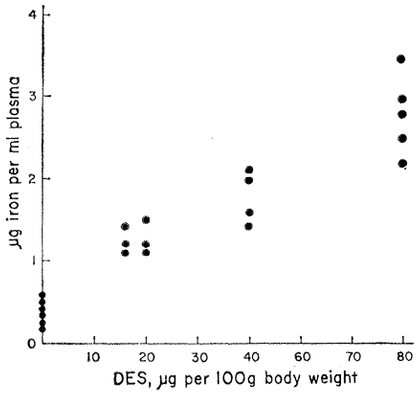

Fig. 1. The concentration of plasma iron in cockerels after the administration of varying doses of diethylstilbestrol (DES).

bath for 10 minutes and homogenized with 1.25 ml of thioglycolic acid (20 percent). After 20 minutes at room temperature, trichloroacetic acid (0.75 ml of a 30-percent solution) was added, the mixture was centrifuged, the supernatant was neutralized with concentrated ammonium hydroxide, and the intensity of the resulting purple color was measured at 520 mμ. The method with bathophenanthroline as the iron reagent (*8*) gave similar results, but since there was ample blood available the simpler though less sensitive thioglycolate method was adequate.

Forty-eight hours after being given a single dose of diethylstilbestrol, cockerels incorporate P^{32} into their plasma protein at a rate approaching that in the laying hen (*2*). Correspondingly, the concentration of alkali-labile protein-bound phosphorus is insignificant in plasma of untreated cockerels and high in that of laying hens or cockerels after estrogen treatment (Table 1). Untreated laying hens have a high plasma-iron content and treatment with estrogens causes a tenfold increase in the plasma-iron content of cockerels. The source of this iron, or, in general, the interrelation between the iron content of plasma, liver, and other organs of fowl and the effect of sex hormones is not clear. Although in several vertebrate species including the chicken the female can store more iron in its liver than the male (*9*), treatment of immature pullets with estrogen for 12 days did not increase the concentration of iron in liver (*10*).

Table 1. The phosphoprotein and iron content of the plasma of hens, untreated cockerels, and cockerels treated with estrogen. Forty-eight hours after the subcutaneous inoculation of the indicated substances, the protein was isolated from one sample of plasma and its alkali-labile phosphorus (P) content was estimated (*2*). The iron content of this preparation (shown in parentheses) and of another sample of whole plasma was determined as described in the text. Each value is the mean of duplicate assays on plasma of three animals. DES, diethylstilbestrol; estradiol, estradiol benzoate (Progynon, Schering Corp.).

Substances administered (per 100 g body wt)	Alkali-labile P (μg/ml plasma)	Iron (μg/ml plasma)
	Cockerel	
None	0.5	0.5 (0.7)
	Laying hen	
None	122.5	7.8 (8.0)
	Cockerel	
DES, 5 mg	82.5	5.9 (5.6)
	Cockerel	
Estradiol, 0.3 mg	59.5	5.6 (5.2)
	Cockerel	
Estradiol, 8 μg		2.2

The plasma iron is protein-bound; extraction of the proteins with hot trichloroacetic acid and organic solvents did not remove iron (see numbers in parentheses, Table 1). Prolonged incubation with dilute sodium hydroxide, which splits off the protein-bound phosphate, also removes the iron, whereas dialysis at *p*H 5, which is effective in the case of mammalian transferrin, does not. These observations support the assumption that the accumulated phosphoprotein, by virtue of its iron-binding capacity, is responsible for the increased iron content of plasma in cockerels. Thus, phosvitin appears to become an "iron carrier" while still in the plasma, before it is transferred to the developing egg in the ovary.

To estimate phosphoprotein in plasma, the protein is precipitated and extracted with a series of solvents to remove nonprotein material which might contain phosphorus and is hydrolyzed for 16 hours in dilute alkali. In contrast, estimation of plasma iron is a brief procedure and provides a simple way of evaluating estrogen action. Furthermore, much less estrogen causes a detectable increase in iron than in phosphoprotein.

