bond). The ultraviolet spectrum (hexane) contained maxima at 225.3 mμ [log ε (extinction) = 4.57], 276 mμ (log ε = 4.08), 292 mμ (log ε = 4.31), and 310.5 mμ (log ε = 4.30) and closely resembled that of *cis*-lachnophyllum ester (*3*).

The nuclear magnetic resonance also was in agreement with the structure of *cis*-lachnophyllum ester. Absorption occurred at $\tau$ 8.97 (triplet, 3H), 8.44 (multiplet, 2H), 7.66 (triplet, 2H), 6.27 (singlet, 3H), and 3.90 (singlet, 2 vinyl hydrogens).

To completely identify the ester, we secured a small quantity of authentic *cis*-lachnophyllum ester (*5*). The melting point of our compound mixed with the authentic sample was undepressed.

Sorensen (*1*) has argued that knowledge of the structures of naturally-occurring acetylenes may aid taxonomists in resolving difficult classification problems in the *Compositae*. Morphologically similar species from different genera can be divided according to the type of $C_{10}$ acetylene present. For this reason we feel that an extensive survey of the desert aster and related species for polyacetylene content may reveal interesting taxonomic relationships.

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### References and Notes

1. N. A. Sorensen, in *Chemistry of Natural Products*, Intern. symp., Australia, 1960 (Butterworths, London, 1961), p. 569.
2. V. V. Viljams, V. S. Smirnov, V. P. Goljmov, *Khim. Zhur. Ser. A.* (*Zhur. Obshschei Khim.*) **5**, 1195 (1935).
3. N. A. Sorensen and K. Stavholt, *Acta Chem. Scand.* **4**, 1575 (1950).
4. P. S. Martin and C. Steelink, *Bull. Ecol. Soc. Amer.* **41**, 129 (1960).
5. We thank Prof. N. A. Sorensen, Norges Teknishke Hogskole, Trondheim, Norway, for providing a sample of *cis*-lachnophyllum ester.

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## Thermodynamic Equilibria in Prebiological Atmospheres

Abstract. *The concentrations of a large number of compounds of biological interest which would be present in the atmosphere at thermodynamic equilibrium were computed under many combinations of temperature, pressure, and elemental composition. These computations revealed a possible mechanism for the abiological formation of asphaltic tar and an oxidative threshold at which all but the simplest compounds disappear.*

One of the principal problems in understanding prebiological conditions on the earth and extraterrestrial atmospheres concerns the nature of the complex chemical species to be encountered. Certain complex compounds must have been necessary for the origin, structure, and nutrition of the first living things. These compounds must have been produced by nonliving processes in suitable quantities and proportions. The elucidation of these concentrations is one essential for a detailed rational theory of the evolution of chemical reactions and the origin of life.

Experiments have been performed in which biologically interesting compounds, such as amino acids, were generated in an atmosphere of small molecules (*1*). Electrical discharge, ultraviolet or other radiation, or heat was applied to the gaseous mixtures.

Computations of the equilibrium concentrations of major constitutents in particular reactions have been made previously. The most important of these are summarized by Miller and Urey (*2*) and by Oparin (*3*). Suess (*4*) has considered the equilibrium of five major chemical species in the system C, H, O. He has calculated the compositions at which solid carbon would appear. No unified study including a great many organic compounds in the equilibrium has been undertaken heretofore.

We have computed the thermodynamic equilibrium concentrations of all major constituents and a representative group of over 100 minor compounds containing O, N, H, and C, considering all elemental compositions and a large range of temperatures and pressures. To include many regions of interest to models of the primitive atmosphere, temperatures between 300° and 1000°K and pressures between $10^{-6}$ and 300 atm were used.

The distribution of molecular species at chemical equilibrium is independent of the specific reactions by which that equilibrium was reached. It requires only that there be at least one reversible reaction path from the major constituents that will produce each compound, and depends only on pressure, temperature, and elemental composition. The equilibrium constants for all possible reactions are then simultaneously satisfied. Many of the conditions which might have arisen naturally can be approximated by thermodynamic equilibria (*5*). These approximations are particularly good at elevated temperatures where reactions proceed rapidly in both directions, as for example in the atmosphere of Venus or in volcanic areas of the earth, or in other regions where catalysts are available. In regions where special mechanisms operate to favor the production of certain compounds, there will be also a tendency to form the products required by thermodynamic equilibrium. Compounds which can be formed at equilibrium need no such special mechanism to explain their presence. However, such special mechanisms would have to be sought for those compounds which could not be so produced but which would have been required for the structure and nutrition of the first living things.

