitatively determined by spectrophotometry.

The base composition, elution sequence, and specific activity of the RNA recovered from the column (Fig. 1) establish the presence of four distinct RNA's. The radioactivity profile describes newly formed RNA, whereas the optical-density profile depicts pre-existing RNA.

The base compositions of all four RNA's (Fig. 1) and of the bulk RNA (Table 1) show that adenylic and uridylic acids are the predominant nucleotides in RNA of the *Ilyanassa* embryo. Ribonucleic acids having high adenylate and uridylate contents have also been reported for the bulk RNA of *Drosophila melanogaster* eggs (*7*) and for the nucleolar and cytoplasmic RNA's of the salivary gland of *Chironomus* larvae (*8*). Investigation of invertebrates other than echinoderms may show that RNA's rich in adenylate and uridylate occur throughout the invertebrate phyla. How-