continuum emission. From the initial proportion of H_2S employed, the 5-cm mean light path through the reaction vessel, and the 2537 Å absorption coefficient of H_2S (*5*), we calculate that approximately 10 percent of the radiation emitted at the beginning of the experiment was absorbed by H_2S; and rather less toward the end of the experiment. Thus $\sim 6 \times 10^6$ amino acid molecules were produced per erg absorbed. We compare this efficiency number with the 5×10^{10} per erg found in comparable shock experiments (*16*). Thus it appears that shocks are approximately 10^4 times more efficient than ultraviolet for prebiological organic synthesis. But $\lambda < 2600$ Å ultraviolet was at least 10^3 times more abundant on the primitive Earth. If we allow for the facts that (i) only free amino acids and not the products of acid hydrolysis were analyzed in experiment 2; that (ii) at $\lambda < 2537$ Å the H_2S photolytic products are more energetic than at 2537 Å; and that (iii) other photon acceptors—for example, HCHO—may be effective at $\lambda < 2537$ Å, we conclude that overall ultraviolet and shock energies were of comparable importance in the prebiological synthesis of amino acids. A previous conclusion (*16*) that shocks were more important relied on earlier ultraviolet quantum yields from Hg sensitization experiments (*11*). The quantum yield per 2537 Å photon found in our experiment is $\phi \sim 5 \times 10^{-5}$, about an order of magnitude more than found in (*11*). Assuming all $\lambda < 2600$ Å photons were absorbed by H_2S on the primitive Earth, and that no subsequent destruction of amino acids occurred, this ϕ and the photon flux for the early Sun (*3*) imply approximately 200 kg of amino acids produced per square centimeter in 10^9 years. Destruction, of course, must have occurred, but a very high concentration of organic compounds and conditions quite congenial for the origin of life seem to be suggested.

The principal conclusions of this work follow: (i) H_2S is an acceptable long-wavelength photon acceptor for prebiological organic chemistry. The 1849 Å line is not necessary for the production of amino acids, but initial ethane is. Ethane is a likely product of electrical discharge and short-wavelength ultraviolet irradiation of methane. Temperatures higher than plausible mean surface temperatures are not required. (ii) A quantum yield $\phi > 5 \times 10^{-5}$, and an efficiency number $\gtrsim 6 \times 10^6$ amino acid molecules per erg are implied. Over 10^9 years of ultraviolet irradiation of the early Earth, this is the equivalent of 200 kg of amino acids produced per square centimeter, a huge number suggesting congenial conditions for the origin of life. (iii) Cystine and therefore perhaps other sulfur-containing amino acids can be produced in simulated prebiological conditions.

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12. The most reasonable estimate for the temperature of the primitive Earth is something around room temperature, but there may have been localized times and places when surface temperatures were higher; upper atmospheric temperatures were of course much higher. Methane is not successfully attacked by radicals and hot hydrogen until it is brought to a temperature of some 300°C (*17*). Accordingly in some experiments temperature of 300° to 400°C were employed. Amino acids have been produced by heat alone from a reducing atmosphere, but temperatures of 900° to 1100°C are required (*18*); we show below that temperatures of 300° to 400°C alone will not produce detectable amino acids.
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Countercurrent Chromatography with Flow-Through Coil Planet Centrifuge

Abstract. *We have developed a new method of countercurrent chromatography which employs a vertical helical tube in the centrifugal field. The helical tube is arranged so that it does not rotate as it revolves, thus eliminating the need for rotating seals. When the gyrating tube is filled with either phase and the other phase is introduced into the tube in the proper direction, an equilibrium state results in which the two phases are split into multiple alternating segments within the coil. Each phase oscillates to and fro with the rotation as the moving phase is steadily eluted out through the other end of the tube. Consequently, solutes introduced into the tube are subjected to a rapid partition process, resulting in an efficient chromatographic separation without the complications arising from solid supports. The method is illustrated by the microanalytical separation of dinitrophenyl amino acids and can be used on a preparative scale.*

Helix countercurrent chromatography (*1, 2*) provides various advantages over the liquid partition techniques with the coil planet centrifuge (*3*), because of its continuous flow-through system. The versatility of the method is, however, limited by the fact that the solvent is fed from a ro-

tating syringe and collected through a rotating seal. The method presented here introduces a continuous flow-through system to the coil planet centrifuge without rotating seals.

