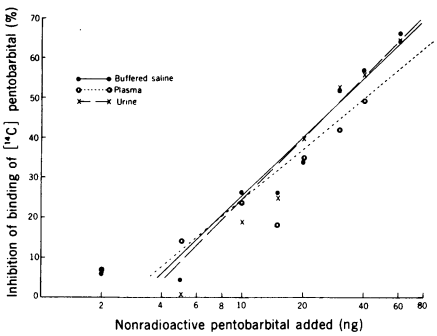

Fig. 3. Inhibition of binding of [^{14}C]pentobarbital to rabbit antiserum by nonradioactive pentobarbital in buffered saline (●——●), plasma (○——○), or urine (×——×). Incubation medium consisted of 0.10 ml of normal rabbit serum, 0.10 ml of rabbit antiserum (0.4 mg of protein), 0.01 ml of [^{14}C]pentobarbital (0.1 nmole), and 0.01 ml of either standard unlabeled pentobarbital (1 to 100 ng) or unknown sample and sufficient phosphate-buffered saline (0.01*M* phosphate, *p*H 7.4) to make a final volume of 0.50 ml. Lines of regression were calculated by the method of least squares.

different substituents at either position 2 or 3 in the barbituric acid ring. Thus, the urea portion of the ring may be critical in determining antibody specificity.

The barbiturate-BGG antigen was effective in eliciting antibodies against barbituric acid derivatives. We believe that this is the first report of the experimental production of antibodies capable of recognizing barbiturates. The radioimmunoassay technique reported here is rapid and extremely sensitive, and should be useful for the determination of barbiturate concentrations in biological tissues and fluids. Theoretically, since metabolic products with changes at the C-5 position may also be detected by the antibody, our procedure, coupled with a solvent extraction, could measure both total concentration of barbiturate and its hydroxylated metabolites. Antibodies directed against steroid haptens (*7*) and digitalis (*8*) have been reported to modify the physiological actions of these agents, and it would be interesting to determine whether antibodies against barbiturates interfere with the pharmacological effects of barbiturates.

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Formaldehyde and Ammonia as Precursors to Prebiotic Amino Acids

Fox and Windsor (*1*) reported synthesis of a number of amino acids when mixtures of formaldehyde and ammonia were heated at 185°C for 8 hours. These reactants were chosen because they have been identified in contemporary interstellar matter, and thus "reaction of the two is more easily visualized as a consequence of this coexistence."

A number of molecules have been found in interstellar clouds, including several diatomic and several polyatomic species (*2*). These discoveries have naturally led to speculation on the relation between the existence of such molecules and the origin of life on earth. However these speculations usually disregard the extremely low volume densities (from 10^{-2} to 10^{-5} molecules per cubic centimeter) and column densities (from 10^{13} to 10^{16} molecules per square centimeter) in the clouds.

For example, consider the experiments cited above. Fox and Windsor used formaldehyde and ammonia concentrations in the moles per liter range. This is some 10^{24} times as great as the maximum expected in interstellar clouds. Mild extended thermal treatment of the type used in the experiments causes the system to approach thermodynamic equilibrium. Rough thermodynamic calculations show, for example, that for the reaction

$$2H_2CO + NH_3 \rightleftarrows \text{glycine} + H_2$$

the equilibrium concentration of glycine is quite high under the experimental conditions, but very low (of the order of one molecule per 1000 km^3) under the conditions existing in interstellar clouds. Equilibrium concentrations of other more complex amino acids would be less. Approach to even the low equilibrium concentrations would be very slow because of the extremely low concentrations of reactants.

The concentrations of formaldehyde and ammonia at the earth's surface could have been increased over those found in the clouds if the solar system, including the earth, formed from condensation of such a cloud. However, in this case most of the molecules would have been destroyed by the high temperatures in the condensing system. Whether or not such destruction occurred, any uncombined formaldehyde or ammonia present early in earth's history would have been lost in the primeval degassing episode that removed the rare gases.