Figure 1 shows the direct correlation between the amount of injected diethylstilbestrol and the amount of plasma iron detected 18 hours later. Estradiol evokes the same response at concentrations of approximately one-fifth of those of diethylstilbestrol (Table 1). As expected, the injection of urine of pregnant women (1 ml) raises the concentration of iron in the plasma of cockerels by at least 400 percent. As a qualitative test, this method is particularly simple; the difference between the very low iron concentration in the plasma of uninjected cockerels and those injected with material containing more than 4 μg of estradiol can be seen with the naked eye upon the addition of an iron reagent.

The aforementioned approach may conceivably be useful for estimating the estrogen content of biological materials in cases where chemical assay is not applicable. In hens, the concentration of plasma iron may provide a biologically meaningful and easily obtainable measure of sexual maturity and reproductive capacity.

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Peptide Synthesis from Amino Acids in Aqueous Solution

Abstract. *Four dipeptides and a tripeptide were formed when an aqueous solution of glycine and leucine was exposed to ultraviolet light in the presence of cyanamide.*

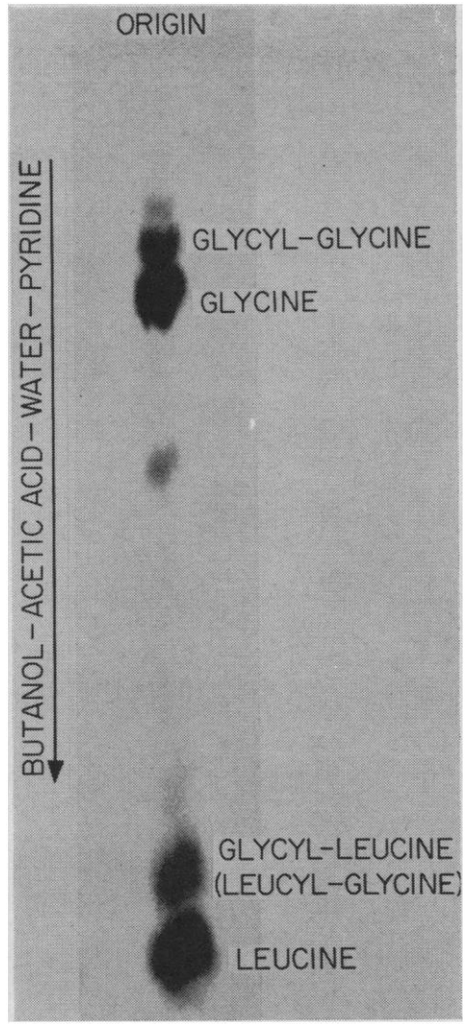
In recent years, several experiments have been performed to test the hypothesis of chemical evolution (*1*). The formation of amino acids in such experiments has been widely reported. In his classic experiment on electric discharge, Miller identified the amino acids glycine, alanine, β-alanine, aspartic acid, and glutamic acid (*2*). Palm and Calvin found glycine, alanine, and as-

partic acid after electron irradiation of methane, ammonia, and water (*3*). Oro reported the presence of glycine, alanine, isoleucine, and proline in his discharge work (*4*). Grossenbacher and Knight produced serine, glutamic acid, glycine, isoleucine, leucine, and lysine by sparking a mixture of methane, ammonia, and water for several days (*5*). More recently, Harada and Fox showed that, when a mixture of methane, ammonia, and water was heated to about 1000°C, 14 of the amino acids commonly present in protein were formed (*6*). The synthesis of most of the amino acids under simulated conditions of the primitive earth thus appears to have been very clearly established.

Other reports indicate that, in addition to amino acids, peptides and polypeptides have been synthesized under abiological conditions. Fox has shown that, under anhydrous conditions, polymers can be prepared by heating a mixture of amino acids to about 180°C. In some cases, polymers having a mean molecular weight of about 300,000 were isolated (*7*). Oro demonstrated that polypeptides can form under aqueous conditions as well. Polyglycines with a degree of polymerization up to 18 were synthesized by heating a concentrated solution of glycine in 2*N* ammonium hydroxide (*8*). Several other possible pathways for polypeptide synthesis have been described by Oro in a recent review (*9*).