Figure 1, A and B, illustrates the general principle of the method. The separation column is a helix (Fig. 1A). Flexible feed and return tubes are supported by the moving disk at the top of the helix and the stationary disk fixed to the center of the upper frame of the centrifuge. The entire helix and the moving disk revolve around the axis of the centrifuge but they are not permitted to rotate with respect to the stationary disk. The fixed orientation of the helix is maintained by coupling (not shown in Fig. 1) a pulley on the helix holder through a toothed belt to a stationary pulley of equal diameter on the axis of the centrifuge drive. This coupling causes a counterrotation ($-\omega$) of the helix to cancel out the rotation (ω) of the helix induced by its revolution. The feed and return tubes do not twist because the moving disk does not rotate with respect to the stationary disk as indicated (Fig. 1B) by the position of the "x" marks shown in successive positions of the relation between the stationary and moving disks. Because the helical column maintains a fixed orientation while it revolves, the radially directed centrifugal force rotates with respect to the column as in the gyrating system described earlier (*2*).

Let us consider the motion of the two immiscible phases, the "upper" and "lower" phases, confined in such a tube without the external introduction of flow (Fig. 1, C–E). For the convenience of illustration, the direction of the centrifugal force which actually rotates is fixed at the bottom, and, instead, the rotation of the coil units is substituted as indicated by the curved arrows. Figure 1C shows the motion of a small amount of the lower (heavier) phase in the upper phase. If the relative centrifugal force $R\omega^2$ (where R is the distance between the center of revolution and the axis of the helix) is strong enough to keep the lower phase near the bottom of the coil unit (position a) at all times, the latter will simply move toward one end of the coil, the head (the other end is called the tail), at an angular velocity ω.

In the system that is described here the centrifugal force usually fails to fix the lower phase which subsequently appears at any portion of the coil unit. When the lower phase appears at the left half of the coil unit (position b), it tends to move toward the head whereas, at the right half (position c), it moves toward the tail. Because the lower phase tends to spend more time in the left half, these two motions do not cancel out and the lower phase moves toward the head with an oscillatory motion at a mean angular velocity smaller than ω. Figure 1D illustrates the motion of a small amount of the upper phase in the lower phase. With a strong centrifugal force, the upper phase could be fixed near the top of the coil unit (position a), constantly moving toward the head of the coil. When the centrifugal force fails to fix the upper phase, this phase moves