Alternatively it might be supposed that the earth swept through a cloud containing the molecules. In this case most of them would have been destroyed by the ultraviolet radiation from the sun before reaching the earth (*3*). However, even if they were protected from photodissociation from ultraviolet light (as they seem to be, to some extent in outer space) and if all the molecules intercepted by the earth remained in its atmosphere, 10^{16} molecules of formaldehyde or ammonia per square centimeter in the cloud would give a partial pressure at the earth's surface less than 10^{-9} atmosphere—about 10^{-12} of the concentration used in the experiment. On the other hand, the molecules could have been generated easily on the primitive earth (NH_3 from outgassing, HCHO from various nonequilibrium energy sources acting on water and methane or other carbon-containing compounds). Ammonia concentrations were probably around 10^{-5} atm (*4*), orders of magnitude greater than would be obtained from a cloud, while formaldehyde gen-

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eration rates could have approached 10^{11} molecules per square centimeter per second (*3*)—a rate orders of magnitude greater than that attainable by deposition from a cloud even if the earth were moving through it at the speed of light. Thus interstellar formaldehyde and ammonia probably had little effect on the composition of the atmosphere of the primitive earth or on prebiological evolution here.

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31 December 1970; revised 10 June 1971

Fox and Windsor (*1*) state that seven amino acids are formed by heating formaldehyde and ammonia, which have been found in interstellar space (*2*). The relevance of Fox's experiments to either interstellar amino acid synthesis or to primitive earth synthesis is questionable. The concentration of formaldehyde and ammonia in space is less than 1 molecule per cubic centimeter (*2*), and Fox's reagents are 5 to 10 molar, an extrapolation of at least 20 orders of magnitude.

Because those experiments were carried out with commercial reagents (*3*), we repeated the experiments with commercial reagents (15 ml of a 37 percent solution of formaldehyde and 5 ml of a 28 percent solution of ammonia) as well as with distilled reagents (15 ml of a 30 percent solution of formaldehyde prepared by distillation of paraformaldehyde from acidic aqueous solution, and 5 ml of a 28 percent solution of ammonia prepared by dissolving distilled liquid ammonia in glass-distilled water). The reaction mixture was heated for 8 hours at 185°C under nitrogen, and the products were cooled, dissolved in 100 ml of constant boiling hydrochloric acid, and refluxed for 24 hours. The liquid was concentrated to dryness, and the residue was desalted in an ion exchange (Dowex 50 × 8) column (0.75 by 40 cm). The amino acids were eluted from the column with 2*N* ammonium hydroxide prepared as above. After being evaporated to dryness, the residue was dissolved to citrate buffer (*p*H 2.2) and analyzed on the amino acid analyzer (*4*) (Table 1, columns 1 and 2). Our results are comparable to those reported by Fox. The distilled reagents give twice the yield of the "dirty" reagents. A control in which the residue was obtained by heating formaldehyde and ammonia without acid hydrolysis did not give any detectable amounts (less than 0.25 μmole per mole of formaldehyde) of amino acids.

Table 1. Yields of amino acids in micromoles per mole of formaldehyde and as the relative yields in percent.

Amino acid	Commercial reagents		Distilled reagents		Acid hydrolysis of hexamethylenetetramine			
					Open vessel		Evacuated vessel	
	Micromoles	Percent	Micromoles	Percent	Micromoles	Percent	Micromoles	Percent
Aspartic acid	0.16	0.54	0.34	0.46	0.82	2.47	0.22	0.15
Threonine*	Trace		Trace		0.13	0.40	0.23	0.16
Serine	0.37	1.22	0.35	0.48	1.36	4.1	0.52	0.34
Glutamic acid†	2.36	7.70	6.0	8.2	3.0	9.0	5.1	3.4
Proline			Trace		Trace		Trace	
Glycine	25.4	82.8	63.4	86.8	23.6	71.2	141.1	94.4
Alanine	0.64	2.1	1.1	1.5	0.39	1.2	1.00	0.67
Valine‡	Trace		Trace		0.17	0.49	0.23	0.15
Isoleucine§	0.14	0.46	Trace		0.13	0.40	Trace	
Leucine§	Trace				0.13	0.40	Trace	
Phenylalanine§			Trace		0.69	2.1	0.31	0.21
Unknown	1.6	5.2	1.78	2.4	2.5	8.3	0.72	0.48
Total yield based on NH_3 (%)								
	0.004		0.008		0.005		0.022	

* We were unable to confirm the presence of threonine in this peak. Fox and Windsor were not able to detect this peak on their amino acid analyzer. † The glutamic acid peak in the amino acid analyzer contains, besides the gultamic acid, an unknown amino acid. ‡ We were unable to confirm its presence because of the extremely low yield. § The isoleucine, leucine, and phenylalanine peaks on the amino acid analyzer were shown by Fox and Windsor (*1*) and by us not to be these compounds.