We have examined whether amino acids at low concentrations combine to give peptides in aqueous solution at a *p*H neither strongly acidic nor too basic. Since deoxyadenosine was readily synthesized from a very dilute aqueous solution of adenine and deoxyribose in the presence of CN^-, Mg^{++}, or cyanamide (*10*), we decided to study these compounds as possible reagents for the synthesis of peptides by dehydration polymerization. Experiments with cyanamide (*11*) are described in this paper. Starting with glycine and leucine, we synthesized the dipeptides glycyl-glycine, glycyl-leucine, leucyl-glycine, leucyl-leucine, and the tripeptide glycyl-glycyl-glycine.

Aqueous solutions of glycine-C^{14} and leucine-C^{14} were exposed to a source of ultraviolet light in the presence of cyanamide. In a typical experiment, 170 μl of a solution containing stoichiometric amounts of glycine, leucine, and cyanamide were used. Their concentrations were of the order of 10^{-2} mole/liter. The specific activity of the amino acids used was approximately 1 mc/mole. The cyanamide, supplied by Eastman Kodak Company, had a melting point of approximately 44°C and was in the form of the monomer. This compound is not to be confused with the dimer (mp 209° to 211°C) also supplied by Eastman as cyanoguanidine.



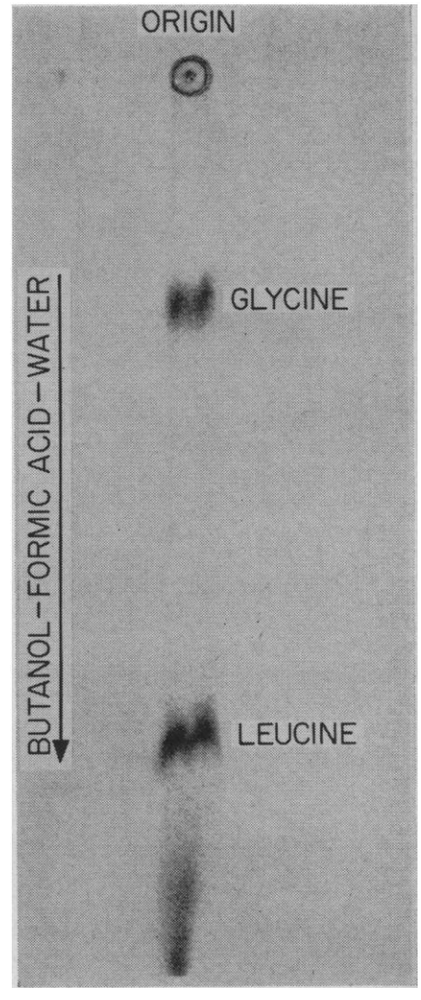


Fig. 1 (left). Chromatogram illustrating formation of peptides from an aqueous solution of glycine and leucine. Fig. 2 (right). Chromatogram showing the products of hydrolysis of the peptide glycyl-leucine.

Dissolved oxygen was removed by bubbling a slow stream of nitrogen through the solution for 10 minutes. The tubes containing the solution were then frozen in liquid nitrogen, evacuated, and sealed. The tubes used for irradiation were made of Spectrosil, which transmits over 95 percent of the ultraviolet light above 1800 Å. The sealed tubes were then exposed for 2 hours to a battery of four ultraviolet lamps (*12*). During the irradiation, the tubes were maintained at room temperature. The *p*H of the solution was about 5.

For identification, we used the coincidence technique of paper chromatography (*13*). Nonradioactive carriers of the suspected resultant products were added to the material chromatographed. In this experiment, we used 10 μg each of the dipeptides glycyl-glycine, glycyl-leucine, leucyl-glycine, leucyl-leucine, and the tripeptides glycyl-glycyl-glycine and leucyl-glycyl-glycine. If the radioactive products are identical with the carriers, the dark spots on the autoradiograph correspond precisely to the bright spots on the shadowgram.

Figure 1 illustrates a one-dimensional chromatogram of the reaction products separated by a mixture of butanol, acetic acid, water, and pyridine (15:3:12:10 by volume). There was perfect coincidence between the carriers glycyl-glycine, glycyl-leucine, leucyl-glycine and material synthesized.

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*).

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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.

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