

binding and in sensitivity to apomorphine (Table 1 and Fig. 2), the effect of haloperidol being similar to that observed in adult rats (13).

The decrease in [³H]spiroperidol binding and apomorphine-induced stereotypy in the offspring of rats treated with haloperidol during pregnancy does not appear to be the result of malnutrition or retarded growth. Inasmuch as haloperidol restricts the access of dopamine to the receptor and α -MT blocks dopamine synthesis, it may be that normal receptor development is contingent on exposure of developing receptor cells to dopamine. When pups receive haloperidol postnatally in the milk of their mothers, they demonstrate supersensitivity on haloperidol withdrawal. This is similar to the effect in mature rats and presumably reflects the effect of blockade of more mature receptors.

The fact that moderately high doses of a neuroleptic administered to pregnant or nursing rat mothers produces prolonged sensitivity changes in their offspring may have implications for the offspring of human mothers treated with neuroleptics.

HELEN ROSENGARTEN
ARNOLD J. FRIEDHOFF

Millhauser Laboratories,
Department of Psychiatry,
New York University School of
Medicine, New York 10016

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10. To prepare caudate tissue for assay, we killed the pups by decapitation, removed brains immediately, placed them on ice, and dissected rapidly both caudates. Pairs of caudates from 14-day-old pups weighed between 48 and 53 mg, increasing to 80 to 90 mg in the 60-day-old animals. After dissection, the caudates from an individual pup were pooled and homogenized in 50 volumes of ice-cold sucrose (0.32M) and centrifuged at 1000g for 10 minutes; the pellet was discarded. The supernatant was recentrifuged at 48,000g for 10 minutes to give a crude P₂ fraction. The pellet was rehomogenized in 50 mM tris-Ringer buffer, pH 7.4, and centrifuged at 48,000g for 10 minutes, resuspended in the same buffer and again centrifuged at 48,000g for 10 minutes; the supernatant was discarded and the pellet resuspended in tris-Ringer buffer, pH 7.4.
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12. Stereotyped behavior was tested at 28 and 35 days postpartum after a single intraperitoneal injection of apomorphine (0.3 mg/kg). Stereotyped behavior was observed for 1 minute at 10-minute intervals for a total of 60 minutes after the injection. Animals were evaluated with a scale for rating the dose-dependent stereotypy that emerges after apomorphine administration. On the original scale (9), stereotypy was rated from 0 to 3 (absent to severe). We used a 5-point scale (0 to 4) with 4 being the most severe. This modification was made because we were able to achieve better discrimination of dose-dependent responses with the expanded range. On our scale, each point was defined as follows: 0, absent or discontinuous sniffing, normal locomotion; 1, continuous sniffing, discontinuous licking, normal locomotion; 2, discontinuous biting, restricted locomotor activity; 3, continuous biting, absent locomotor activity; 4, continuous biting, absent locomotor activity, and absent startle response.
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HCN Did Not Condense to Give Heteropolypeptides on the Primitive Earth

Matthews and co-workers propose that (i) heteropolypeptides are formed directly from hydrogen cyanide (HCN) (1, 2) and (ii) these polypeptides were formed on the primitive earth as well as such extraterrestrial environs as the moon and Jupiter (3). I briefly outline earlier data, which they do not cite, and other results that show their hypothesis is incorrect.

The available experimental evidence in support of the proposal that polypeptides and their precursors are formed directly from HCN is tenuous (1-4). There are two findings (i) that hydrolysis of HCN oligomers gives amino acids and (ii) that HCN oligomers exhibit infrared absorption at a frequency where the carbonyl and imine group is known to absorb. However, many compounds release amino acids on hydrolysis—a notable example is the formation of glycine by hydrolysis of diaminomaleonitrile, a tetramer of HCN, while infrared absorption in the carbonyl region does not prove that peptides are present (5).

Two different laboratories reported experiments that were specifically designed to test for the presence of peptide bonds in the HCN oligomers; these experiments yielded completely negative results, which were not cited by Matthews *et al.* (6, 7). Of particular significance is the absence of oligomer hydrolysis catalyzed by Pronase, an enzyme that will even catalyze the hydrolysis of diglycine to glycine. If peptide links are present in the HCN oligomers, a significant number should be diglycine units, which would be susceptible to Pronase catalyzed hydrolysis. Matthews (3) cites

unpublished work in which the enzymatic hydrolysis of HCN oligomers is claimed (3). This study (3) remains unpublished.