As a further control, hexamethylenetetramine, which is formed from formaldehyde and ammonia (*5*) was subjected to acid hydrolysis. The hexamethylenetetramine was purified by sublimation (135°C, 0.2 torr; this hexamethylenetetramine did not have any detectable amounts of amino acids —less than 0.3 μmole per mole of formaldehyde) and hydrolyzed by refluxing in 6*N* HCl in an open vessel for 24 hours. The product was desalted and analyzed on the amino acid analyzer (Table 1, column 3).

When the acid hydrolysis was carried out in an evacuated tube at 110°C for 24 hours, which is the normal procedure for acid hydrolysis of peptides and proteins (*6*), much greater amounts of amino acids were formed (Table 1, column 4) than in the hydrolysis by reflux used by Fox and Windsor.

The identities of the amino acids were confirmed by use of one-dimensional high-voltage electrophoresis (10 percent acetic acid, 2 percent formic acid, 10 volt/cm), one-dimensional paper chromatography (*n*-butanol : acetic acid : water 4 : 1 : 5). The presence of the following amino acids was confirmed: aspartic acid, serine, glutamic acid, proline, glycine, and alanine.

The acid hydrolysis of hexamethylenetetramine accounts for the entire yield of amino acids reported (*1*). Indeed, it had been shown previously that amino acids can be generated by acid hydrolysis of mixtures of formaldehyde and other nitrogen compounds (*7*).

These results show that heating formaldehyde and ammonia is not a prebiotic synthesis of amino acids, even neglecting the extrapolation of 20 orders of magnitude in the concentration. In addition, since formaldehyde, ammonia, and some other potential amino acids precursors (such as acetaldehyde and HCN) are lunar module exhaust products (*8*), it is possible that some of the amino acids reported by Fox and co-workers (*9*) for the hydrolyzates of water extracts of the lunar samples are also artifacts of the acid hydrolysis. This is further supported by the fact that no amino acids in unhydrolyzed water extracts were detected by Fox *et al.* (*10*).

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We agree with much of Hulett's chemical logic within the limits that he selects (*1*), but not with his presumption of all of those limits. Similarily, we did not confine our thinking to formaldehyde and ammonia as only, or principal, precursors of amino acids on the earth in its geologic past. We were also not proposing abiotic synthesis of amino acids within clouds of interstellar matter. That postulate is, however, in our view worthy of investigation, especially since analytical studies of lunar dust suggest the possibility of such a synthesis at the surface of the moon (*2*).

Among our essential assertions are (i) experiments have a newly plausible basis in intermediates demonstrated to have cosmic occurrence, and (ii) experiments that simultaneously yield a number of the α-amino acids of protein are most germane to evolutionary theory (*3*). Conceptually, exceedingly small amounts of truly evolvable precursors were all that were needed to begin organic evolution on earth. The early biosphere, insofar as we know, required no more than one spontaneously originating protobiogenetic seed capable of converting related organic matter in the environment into nutritive stuff for further proliferation through the first cells (*4*). A number of questions for the successive transformations between galactic organics and terrestrial amino acids have not yet been answered, but we can be sure that physical and chemical condensations have occurred in the past. We can also be sure that a wide range of physical conditions existed during such condensations.

Another way in which Hulett's premises are too limiting for adequate experimental exploration stems from his postulation of reactions in the atmosphere. The importance of the lithosphere has been indicated, for example, by the reliance of contemporary organisms on metal atoms (*5*). This is one aspect of the meaning of our statement (*3*) that "the syntheses from formaldehyde proceed from nongaseous intermediates." No reason is apparent for ruling out the locale of the lithosphere, which is the phase most likely, for example, to occlude hexamethylenetetramine (HMT), of sublimation point 263°C, as a solid intermediate. Formaldehyde and ammonia may have been more abundant on the primitive earth than in interstellar matter, as Hulett proposes, but this is a presumption; colossal clouds of galactic formaldehyde have a contemporary factual basis (*6*).

