those reported in the adult frog for classes 2, 3, and 4 (*9*). The major differences between tadpole and frog were (i) the absence of class 1 fibers in the tadpole (*10*), and (ii) the absence of class 2 fibers in the periphery of the tadpole retina (class 2 responses were only present at the equator of the retina, whereas the adult frog exhibits responses in all regions of the retina).

We next attempted to correlate these physiological differences with anatomical findings. In Golgi silver stains (*11*) of 22 tadpole retinas we found only three of the four ganglion cell types in the adult frog—the "E" type, the "H" type, and the "broad" type (Fig. 2); the constricted ganglion cell tree of the frog was absent in the tadpole. This result coincides with the absence of class 1 responses (Fig. 1B). Also, the E type cells were absent from the periphery of the tadpole retina (that is, they were only found at the equator of the tadpole retina). This result matches the physiological distribution of class 2 responses (Fig. 1B). Finally, the H and broad-type ganglion cells were both found in all regions of the tadpole retina, which correlates with the distribution of physiological responses of classes 3 and 4 in tadpoles (Fig. 1B). In contrast, all four types of ganglion cells and classes of responses are uniformly distributed throughout the retina of the adult frog (Fig. 1A). Thus the development of the four classes of visual form detectors matches the growth of each type of dendritic tree. These results strongly support the hypothesis of Fig. 1A, especially for classes 1 and 2. Our data are also consistent with that hypothesis for classes 3 and 4, although we cannot pinpoint class 3 to H trees and class 4 to broad trees (see *12*).

We therefore conclude that the shapes of the dendritic trees of the ganglion cell provide a code for visual recognition of form; the code probably determines the convergence and summation onto the ganglion cell from dozens of bipolar and amacrine cells in the outer layers of the retina (*12*). Since bipolar and amacrine cells also have a pronounced field organization, the code in the ganglion cell must be composed of the summation of the codes from earlier stages. Our work, in conjunction with the network analysis of outer layers of amphibian retina (*13*), should lead to a complete description of vision in terms of network anatomy and physiology. Only in this manner can we hope to work out the neural basis for understanding behavior (*14*).

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5. Class 5 responses, which were reported in the adult frog [H. R. Maturana, J. Y. Lettvin, W. S. McCulloch, W. H. Pitts, *J. Gen. Physiol.* **43** (Suppl.), 129 (1960)] will be omitted from this report, because they were rarely found in the adult frog and were completely absent from our results in the tadpole frog.
6. The correlation by Lettvin *et al.* (*3*) of frog anatomy and physiology was based on three arguments. (i) There were four types of ganglion cell trees and four classes of responses. Since it was impossible to study the anatomy and physiology of the same cell, they could only attempt correlations based on (ii) and (iii). (ii) Conduction velocity of each class matched the soma size of each ganglion cell type; however, there was much overlap of velocities and cell size was a crude estimate. (iii) The size and shape of receptive fields of each class matched the spread of the dendrites of each ganglion cell type; however, amacrine cells have recently been shown to have a marked effect on receptive field size of ganglion cells [J. E. Dowling, *Proc. Roy. Soc. London, Ser. B* **170**, 205 (1968); F. S. Werblin and J. E. Dowling, *J. Neurophysiol.* **32**, 339 (1969)]. Also measurement of field size is highly inaccurate [G. H. Jacobs, *Brain Res.* **14**, 553 (1969)].
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10. It is improbable that our electrodes simply missed small class 1 fibers because of size, since we easily recorded from the equally small fibers of class 2 (conduction velocity 0.1 to 1.0 m/sec).
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12. In general, the lack of silver staining of a cell type is not absolute proof of the absence of this cell in the tissue. However, in our experiments, the close correlation of anatomical and physiological distributions indicates that the lack of staining does indeed reflect the absence of a cell type.
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14. An account of tadpole retina outer layers will be presented elsewhere (B. Pomeranz, in preparation).
15. Supported by National Research Council of Canada, grant 302-120-25. We thank Manuel Uy for histological assistance.