Matthews' recent papers (1, 2) do not even acknowledge that the formation of heteropolypeptides has been disputed. The hydrolysis of the reaction product of poly(α -cyanoglycine) and H¹³CN to yield ¹³C-labeled amino acids is discussed (2). But, since poly(α -cyanoglycine) already contains peptide links, no data concerning the presence of peptides in the HCN oligomers can be obtained from these experiments. The incorporation of deuterium in the amino acids released on hydrolysis of the HCN oligomers with DCI in D₂O is also reported (1). These data do not provide evidence for peptides or peptide precursors since it is noted that deuterated glycine is released on deuterolysis of diaminomaleonitrile [(reference 14 in (1))]. Since diaminomaleonitrile has a central role in the formation of HCN oligomers (see below), it is not surprising that deuterolysis of the HCN oligomers yields deuterated glycine.

Azacyclopropenyldienimine, a dimer of HCN, is claimed to be the monomer unit that condenses to give the HCN oligomers (1, 2, 8). First, no mention was made of the iminoacetonitrile structure for the HCN dimer, which has been shown to be the most stable dimer of hydrogen cyanide by a variety of theoretical calculations (9). Second, since only a low steady-state concentration of the HCN dimer, be it iminoacetonitrile or azacyclopropenyldienimine, will be produced from HCN it is highly unlikely

that this dimer will undergo self-condensation reactions. Instead this dimer will undergo addition reactions with the large excess of cyanide that is present to generate diaminomaleonitrile. The addition of cyanide to *N*-alkyl derivatives of iminoacetonitrile, yielding *N*-substituted diaminomaleonitrile derivatives, was shown to proceed more rapidly than their oligomerization (10).

It might be argued that, even though diaminomaleonitrile forms spontaneously from HCN, there are sufficiently large amounts of HCN dimer and trimer in equilibrium with it for the proposed self-condensation of HCN dimer (1, 2). We find that the formation of diaminomaleonitrile from the dimer and trimer is virtually irreversible (11). Incubating $H^{13}CN$ with diaminomaleonitrile at pH 7 and pH 8.5 for 3 to 10 days at 30°C resulted in no detectable increase in the ^{13}C content of the recovered diaminomaleonitrile. Since trimer formed at equilibrium would react rapidly with $H^{13}CN$ to generate [^{13}C]diaminomaleonitrile, this result demonstrates that there is essentially no trimer (and therefore no dimer) in equilibrium with diaminomaleonitrile.

Diaminomaleonitrile, and not a HCN dimer, must be the direct precursor to the HCN oligomers since it has been established that the formation of diaminomaleonitrile from HCN dimer and trimer is essentially irreversible. The structures of the hydrolysis products of the HCN oligomers, glycine, diaminosuccinic acid, aspartic acid, 4,5-dihydropyrimidine, and oxalic acid are readily understood if it is assumed that they are derived from diaminomaleonitrile structural units incorporated into HCN oligomers (12).

Gas phase condensation reactions of HCN are postulated to have been important sources of biomolecules in extraterrestrial environments as well as on the primitive earth (1). The thermal, gas phase condensation of HCN has not been experimentally defined. The photolysis of HCN eventually generates HCN oligomers but this is probably due to the base-catalyzed condensation of HCN on the walls of the reaction vessels since oligomerization continues after the irradiation is stopped (13). The products formed by direct photolysis— $(CN)_2$, H_2 , CH_4 , NH_3 , CH_3NH_2 , and the like—are unrelated to any postulated by Matthews.

The incorporation of carbon-bound deuterium in the glycine obtained by extraction of the Murchison meteorite with D_2O (14) was cited as evidence for the presence of HCN oligomers in this meteorite and other extraterrestrial environ-

ments (1). Subsequent studies not cited by Matthews established that the deuterium incorporation is due to hydrogen-deuterium exchange and not to the synthesis of carbon-deuterium bonds by the deuterolysis of HCN oligomers or other precursors (15).