We see internal inconsistencies, however, within the interpretations of Wolman *et al.* (*7*). Since formaldehyde and ammonia are intermediates for HMT, and hydrolysis of HMT yields the set of amino acids we and they have obtained from formaldehyde and ammonia by heating, basic logic specifies that formaldehyde and ammonia and HMT are all intermediates in a sequence yielding amino acids. If $A \rightarrow B$ and $B \rightarrow C$, then $A \rightarrow \rightarrow C$. This result is thus relevant to attempts to model prebiotic synthesis of amino acids; Wolman *et al.* can be credited with identifying one intermediate (*8*).

Discussion by Wolman *et al.* of the concentrations of reagents is not, however, relevant; the HMT and other material formed from formaldehyde and ammonia are solid products that are less volatile than water or excess ammonia. The boiling off or evaporation of water are well known as either laboratory or geochemical processes. Temperatures sufficiently high to activate reactions often cause volatilization *in open systems*.

One reason, in fact, that we did not cite the work of Oró *et al.* (*9*) is that it was not carried out in an open system of the kind that is evidently geologically relevant. Hermetic reactions in dilute aqueous solution are sometimes appropriate for the purpose of preparing compounds in the laboratory —but not for simulating geochemical events (*4*). Prebiotic geological reactions could not have occurred in glassware (*4*), as we know it, or totally in the absence of the lithosphere ("dirt") of various kinds.

The statements by Wolman *et al.* about lunar exhaust products do not take into account evidence to the contrary from varied studies (*2*, *10*). Their assertion that no free amino acids were found in unhydrolyzed water extracts of lunar extracts is partially incorrect (*10–12*).

Development of a valid theory of origins of amino acids benefits from the use of factually established intermediates in experiments refined to increasingly geological conditions; an observed tendency to ignore gravity and the fact that the geochemical realm is predominantly open is not beneficial. Water, ammonia, diatomic hydrogen, and other fugacious substances in the open seldom or never remain in situ at elevated temperatures in the real world, in contrast to their behavior in closed laboratory apparatus.

Meaningful laboratory investigation seems to call for numerous experiments and observations based on the new, incomplete information on organic matter in interstellar clouds followed by Darwinian selection from alternative inferences, unfettered by old experiments based on more highly hypothetical, or unduly limited, conditions. We believe that refinement of experiments toward increased geological relevance can occur when they are carried out in open systems, so that, in part, concentrations can increase, and that at least some of the experimental repetitions need to be conducted in the presence of lithospheric material (*13*).

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10. We did in fact report free amino acids from Apollo 11 fines [see P. E. Hare, K. Harada, S. W. Fox, *Proc. Apollo 11 Lunar Sci. Conf.* **2**, 1799 (1970)] as did M. E. Murphy, V. E. Modzeleski, B. Nagy, W. M. Scott, M. Young, C. M. Drew, P. B. Hamilton, H. C. Urey (*ibid.*, p. 1879). However, another explanation for their being obtained in the free state is that the free amino acids resulted from partial hydrolysis of precursors during extraction of lunar dust with hot water.
11. The reference by Wolman *et al.* (*7*) to our Apollo 12 work is incorrect; we have not submitted a paper for the volume they refer to. That study has been reported in *Science* (*2*).
12. We would have been pleased to report, from actual analyses, in conformance with the conjecture of Wolman *et al.*, that HMT is present in lunar dust. Such occurrence would have signified to us the presence of lunar formaldehyde and ammonia, as intermediates of nonterrestrial origin in the moon's crust. Indeed, we found a peak in the basic amino acid region of hydrolyzates, with RT corresponding to that of HMT (S. W. Fox, P. E. Hare, K. Harada, C. R. Windsor, *Proc. Int. Ass. Geochem. Cosmochem. Tokyo, September 1970*, in press). The peak is however present in some acidic hydrolyzates, whereas it is absent in the extract prior to hydrolysis. The behavior of HMT in the pure state is opposite to that observed for this peak; the postulate of the presence of HMT in lunar dust is thus not supported.
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13 October 1971 ■