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Synthesis of Amino Acids by the Heating of Formaldehyde and Ammonia

Abstract. *The heating of formaldehyde and ammonia yields a product that, on hydrolysis, is converted into seven amino acids: aspartic acid, glutamic acid, serine, proline, valine, glycine, and alanine. Glycine is the predominant amino acid. Inasmuch as formaldehyde and ammonia have been identified as compounds in galactic clouds, these experimental results are interpreted in a cosmochemical and geochemical context.*

The finding of formaldehyde (*1*) and ammonia (*2*) as interstellar molecules provides a relatively factual basis for the existence of precursors of amino acids in the galaxy. In some of the sources of microwave radiation identified, both formaldehyde and ammonia are present (*3*); reaction of the two is more easily visualized as a consequence of this coexistence. The conversion of formaldehyde and ammonia to glycine and alanine by ultraviolet radiation has been reported by Reid (*4*), whereas the formation of serine, glycine, glutamic acid, alanine, valine, phenylalanine, and isoleucine from ammonium chloride and formaldehyde by ultraviolet radiation has been reported by Pavlovskaya and Pasynski (*5*). The Russian authors reported also "basic amino acids," but these latter might have been amines including hexamethylenetetramine (*4*).

Experiments in producing amino acids by subjecting formaldehyde and ammonia to heat instead of high-energy radiation were undertaken by us for several reasons. Heat is generally less destructive than high-energy radiation and is easily controlled, nearly always being used in the organic chemical laboratory for this reason (*6*). Heat has been the form of energy which has yielded a relatively comprehensive the-

ory of the origins of macromolecular and cellular systems (*7*). Moreover, volcanic temperatures in beds of silica can convert a mildly "reducing atmosphere" of methane, ammonia, and water (*8*) into compounds hydrolyzable to most of the amino acids found in contemporary proteins and to virtually none not found in such polymers. Formaldehyde, as a partly oxidized methane, was therefore used to permit a comparison of necessary temperatures and the nature of the products from two sources of carbon.

Reactions between ammonia and formaldehyde were tried at various ratios; a ratio of 1 to 3 was studied in detail. Fifteen milliliters of 37 percent formaldehyde and 5 ml of 28 percent ammonia were placed in an interjoint glass tube (25 by 3.75 cm). Under a slow flow of nitrogen, the tube was half immersed into a bath of Blue M HTF-100 Ucon fluid at 185°C for 8 hours (initial heating to 185°C from a cold bath required 30 minutes). The products failed to give biuret tests. The reaction tube was allowed to cool, 100 ml of 6*N* redistilled HCl was added, and the mixture was refluxed for 24 hours. The liquid was then concentrated to dryness, and the residue redissolved in citrate buffer for examination on the automatic amino acid analyzer (*9*). For confirmation of the identities of the amino acids, samples were desalted (*10*) and examined by thin-layer chromatography (*11*) (Table 1).

The conversion of formaldehyde into the amino acids in Table 1 constitutes a yield of 0.002 to 0.007 percent. Controls in which formaldehyde alone was heated yielded no amino acids. Most of the product is hexamethylenetetramine, as established by chromatography of the authentic base. On the analyzer column, the hexamethylenetetramine runs near lysine. This mobility suggests that Pavlovskaya and Pasynski (*5*) may have mistaken this amine for "basic amino acids" in their paper chromatography, since the substance of their unconfirmed spot ran behind the neutral and acidic amino acids.

Identification on the amino acid analyzer was in large part confirmed by thin-layer chromatography. The fact that a peak with the R_F of isoleucine accounts for 9.3 percent of the product, whereas none was observed in thin-layer chromatography, suggests that the compound is not isoleucine. Proline is, however, present in substantial proportion and has appeared from the product of gases heated over silica (*8, 12*). Proline may have been missed in qualitative paper chromatograms because of its relatively faint yellow color reaction with ninhydrin. The amino acids found are very similar to those of Pavlovskaya and Pasynski (*5*).