Amino acids, purines, and pyrimidines are released on hydrolysis of the HCN oligomers (12). It is reasonable to extrapolate from these data that HCN was a likely source of these biomolecules on the primitive earth and possibly some extraterrestrial environments. Also some of the amino acids released in spark discharge experiments may have resulted from the hydrolysis of HCN oligomers. But, the postulate that heteropolypeptides are formed by the gas phase condensation of HCN dimers is incompatible with a large body of experimental data.

JAMES P. FERRIS

Department of Chemistry,
Rensselaer Polytechnic Institute,
Troy, New York 12181

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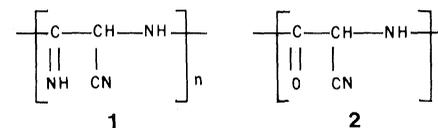
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It has been proposed (1, 2) that the original heteropolypeptides on Earth were synthesized spontaneously from hydrogen cyanide and water without the intervening formation of α -amino acids. A key step was the direct vapor-phase polymerization of HCN to polyaminomalnonitrile (1), after the initial production of hydrogen cyanide in the upper atmosphere by photolysis of meth-

ane and ammonia (or other reducing combination of gases). Further cyanide interaction with the nitrile side chains then yielded heteropolypeptides, which became heteropolypeptides on contact with water.

The model predicts that mixtures of α -amino acids (not just glycine) would be formed by hydrolyzing polymeric products resulting from each of the following types of reactions: (i) liquid phase polymerization of HCN in the absence of water (3); (ii) electric discharge experiments yielding HCN from methane-ammonia mixtures (1); (iii) alkaline treatment of aminoacetonitrile (4), aminomalnonitrile (HCN trimer) (5), and diaminomalnonitrile (HCN tetramer) (5), all of which are ready sources of HCN after decomposition; (iv) $H^{13}CN$ modification of the reactive nitrile side chains of poly- α -cyanoglycine (2), the polyamide analog of polyaminomalnonitrile (6); and (v) further, deuterolysis (D_2O/DCI) of each of the above polymeric products would be expected to yield perdeuterated glycine as well as other deuterated amino acids (7).



My co-workers and I have verified these predictions (1-7). What is also needed to fully establish the validity of the model is the unambiguous isolation and characterization of heteropolypeptides from such experiments. Here we have been less successful, mainly, I believe, because of the inherent drawbacks of condensed-phase studies (cross-linking), vapor-phase experiments (wall effects) and, especially, reactions in water (hydrolysis). In general, we obtained water-soluble, yellow-brown solids, in many cases separated by Sephadex into fractions, each of which yielded mixtures of α -amino acids after acid hydrolysis. These polymers probably consist of polypeptide fragments linked to several kinds of other segments (1, 7). Ambiguous results are therefore not surprising when they are subjected to standard biuret and enzyme tests for proteins, particularly when cyanides are released during testing (2). Altogether, this body of work suggests that crude polymers with some peptide character are readily formed from HCN reactions over a wide range of physical conditions, including those prevailing within carbonaceous chondrites and, perhaps, the moon (2, 7). Hydrogen cyanide polymerization could account, too, for the yellow-

low-orange-brown colors of Jupiter with its reducing environment, a continuing reminder that optimum conditions for heteropolyamidine synthesis might well have existed when Earth was a young planet with an upper atmosphere rich in reduced carbon and nitrogen. As these cyanide polymers settled onto Earth's surface—land and sea—together with other products of atmospheric photochemistry, a proteinaceous matrix developed able to take part in and promote the chemistry leading to the emergence of life (2, 8).

A more widely accepted view of the origin of proteins starts with the prior synthesis of α -amino acids from intermediates such as HCN oligomers or aminoacetonitriles (9, 10). How the thermodynamic barrier to spontaneous polymerization of these monomers is overcome, however, remains a question that generates much difference of opinion (9). With a "dilute soup" model in mind (10), Ferris has critically assessed our research and concluded that our new paradigm of innate protein structure is incorrect.