20-Hydroxyecdysone, What It Can Do

Wright *et al.* (*1*) showed that 20-hydroxyecdysone inhibits egg maturation in the stable fly. This same inhibition occurs in other species of insects (*2*). Application of [^{3}H]uridine to ecdysone-treated adult females and subsequent autoradiography has revealed that the role of the nurse cells in egg maturation has been impaired by the hormone treatment. Wright *et al.*, without further study, come to the conclusion: "In contrast, the oocytes within the ovarian follicle of the controls changed significantly from a spherical shape (follicle stage 6) to an elongated shape, which indicated that protein was synthesized in the nurse cell cytoplasm for vitellogenesis." A few lines further down, without giving additional data they write: "The 20-hydroxyecdysone thus prevented the synthesis of the lipid material necessary for vitellogenesis and final egg maturation."

Demonstration of impaired transport of RNA out of the nurse cell nucleus of an egg follicle is not evidence for impaired protein synthesis, and the change in shape of a growing egg taken as proof for protein synthesis is reminiscent of 18th-century biology. No data are given to demonstrate that 20-hydroxyecdysone prevented lipid synthesis. Impaired RNA transport is no evidence for prevented lipid synthesis.

The inhibitory role of ecdysones on egg maturation in insects has not only been shown in the species cited by the authors (*1*, *2*) but also in *Leucophaea maderae*, an ovoviviparous cockroach (*3*). In this case either implantation of active prothoracic glands or injections of α-ecdysone into adult females inhibited egg maturation; with graded doses of α-ecdysone a graded response was obtained.

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20 September 1971 ■

Stability in Zoological Nomenclature

One of the most frequently expressed criticisms of the rules of zoological nomenclature has been that the procedure for protecting a well-established name against the revival of previously forgotten older names is so cumbersome. To meet this criticism the International Zoological Congress at London (1958) adopted a *Statute of Limitation* (Article 23b of the Code) (*1*) giving automatic protection to names that had been in unchallenged use for 50 or more years. The wording of Article 23b, as originally adopted, was somewhat ambiguous and raised questions. Some zoologists, particularly entomologists, considered it unworkable. Nevertheless, it was confirmed by a small majority at the International Congress in Washington (1963). However, the Congress instructed the International Commission of Zoological Nomenclature to prepare a Declaration that would clarify the provisions of the Statute. In November 1969 the Commission adopted by more than two-thirds majority (16 to 7) an improved wording of Article 23b, to be issued as a Declaration (*2*). The wording that was adopted is as follows:

(b) *Limitation.* A name that is in general current use and has been available for at least 50 years shall not be displaced after 1960 by an unused senior synonym.

(i) A name is to be considered as in general current use when, in the immediately preceding 50 years, it has been applied to a particular taxon, as its presumably valid name, by at least five different authors and in at least ten publications.

(ii) A senior synonym is to be considered unused when, during the immediately preceding 50 years, it has not once been applied to a particular taxon as its presumably valid name. An unused senior synonym employed after 1960 in violation of the provisions of Article 23b, whether explicitly to replace the junior synonym or not, does not thereby lose its status as an unused name.

(iii) The mentioning of a name in a synonymy or its mere listing in an abstracting publication, or in a nomenclator or other index or list of names does not constitute usage in the sense of Article 23b.

(iv) Each citation of a name is to be considered on its own merits regardless of the nature or the title of the work in which the name appears.

(v) A zoologist who considers the existence of an unused senior synonym in the literature a source of confusion may apply to the Commission to place the name on the appropriate Official Index [of rejected and invalid names].

(vi) A zoologist who considers that an unused senior synonym should displace a junior synonym that is in general current use, may apply to the Commission for a ruling under the plenary powers.

(vii) Nothing in Article 23b affects the question of the Law of Homonymy. A name rejected under the provisions of Article 23b is rejected for the purposes of the Law of Priority but not for those of the Law of Homonymy.

(viii) An unused senior synonym rejected under the provisions of Article 23b is termed a nomen oblitum.

Article 23b, in its new version, continues to be an integral part of the International Code of Zoological Nomenclature. The preceding improved rewording of the Article, having been adopted by more than two-thirds of

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Formaldehyde and Ammonia as Precursors to Prebiotic Amino Acids

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