Table 1. Amino acids produced by hydrolysis of the thermal reaction product of formaldehyde, ammonia, and water. In experiment 1, a 1 : 1 ratio of 37 percent formaldehyde and 28 percent ammonia was used; in experiments 3 to 5 a 3 : 1 ratio was used. Experiment 6 consisted of thin-layer chromatography of the products of experiment 5 (the + denotes that the amino acid was present). The results are expressed as mole ratios in percent, and were calculated without ammonia and without hexamethylenetetramine, which is the most abundant product. Trace, tr.

Amino acid	Experiment				
	1	3	4	5	6
Aspartic acid		1.3	8.3	2.3	+
Serine		4.0	6.3	1.7	+
Glutamic acid	9.9	1.6		1.9	+
Proline		5.0	tr.	1.4	+
Glycine	90.1	39.5	45.8	81.3	+
Alanine		5.0	7.3	9.5	+
Valine		0.4	9.4	1.9	+
Isoleucine		9.4			
Leucine		0.5	3.1		
Phenylalanine		1.2			
Unknowns*		32.2	19.8		

* Equivalent to leucine.

The dominance of glycine and alanine in the products recalls the glycine and alanine found by Reid (*4*), the principal appearance of these two in the thermal synthesis from methane, ammonia, and water (*8, 13*), and the almost entire dominance by these two of the amino acids produced by Miller in electric discharge experiments (*14*). Moreover, glycine and alanine are the principal amino acids found in geological samples (*15*) and in samples of the fine dust of the moon (*16*) (although the possibility of atypical terrestrial contamination is not excluded in this last case).

The choice of reactants in accord with the premise of a weakly reducing atmosphere in the experiments performed at volcanic temperatures (*8*) was in part dictated by the objective of producing hydrogen-poor amino acids such as phenylalanine and tyrosine. These two benzenoid amino acids did result, as well as at least ten other amino acids. Nine of these amino acids were confirmed by ionophoretic analysis (*12, 17*). Noteworthy is the fact that the formaldehyde mixture contains a partly oxidized form of carbon and that seven to nine kinds of amino acid result.

A number of presumably prebiological syntheses have now been shown to yield families of amino acids (*8, 18*); these families closely resemble an array of many of the individual monomers of contemporary protein. This multifold result is for this reason more conceptually plausable in an evolutionary context than would be a single pure amino acid such as might be sought in conventional organic chemistry. Noteworthy also is the fact that the syntheses from formaldehyde proceed from nongaseous intermediates, and in an open system.

The negative biuret tests suggest that the polymer is the "HCN polymer" (*19*) rather than a polyamino acid. The product is easily hydrolyzed. The resultant amino acids in a primordial situation (*20*) could react geologically to form polyamino acids at the same temperature that would bring about hydrolysis of the original material and subsequent concentration to a solid residue (*7*) prior to polymerization at the same temperature.

The origin of a sufficient number of types of amino acid are thus accounted for to place the total sequence of simple compounds → amino acids → proteinoids → microspheres (*7*) on a relatively factual basis. The formation of proteinoids and microspheres is carried out in the laboratory under conditions that are actual in that those conditions exist in moderate and extreme abundance, respectively, on the contemporary earth (*7*). The present results describe amino acids in measurable amounts from intermediates identified in contemporary interstellar matter, rather than from postulated primitive atmospheres. Moreover, the entire sequence can occur at temperatures well below 200°C.

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Vascular Lesions: Possible Pathogenetic Basis of the Generalized Shwartzman Reaction

Abstract. *Evidence of vascular injury was found in rabbits after a single small dose of endotoxin from* Escherichia coli. *Eighty percent of the treated animals developed circulating endothelial cells, leukopenia, and thrombocytopenia, and 50 percent had aortic endothelial lesions as determined by electron microscopy. Prior anticoagulation with heparin did not prevent this response. No control animals showed these abnormalities.*