1) He quotes negative evidence for the presence of peptides in HCN products, but fails to mention the rigorous work of Draganic and Draganic (11) who combined a modified biuret procedure with infrared absorption spectroscopy to show the presence of peptide links and nitrile groups in products formed by ionizing radiation in aqueous cyanides. Subsequent hydrolysis yielded several α -amino acids.

2) The point of our work on poly- α -cyanoglycine was not to demonstrate HCN polymerization, but rather to show that HCN (and H₂O) could convert a homopolymer to a heteropolymer possessing protein side chains (6). Ferris proposes no alternative mechanism to account for side chain formation in HCN reactions.

3) It is conventionally assumed that HCN dimer is iminoacetonitrile. Since the HCN dimer has not yet been isolated we have suggested that other more reactive structures such as aminocyanocarbene or azacyclopropenylideneimine might actually be involved in HCN polymerization (12), particularly within clouds of hydrogen-bonded HCN molecules. The *N*-alkyl derivatives of iminoacetonitrile studied by Ferris (13) are so stable that they tell little about the nature of the elusive dimer of HCN.

4) Ferris presents no direct evidence that HCN oligomers are formed from diaminomaleonitrile, (HCN)₄. He reasons (14) that the steady state concentrations of (HCN)₂ and (HCN)₃ are ex-

ceedingly low in dilute aqueous solutions of HCN, as shown by the absence of exchange of H¹³CN with (HCN)₄ when incubated in alkali. However, H¹³CN would also be expected to yield some (H¹³CN)₄, which apparently was not detected. If conditions did not favor (H¹³CN)₄ formation, why should exchange of H¹³CN with (HCN)₄ take place? Indeed, both exchange and polymerization (as shown by the solution becoming black) evidently occurred in a parallel experiment carried out in DMSO. The aqueous experiments are clearly not conclusive and, in any case, are hardly relevant to our nonaqueous model. In our original studies (3), we were careful to remove (HCN)₄ from polymeric products before hydrolyzing them to α -amino acids. Perdeuterated glycine obtained from HCN polymers could not therefore be due only to the presence of (HCN)₄ (7).

5) We agree with Ferris that the products of HCN photolysis—and, we would add, of spark discharge experiments—probably arise mainly from base-catalyzed reactions of HCN condensed on the walls of the reaction vessels. Such experiments may be significant, but not as models for atmospheric chemistry.

6) Regarding the components of carbonaceous chondrites, we continue to believe that the incorporation of carbon-bound deuterium in the glycine obtained by extraction of the Murchison meteorite with D₂O is evidence for the presence of HCN polymers. Our current GC-MS studies (mass fragmentography) (15) lead us to question Lawless' concept of hydrogen-deuterium exchange brought about by "selective catalytic activity" of the meteorite (16).

Far from refuting the hypothesis that

polyaminomalononitrile was the original ancestor of all proteins, it seems to us that the research of Ferris and his co-workers is too narrowly restricted to aqueous cyanide chemistry to have much bearing on the issues involved. We are encouraged to persist in our reinvestigation and reinterpretation of chemical evolution studies on the origin of proteins (7, 8).

CLIFFORD N. MATTHEWS

Department of Chemistry, University of Illinois at Chicago Circle, Chicago 60680

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Infant Perception of Visually Presented Objects

Dodwell *et al.* (1) have published data indicating that human infants in the newborn period do not discriminate between real objects and pictures of objects. However, the experiment that produced the result is flawed.

Dodwell *et al.* used an experimental design in which they intended to present babies with an object and a representation of an object. They then intended to determine whether the babies reached as much for the one as the other. Unfortunately, rather than presenting the babies with a representation and an object, they presented two objects. To be sure, one of the objects was a photograph, but a photograph is an object; it has parallax

variables around its edges. If one is to use a photograph to present representations, one must obliterate these object-specifying variables. This can be done either by using a very large photograph whose edges are out of the visual field or by presenting the photograph flush against a background (2).

Dodwell *et al.* thus presented the babies with two objects, one with a representation in its center, the other with another object in its center. It is not at all certain that the infant would see the representation or the small object (3); a demonstration that they could would itself be significant. In principle, Dodwell *et al.* could have determined this if they