The difficulties previously limiting the computation of concentrations of many organic compounds were overcome by using a high-speed computer. Our program for the IBM 7090 computer minimizes the free energy of the system and yields equilibrium compositions of the compounds by means of the very general method developed by White, Johnson, and Dantzig (*6*). The solution is approached by an iterative process. At each step, $M + 1$ linear equations are solved, where $M$ is the number of elements in the system. The atmosphere was assumed to be a mixture of perfect gases. Twenty-five elements and over 500 compounds can be handled by the program.

The standard free energies of formation for the compounds were obtained from National Bureau of Standards tables (*7*) or computed by summing the free energies of their component groups by Van Krevelen's method (*8*). Values for the major constituents such as $CO_2$, $CH_4$, $H_2O$, and CO are known accurately. For the biologically interesting compounds, the free energies are known to an accuracy of about 2 kcal/mole, although differences within a series of homologous compounds are less. If the free energy used for a compound is too high by 1 kcal, the concentration calculated will be too small by a factor of 0.36 at 500°K and 0.60 at 1000°K.

Only compounds having molecular weights less than 300 were included in this initial study. The free energies of an extended list of biologically interesting compounds are being prepared with the aid of a computer program, for inclusion in the computations. The partition of compounds between the atmosphere and liquid and solid phases, under a variety of steady-state conditions, still needs to be explored. The existence of multiple phases will limit the regions of the gaseous equilibrium diagram which can be reached. However, in the allowed regions, the balance of species will not be affected.

So that all possible compounds could be considered, a method was developed for combining all structurally similar isomers into a single computation. The concentration of this composite is equal to the sum of the concentrations of the isomers. It can be shown that its standard free energy, $F_L$ in terms of the free energies of the $N$ isomers, $F(i)$ is given by

$$F_L = -RT \ln \sum_{i=1,N} e^{-F(i)/RT}$$

In the case where the free energies of all isomers are equal

$$F_L = F(i) - RT \ln N$$

For a saturated hydrocarbon of 20 carbon atoms, there are over 1 million isomers. The concentration of any one is so small that even a million of them have a negligible total concentration. However, the number of isomers builds up very rapidly with increasing size so that eventually the extremely large compounds in a family would predominate. However, it seems impossible ever to reach this state as some of the necessary intermediates in their synthesis have negligible concentrations. This is the situation with all of the families we have considered except the polynuclear aromatics in the asphalt region (Fig. 1).

Figure 1 shows the phase diagram of the system C, H, O. Equilibrium calculations indicate that the free energy of carbon in the gaseous mixture becomes equal to that of graphite along the lines shown for temperatures 500° and 700°K. These lines confirm the work of Suess (*4*). Since the activation energy required to form solid carbon is very high, under many experimental conditions an accumulation of tars and asphalts precedes the formation of carbon. We have therefore also extensively investigated the region beyond these lines. A composite compound of 100 isomers with the formula $C_{22}H_{12}$ was used to approximate the polynuclear aromatics up to six benzene rings. The vapor pressure becomes sufficiently high to form a liquid phase as shown by the dashed lines of Fig. 1 at 500° and 700°K and 1 atm total pressure. (Excluding solid carbon formation is analogous to computing some physical constant for a supercooled liquid. It makes possible a computation in a region which would otherwise be "forbidden," and leaves open the question of whether or not such a region could ever actually exist.)

Table 1 shows the concentrations of many organic compounds computed at equilibrium in the gas phase at 1 atmosphere pressure and 500°K at the four points marked by asterisks on the phase diagram in Fig. 1. These are illustrative examples and we do not mean to imply that the evolution of the atmosphere passed through these points. The nitrogen concentration, a total of 1 mole, was made equal to that of carbon for these computations. The nitrogen is present entirely as $N_2$ except for trace components so that the concentrations of the carbon-, hydrogen-, and oxygen-containing compounds are only slightly affected.

Point *A* is located just outside the region where solid carbon might form. Point *B* is between the solid carbon and asphalt thresholds. Point *C* is located beyond the asphalt threshold and shows a very profound increase in the concentrations of a number of interesting compounds. Point *D* is in the highly reducing region where organic compound concentrations are relatively low.