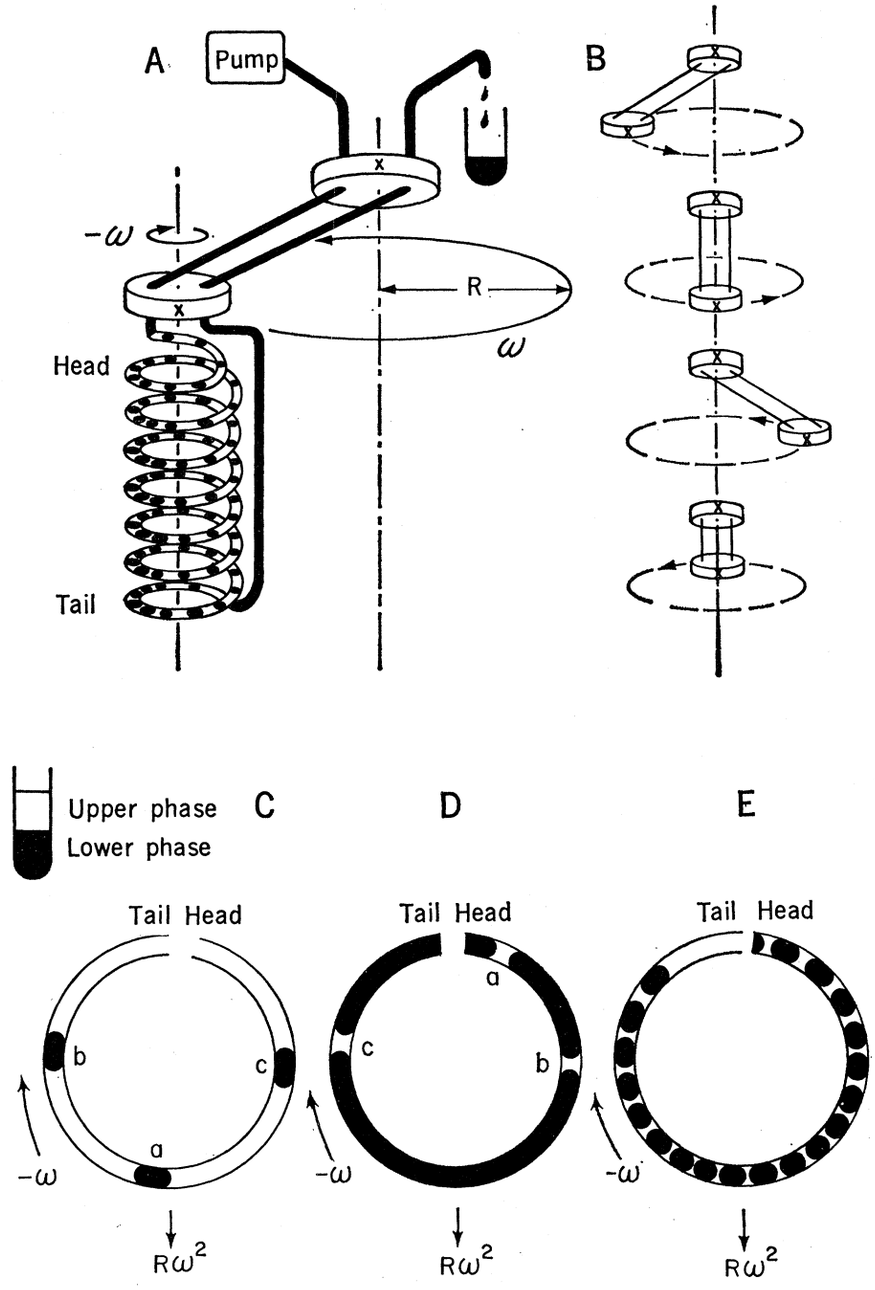

Fig. 1. Schematic illustration of the general principle of the method and the motion of the two phases in a coiled tube undergoing planetary motion. (A) Helical separation column with flexible feed and return tubes supported by the moving disk and the stationary disk. (B) Successive positions of the stationary and moving disks. (C) Motion of the lower (heavier) phase in the upper phase. (D) Motion of the upper phase in the lower phase. (E) Equilibrium state established after the introduction of approximately equal volumes of the two phases.

toward the head at the right half (position b) and toward the tail at the left half (position c) of the coil unit. However, these motions again do not cancel out and the upper phase also moves toward the head. When approximately equal volumes of the two phases are introduced into the tube, the motion of the phases becomes quite complex but finally reaches an equilibrium state, illustrated in Fig. 1E. At equilibrium the multiple segments of the two phases (*4*) are alternately arranged from the head to the tail side and any excess of either phase remains at the tail. Consequently, this phenomenon determines the proper direction of the elution. When the tube is filled with either phase and the other is introduced from the head, the equilibrium state is quickly established from the head through the tail as elution proceeds and a given volume of the stationary phase is held within the equilibrated coil (Fig. 1A). On the other hand, introduction of the flow through the tail results in a steady carryover of the stationary phase until the moving phase fills the entire column. Thus the moving phase should be fed from the head end, determined by both the handedness of the coil and the direction of planetary motion. Consequently, a sample solution introduced into the tube is subjected to a partition process between the oscillating alternate segments of the two phases and finally eluted out through the tail end of the coil.

In order to form the two phases into multiple segments in a relatively small-bore tubing without plug flow, an adequate centrifugal force is provided by the relatively long radius of revolution. We have constructed a test system with the radii adjustable to 30.7, 20.2, 13.5, and 8.6 cm by modifying a conventional centrifuge (International Equipment Company model II). A stationary tube (60 cm long) is mounted to the motor housing and the motor shaft extended through a bearing at the top of the stationary tube. It is then connected to a rotating tube that fits freely over the stationary tube with another bearing at the bottom. A pair of arms fixed to the top and bottom of the rotating tube, 50 cm apart, hold the rotating rods or coil holders with bearings. When a pair of toothed pulleys of the same size, one fixed to the bottom of the stationary tube and the other at the bottom of the coil holder, are connected by a toothed belt, revolution introduces the desired planetary motion to the coil holder, that is, one rotation per revolution in the opposite direction. The column is made either by winding the Teflon tubing onto the coil holder or by arranging multiple column units interconnected in a series (tail-head connection) or several separate parallel columns around the holder. Both feed and return tubes are passed through the center hole at the top of the holder and then supported at a height of 25 cm above the center of the apparatus. These tubes, protected with a piece of silicone rubber tubing at the hole, have not failed or stretched appreciably even after having been used many times.