The generalized Shwartzman reaction has long been thought to result from the activation of blood coagulation factors, with consequent fibrin formation (*1*). The lesions have been considered essentially embolic in nature, with the trapping of fibrin and platelets in small vessels. As an alternate hypothesis it is proposed that the generalized Shwartzman reaction is primarily a vascular reaction, with in situ thrombi forming at areas of endothelial cell detachment and exposed basement membrane (*2*). Such multiple localized areas of endothelial desquamation could account for the widespread consumption of platelets and labile clotting factors, which would lead to their depletion in the generalized Shwartzman reaction. The studies to be described provide support for the above hypothesis by demonstrating the presence of circulating endothelial cells in the blood of rabbits injected with endotoxin (*3*), and the presence of corresponding endothelial lesions in major vessels of these animals. These abnormalities were not prevented by concurrent anticoagulation with heparin.

Adult male New Zealand rabbits (2.5 to 4.0 kg) were used. Each rabbit in group A was given a single intravenous dose of *Escherichia coli* 0127:B8 endotoxin (Difco; 40 to 120 μg/kg) which was suspended in pyrogen-free isotonic saline, at a concentration of 40 μg/ml. Group B was similarly treated, but each rabbit was given 1.0 to 1.5 ml of intravenous sodium heparin (Liquaemin Sodium "10," Organon, Inc.; 1000 U.S.P. units per milliliter) immediately before the endotoxin. Rabbits in group C received sterile saline solution in volumes equivalent to those used in group A, and group D rabbits were given both heparin and saline.

The rabbits were anesthetized with intravenous sodium pentobarbital supplemented with ether. Blood was collected by free flow from the aorta. This maneuver was designed to preclude aspiration of endothelium at the tip of the needle. The first 3 to 5 ml of blood was discarded to remove any endothelial cells obtained from the vessel wall at the site of puncture. The optimum time for collection of specimens for circulating endothelium was 30 to 60 minutes after injection of endotoxin.

Blood treated with sodium ethylenediaminetetraacetic acid to inhibit coagulation was examined for total leukocyte count with a Coulter-S counter. Mean normal values were 8300 cell/mm³ in 33 rabbits. Platelets counted by the method of Brecher-Cronkite (*4*) had a mean value of 321,000/mm³ in 57 rabbits. Coagulation times for whole blood in glass were determined as described by Lee and White (*5*).

The presence of circulating endothelium was determined by light and electron microscopy (*6*, *7*). Briefly, 5 ml of blood was collected into 20 ml of a buffered, citrated solution containing approximately 1 percent formaldehyde (*6*). Red blood cells were hemolyzed with a 1 percent saponin solution, and the nucleated cells were collected by centrifugation for 10 minutes at 340*g*. For light microscopy, small portions of the "leukoconcentrate" (that is, the elements remaining after saponization) were smeared on glass slides and stained with Wright's solution and then by Giemsa stain. Endothelial cells were selected for electron microscopic examination as described (*7*). Leukoconcentrates were suspended in a few drops of buffer and were immediately fixed in a large volume of phosphate-buffered glutaraldehyde. During osmium fixation, dehydration, and

Table 1. Changes in mean maximum count of white blood cells (WBC) and platelets, changes in aortic endothelium as determined by electron microscopy, and presence of endothelial cells in the circulating blood of rabbits. Group A was treated with *E. coli* endotoxin; group B with endotoxin and heparin; group C with saline solution; and group D with saline and heparin. Data were analyzed by the Wilcoxon Rank Sum Test (*19*) for differences between the means. Grading the condition of aortic endothelium was as follows: −, negative; ±, equivocal; and +, positive.

Group	WBC		Platelets		Animals with aortic endothelial lesions			Animals with circulating endothelium	
	Animals (No.)	Change (%)	Animals (No.)	Change (%)	− (No.)	± (No.)	+ (No.)	− (No.)	+ (No.)
A	16	−57*	24	−44*	3	0	6	8	30
B	15	−40*	26	−34	0	3	2	4	19
C	12	− 4	12	+ 1	5	4	0	13	0
D	3	0	4	+ 2	1	0	0	4	0

* Difference between mean count prior to and after treatment is significant ($P < .005$).