Because of the structure of the computer, any number smaller than $10^{-38}$ is recorded as zero. From an estimate of the total amount of carbon in the earth's crust, one can compute that a mole fraction of this order would represent less than one million molecules in the entire atmosphere. Unless the particular molecule were of unique importance, for example a special catalyst, this quantity can be considered to be effectively zero. A concentration of $10^{-20}$ would represent about one molecule per droplet of condensate, without assuming enrichment by phase partition. In the experiments with primitive atmospheres, concentration in a condensed phase is used to accumulate the products produced in the gas. Such a process would also have occurred on the primitive earth.

Table 1 shows that there is a wide distribution of organic compounds which have concentrations large enough to be important in any discussion concerning the origin of life. The appearance of small biologically important compounds (such as formic, acetic, lactic, and pyruvic acids, pyridine, glycine, and various alcohols) shows that no special mechanism is necessary to explain their presence in a primitive atmosphere other than the assumption of conditions enabling the establishment of partial or complete thermodynamic equilibrium. On the other hand, there are compounds that are critical to life—such as ribose, adenine, cytosine, and glycylglycine and presumably other compounds of a similar level of complexity and energy—which, under all elemental compositions tried, are extremely scarce.

Figure 2 shows the concentrations of selected compounds at 500°K along the line of dots in Fig. 1 (chosen arbitrarily) through typical solid carbon and liquid aromatic thresholds. The evolution of the earth's atmosphere probably crossed this line at some point. As the proportion of carbon is increased, the aromatic compounds become important. First benzene and then, successively, naphthalene and the higher aromatic compounds rapidly increase in concentration. Eventually, a liquid phase of polynuclear aromatics is formed.

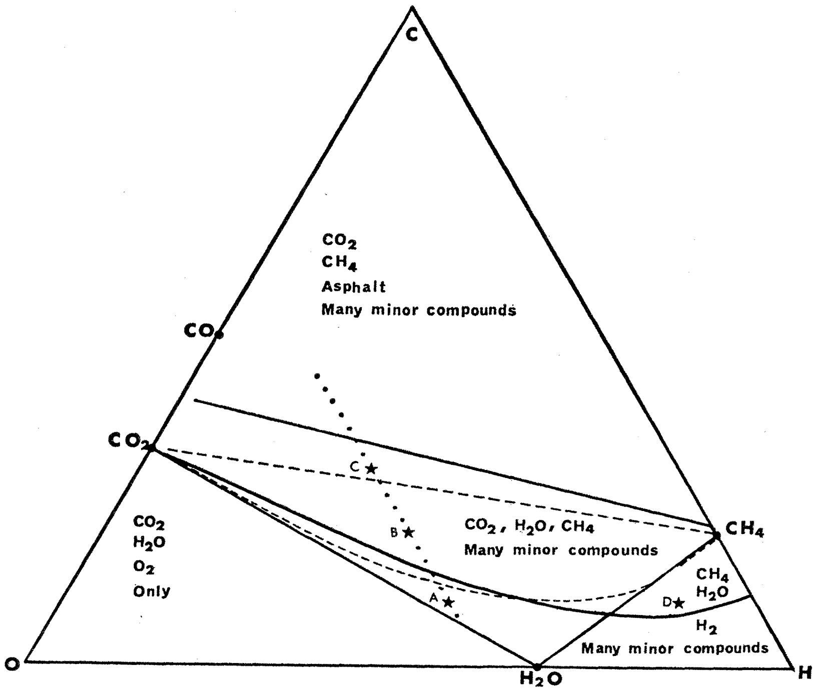
A similar study at 100 atm shows that the concentrations of the larger compounds are favored by the increased pressure. The carbon and asphalt lines are not changed appreciably.