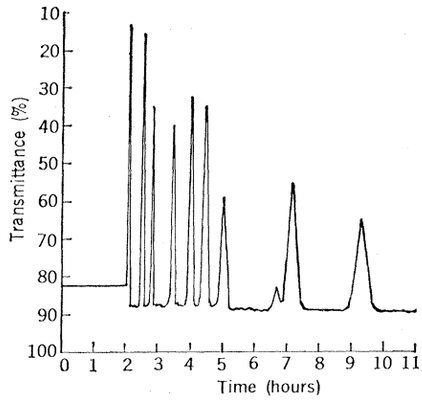

Fig. 2. Results of the separation of nine DNP amino acids. Recording was made by means of an ultraviolet monitor (LKB Uvicord II) at 280 nm. Peaks identified in order of elution and their partition coefficients from left to right are: *N*-dinitrophenyl-δ-L-ornithine (> 100); *N*-dinitrophenyl-L-aspartic acid (3.8); *N*-dinitrophenyl-L-glutamic acid (1.9); *N,N'*-dinitrophenyl-L-cystine (0.94; *N*-dinitrophenyl-β-alanine (0.71); *N*-dinitrophenyl-L-alanine (0.56); *N*-dinitrophenyl-L-proline (0.45); *N*-dinitrophenyl-L-valine (0.26); *N*-dinitrophenyl-L-leucine (0.18).

We have estimated the efficiency of the method for the separation of dinitrophenyl (DNP) amino acids, using a phase system consisting of chloroform, glacial acetic acid, and 0.1*N* HCl (2 : 2 : 1). Figure 2 shows the separation for nine DNP amino acids on a column of Teflon tubing (0.30-mm bore 100 m long) (Zeus Industrial Products, Inc., Princeton, N.J.) with a helix diameter of 5 mm and a total capacity of about 8 ml. The volume of sample is 10 μl (each component is present at a concentration of about 1 percent where solubility permits). The upper phase is fed with a metering pump (Chromatronix type CMP) at a rate of 2.4 ml per hour. The apparatus is spun at 550 rev/min with a radius of 30.7 cm, and the centrifugal force at the helix is 100*g*. The measured equilibrium feed pressure is approximately 13.5 atm. The efficiency calculated from the formula used for gas chromatography (*1*) ranges between 10,000 and 3000 theoretical plates, showing a tendency to decrease with increased retention time.

The potential of the method for work on a preparative scale has been examined on a column prepared from Teflon tubing (1.2-mm bore, 100 m long) with a helix diameter of 1 cm and a total capacity of about 120 ml. At 520 rev/min with a radius of revolution of 8.6 cm and a flow rate of 24 ml per hour, a 1-ml sample (*5*) can be eluted out within 13 hours at an efficiency ranging between 4000 and 1000 theoretical plates.

The method reported here is reproducible with minimum carryover of the stationary phase and provides the following advantages over helix countercurrent chromatography: (i) the system has no rotating seals; (ii) the sample can be introduced into the running column when desired; and (iii) gradient or stepwise elution is applicable. Similar efficiency is obtained for a much shorter elution time under reduced pressure. Multiple columns can be arranged parallel to each other for simultaneous runs.

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4. With a relatively large-bore tubing, a similar treatment may produce multiple droplets of either phase in the other. The picture of the hydrodynamic phase equilibrium may be modified by various factors such as viscosity, interfacial tension, or the density difference of the two phases, and for some solvent systems the ideal condition may be obtained by using an even greater radius of revolution.
5. For the convenience of recording, the sample solution was prepared by diluting 100 μl of the original sample with either phase.
6. We thank H. Chapman for fabricating the instrumentation and Miss J. Friedlander for preparing the manuscript.

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