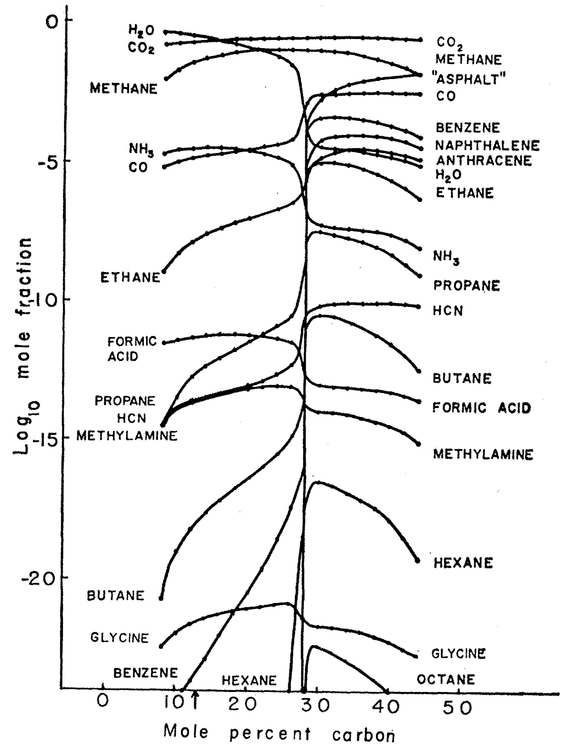
It should be noted that the amounts of the paraffinic hydrocarbons which are produced under the same conditions are negligible. In fact, we have not been able to find any combination of pressure, temperature, and elemental proportions which would favor the production of aliphatic petroleum compounds in an "ideal" gas atmosphere.

Whether any considerable quantities of asphalt could actually have been formed by this process in any part of the atmosphere of the primitive earth would depend on the existence of a suitable temperature and on a favorable balance between the rates of addition of water from the lower layers and of the loss of hydrogen into space. If such a process would have begun, it would be expected to have continued in a steady state until a large proportion of the available carbon had been

Table 1. Concentrations in moles per mole of total carbon of selected compounds at equilibrium in atmospheres of various compositions at 500°K and 1 atmosphere pressure. The four compositions *A, B, C,* and *D* are shown in Fig. 1. In addition to the elements carbon, hydrogen, and oxygen, an amount of nitrogen equal to that of carbon is included. Composition *C*, in the "asphalt" region, has generally much higher amounts of each compound than the other compositions. Composition *D*, in the region of excess hydrogen, has much lower concentrations. Many compounds that are critical to life such as ribose, adeninie, cytosine, glycylglycine, glycerol, and serine were found in concentrations less than $10^{-35}$ at all these compositions.

| Compound | A | B | C | D |
|---|---|---|---|---|
| Water | 2.24 | 0.49 | $0.79 \times 10^{-4}$ | 1.00 |
| Carbon dioxide | .88 | .75 | .66 | $.85 \times 10^{-6}$ |
| Nitrogen | .50 | .50 | .50 | .49 |
| Methane | .12 | .25 | .24 | 1.00 |
| Hydrogen | $.27 \times 10^{-1}$ | $.12 \times 10^{-1}$ | $.13 \times 10^{-3}$ | .95 |
| Ammonia | $.14 \times 10^{-3}$ | $.72 \times 10^{-4}$ | $.11 \times 10^{-6}$ | $.30 \times 10^{-1}$ |
| Carbon monoxide | $.54 \times 10^{-4}$ | $.89 \times 10^{-4}$ | $.53 \times 10^{-2}$ | $.41 \times 10^{-8}$ |
| Ethane | $.27 \times 10^{-7}$ | $.27 \times 10^{-6}$ | $.23 \times 10^{-4}$ | $.55 \times 10^{-7}$ |
| Acetic acid | $.29 \times 10^{-9}$ | $.97 \times 10^{-9}$ | $.12 \times 10^{-8}$ | $.26 \times 10^{-14}$ |
| Formic acid | $.20 \times 10^{-10}$ | $.14 \times 10^{-10}$ | $.18 \times 10^{-12}$ | $.72 \times 10^{-15}$ |
| Methanol | $.75 \times 10^{-11}$ | $.80 \times 10^{-11}$ | $.11 \times 10^{-12}$ | $.81 \times 10^{-12}$ |
| Formaldehyde | $.30 \times 10^{-11}$ | $.39 \times 10^{-11}$ | $.36 \times 10^{-11}$ | $.84 \times 10^{-14}$ |
| Ethylene | $.81 \times 10^{-13}$ | $.10 \times 10^{-11}$ | $.57 \times 10^{-8}$ | $.44 \times 10^{-14}$ |
| Hydrogen cyanide | $.58 \times 10^{-13}$ | $.23 \times 10^{-12}$ | $.14 \times 10^{-9}$ | $.21 \times 10^{-14}$ |
| Methylamine | $.57 \times 10^{-13}$ | $.15 \times 10^{-12}$ | $.21 \times 10^{-13}$ | $.31 \times 10^{-11}$ |
| Acetaldehyde | $.40 \times 10^{-14}$ | $.26 \times 10^{-13}$ | $.21 \times 10^{-11}$ | $.28 \times 10^{-17}$ |
| Ethanol | $.33 \times 10^{-15}$ | $.17 \times 10^{-14}$ | $.21 \times 10^{-14}$ | $.86 \times 10^{-17}$ |
| Ketene | $.36 \times 10^{-17}$ | $.29 \times 10^{-16}$ | $.15 \times 10^{-12}$ | $.65 \times 10^{-22}$ |
| Acetone | $.12 \times 10^{-17}$ | $.39 \times 10^{-16}$ | $.28 \times 10^{-12}$ | $.21 \times 10^{-21}$ |
| Methyl ether | $.78 \times 10^{-19}$ | $.41 \times 10^{-18}$ | $.51 \times 10^{-18}$ | $.20 \times 10^{-20}$ |
| Pyruvic acid | $0.42 \times 10^{-20}$ | $0.44 \times 10^{-19}$ | $0.45 \times 10^{-17}$ | $0.31 \times 10^{-29}$ |
| Formamide | $.20 \times 10^{-20}$ | $.33 \times 10^{-20}$ | $.45 \times 10^{-21}$ | $.36 \times 10^{-22}$ |
| Glycine | $.42 \times 10^{-21}$ | $.17 \times 10^{-20}$ | $.31 \times 10^{-21}$ | $.24 \times 10^{-25}$ |
| Acetylene | $.22 \times 10^{-22}$ | $.34 \times 10^{-21}$ | $.12 \times 10^{-15}$ | $.31 \times 10^{-25}$ |
| Lactic acid | $.24 \times 10^{-23}$ | $.20 \times 10^{-22}$ | $.32 \times 10^{-22}$ | $.66 \times 10^{-31}$ |
| Ethylene glycol | $.13 \times 10^{-23}$ | $.34 \times 10^{-23}$ | $.63 \times 10^{-25}$ | $.43 \times 10^{-27}$ |
| Acetamide | $.86 \times 10^{-24}$ | $.69 \times 10^{-23}$ | $.83 \times 10^{-22}$ | $.38 \times 10^{-26}$ |
| Benzene | $.52 \times 10^{-24}$ | $.71 \times 10^{-20}$ | $.66 \times 10^{-3}$ | $.18 \times 10^{-32}$ |
| Alanine | $.33 \times 10^{-27}$ | $.66 \times 10^{-26}$ | $.10 \times 10^{-24}$ | $.45 \times 10^{-32}$ |
| Pyridine | $.91 \times 10^{-28}$ | $.31 \times 10^{-24}$ | $.49 \times 10^{-10}$ | $.81 \times 10^{-35}$ |
| Cyanogen | $.61 \times 10^{-30}$ | $.22 \times 10^{-28}$ | $.74 \times 10^{-21}$ | $.24 \times 10^{-34}$ |
| Pyrrole | $.59 \times 10^{-30}$ | $.33 \times 10^{-27}$ | $.91 \times 10^{-17}$ | $.82 \times 10^{-35}$ |
| Furan | $.31 \times 10^{-30}$ | $.74 \times 10^{-28}$ | $.20 \times 10^{-18}$ | $< 10^{-38}$ |
| Benzoic acid | $.50 \times 10^{-31}$ | $.11 \times 10^{-26}$ | $.13 \times 10^{-9}$ | $< 10^{-38}$ |
| Phenol | $.26 \times 10^{-31}$ | $.18 \times 10^{-27}$ | $.25 \times 10^{-12}$ | $< 10^{-38}$ |
| Pyrimidine | $.16 \times 10^{-31}$ | $.14 \times 10^{-28}$ | $.37 \times 10^{-17}$ | $.37 \times 10^{-37}$ |
| Xylene | $.40 \times 10^{-33}$ | $.13 \times 10^{-27}$ | $.94 \times 10^{-7}$ | $< 10^{-38}$ |
| Benzaldehyde | $70 \times 10^{-36}$ | $.30 \times 10^{-31}$ | $.24 \times 10^{-12}$ | $< 10^{-38}$ |
| Naphthalene | $< 10^{-38}$ | $.86 \times 10^{-34}$ | $.14 \times 10^{-3}$ | $< 10^{-38}$ |
| Anthracene | $< 10^{-38}$ | $< 10^{-38}$ | $.29 \times 10^{-4}$ | $< 10^{-38}$ |
| Oxygen | $< 10^{-38}$ | $< 10^{-38}$ | $< 10^{-38}$ | $< 10^{-38}$ |





Figs. 1 and 2. Fig. 1 (left). Thermodynamic equilibrium in atmospheres of varying elemental proportions. The ternary diagram shows all compositions of systems containing carbon, hydrogen, and oxygen (every point on the diagram represents a total of 100 percent of the three components). The points corresponding to the major compounds are indicated, and lines marking the regions where different ones become important are shown. The lower curves indicate the potential formation of solid carbon if equilibrium could be attained. The dashed curve holds at 500°K, the continuous one at 700°K. The upper lines indicate the "asphalt threshold," the dashed one at 500° and the continuous one at 700°K. Above this threshold, thermodynamic equilibrium favors the formation of large proportions of polycyclic aromatic compounds ("asphalt"), and a lesser increase of most of the other families of compounds. The dots indicate the points used in the computations for Fig. 2. The lettered asterisks indicate the points illustrated in detail in Table 1. Fig. 2 (right). Equilibrium concentrations in mole fractions of selected compounds at 500°K and 1 atm pressure with composition 40 percent oxygen, the indicated percentage of carbon, and the rest hydrogen. To this basic composition is added an amount of nitrogen equal to the amount of carbon. The nitrogen remains primarily as $N_2$ but produces significant quantities of some interesting compounds. The free energy of carbon in the system equals that of graphite at the composition indicated by the arrow. At this point solid carbon would be precipitated if it could be formed; there is no inflection of the curves at this point. The "asphalt threshold" is shown as a very sharp inflection in the curves, sharpest of all for the aromatic and related heterocyclic compounds. If an atmosphere such as this were to condense, there would be about one molecule of glycine per droplet of condensate.

converted to aromatic hydrocarbons and carbon oxides.

Another critical composition occurs as the amount of oxygen increases. For all C to H ratios, there is a sharp threshold of oxidation at which most of the biologically interesting compounds can no longer occur in the equilibrium mixture. For example, in a C—H—O composition in the ratio of 1 : 4 : 4 at 1 atmosphere and 500°K, the carbon and hydrogen are present as carbon dioxide and water. The free oxygen mole fraction is $10^{-4}$. The only organic compound present is formic acid, at $0.2 \times 10^{-29}$ mole fraction.

We believe that studies such as these are needed in order to separate problems of primordial chemical evolution into those which depend on thermodynamic equilibrium and those which depend on irreversible processes. Previous discussions of prebiotic evolution have included a number of special mechanisms, including heat to energize and catalyze various reactions. Even in the presence of a special mechanism which favors the production of particular compounds, however, there will also be a tendency to form all the products required by thermodynamic equilibrium. The concentration of these particular compounds must be maintained in spite of the possible presence of any broad specificity catalysts which might permit the establishment of equilibrium. In a detailed study of biological conditions on terrestrial planets, not only the presence of certain key compounds is important, but also the relative concentrations of other related compounds which could compete with them in the special reactions postulated for the origin of life. Information about equilibrium states is, in general, a necessary prerequisite for the study of nonequilibrium states and irreversible processes.

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### References and Notes

1. The bibliography on this subject is much too extensive to be given here. For a brief review and bibliography see S. W. Fox, *Science* **132**, 200 (1960).
2. S. L. Miller and H. C. Urey, *ibid.* **130**, 245 (1959).
3. A. I. Oparin, *The Origin of Life on the Earth*, A. Synge, Trans. (Academic Press, New York, 1957).
4. H. E. Suess, *J. Geophys. Res.* **67**, 2029 (1962).
5. The concept of beginning the investigation of the origins of life with a study of those compounds that can arise from thermodynamic equilibrium was first suggested to us by Prof. Harold J. Morowitz.

→ W. B. White, S. M. Johnson, G. B. Dantzig, *J. Chem. Phys.* **28**, 751 (1958).

7. *Selected Values of Chemical Thermodynamic Properties* (National Bureau of Standards, Washington, D.C., 1952).
8. D. W. Van Krevelen and H. A. G. Chermin, *Chem. Eng. Sci.* **1**, 66 (1951). See also previous work by J. L. Franklin, *Ind. Eng. Chem.* **41**, 1070 (1949).
9. This work was supported by grant GM 08710 from NIH to, and NASA contract No. 21-003-002 with, the National Biomedical Research Foundation.

10 September 1964

## D-Malate: Effects on Activity of L-Malate Dehydrogenase in Developing Sea Urchin Embryos

Abstract. *Sea urchin embryos grown in salt water containing* L-*malate showed a pattern of development of* L-*malate dehydrogenase which did not differ from that of embryos grown in salt water. However, embryos grown in* $10^{-3}$M D-*malate for 6 or 12 hours had one additional band of* L-*malate dehydrogenase that was not present in control embryos of the same age.*

The regulation of cytodifferentiation in embryonic stages immediately after fertilization has received little attention because of the inherent experimental difficulties (*1*). The facts that eggs of sea urchins are available in quantity, can be fertilized in vitro under controlled conditions, can undergo synchronous development, and are relatively permeable to exogenous materials make them an ideal system for studying the regulation of macromolecule formation (*1*).

Moore and Villee (*2*) have shown that the unfertilized sea urchin egg contains five electrophoretically separable L-malate dehydrogenases (L-MDH). The number decreased to three in whole embryos early in development and then increased to four in the 12-hour embryo. Furthermore, when sea urchin embryos at the 64-cell stage (4-hour embryos) were dissociated and separated into large and small blastomeres, the large blastomere showed two and the small blastomere showed three bands of L-MDH activity by disc microelectrophoresis (*3*). It was of interest to determine whether this pattern of malate dehydrogenase (MDH) development could be altered by growing embryos in sea water containing D- or L-malate.

Embryos were collected (*2*) and homogenized in glass-distilled water with a Ten-Broeck hand homogenizer until microscopy showed that at least 95 percent of the embryos were disrupted. The homogenate was centrifuged, and the supernatant fluid was decanted and stored overnight at 4°C. Portions were analyzed spectrophotometrically for MDH activity (*4*), using NAD (*5*) and its analogues as hydrogen acceptors; other portions were subjected to disc-microelectrophoretic analysis on polyacrylamide gel as has been described (*3*). The electrophoresis was conducted in tris buffer at *p*H 8.3 instead of in glycylglycine buffer at *p*H 7.4, and cyanide was omitted from the reaction mixtures. Approximately the same total amount of activity was applied to each sample gel for microelectrophoresis in order to compare the MDH patterns of embryos at different stages of development and of embryos grown in sea water, L-malate, or D-malate.

Echinochrome content of the enzyme preparations inhibits polymerization of the sample gel; hence, the layer of the polyacrylamide that contained the sample was prepared by mixing 1 part each of enzyme preparation and water and 1.5 parts each of "upper gel" and 2.2*M* sucrose solution. The buffer layer for the cathode was applied slowly to prevent "wash-out" of the sample before the electrophoresis began.

Unfertilized eggs have six bands of L-MDH activity and perhaps a seventh which appeared as a thin band (Fig. 1); the number of bands decreased to three in 4- and 6-hour embryos before increasing to four in 12-hour embryos. The major bands of MDH activity in unfertilized eggs appeared to be Nos. 3, 5, and 6, but activities were not determined quantitatively. The ratio of L-MDH activity with APAD to activity with NAD (as hydrogen acceptors) was less than 1 for preparations from both unfertilized eggs and embryos, a figure which agrees with the observations of Moore and Villee (*3*).

The effect of varied concentrations of L- and D-malate on fertilization and development of the eggs was investigated. D-Malate at $10^{-1}M$ or $10^{-2}M$ inhibits fertilization; $10^{-3}M$ D-malate inhibits neither fertilization nor development. In $10^{-1}M$ L-malate, the percentage of eggs fertilized ranged from 50 to 100 percent, but in $10^{-2}M$ L-malate both fertilization and development were normal.

Embryos grown for 4 hours in sea water, $10^{-2}M$ L-malate in sea water, or $10^{-3}M$ D-malate in sea water had iden-