ing capacity on biological surfaces due to fixed charges and their possible displacement according to their affinity is of considerable importance. Our knowledge of the ion binding capacity for polyelectrolyte structures is recent[5,6]. One highly interesting hypothesis for biological structures is that cations form a mobile monolayer[7,8] around the dissociable groups of polymer chains.

The constants of Na^+ and K^+ found for the cell-wall, are of the order of magnitude for weak interaction. It seems reasonable to regard the monovalent counterions as forming a very highly mobile monolayer on the polymer skeletal surface of cell-wall.

On the other hand the association constants of divalent and trivalent cations are clearly for stronger interactions. This result is consistent with the concept of the counterions closely bound by electrostatic interactions.

Riassunto. Sono state studiate le costanti apparenti di associazione di vari cationi per il cell-wall isolato di cellule di *Staphylococcus aureus*. E' risultato che gli ioni monovalenti presentano una bassa costante di associazione mentre gli ioni bivalenti e trivalenti presentano valori nettamente superiori delle costanti di associazione. Si conclude che mentre i cationi monovalenti sono legati da deboli legami elettrostatici, i cationi di valenza superiore formano con i gruppi dissociabili legami ionici stabili.

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Institute of Microbiology, University of Napoli (Italy), 25 July 1967.

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Cell-Like Structures from Simple Molecules under Simulated Primitive Earth Conditions

Over 2 decades ago a group of experiments on the origin of life was performed by HERRERA using ammonium thiocyanate and formaldehyde[1]. This work has become relevant since MILLER's synthesis[2] of formaldehyde in a prebiological system and HEYNS' extension[3] which yielded ammonium thiocyanate.

We chose an experiment[4] in which it was claimed that simple mixing of 7 ml 37% formaldehyde and 3 g ammonium thiocyanate resulted in 'life-like forms'. After mixing the materials, the colorless liquid became slightly red in a few sec, and finally a golden yellow after 1 h. Microscopic examination (without a cover glass) revealed a high density of spheres 1–5 μ in diameter (Figure 1). A drop of water was applied so it slightly overlapped onto the drying mixture (Figure 2). Vigorous streaming of the reaction mixture into the water resulted. The spheres darkened considerably and structures of a greater size and complexity, 10–100 μ in diameter are seen (Figure 1). 0.1 M acetic acid and 0.1 M NaH_2PO_4 gave the same results when used as rehydrants. When the drying spheres were rehydrated with 1% solutions of methylene blue, trypan blue or Ponceau S, they concentrated the stain.

Internal fluid regulation is one of the primary properties of life. The contractile vacuole is used by some unicellular organisms for this purpose[5]. In many cases we observed motion of an internal vacuole-like structure toward the boundary and its subsequent extrusion. The boundary then closed, reformed, and resumed its circular shape (Figure 3).

The yellowing liquid became progressively less abundant as a thin layer of solid formed on its surface and much solid deposited at the bottom. Little supernatant was left after 48 h at room temperature.

UV-radiation is assumed to have been an important energy source for chemical evolution[6]; so we considered its effect on the reaction. We used dilute reactant solutions to test for the catalytic effect of UV-radiation on concentrations more likely to have occurred in a primitive earth environment. A typical starting mixture consisted of equivolumes of 0.5 M NH_4SCN and 1.4 M formaldehyde. Samples were subjected to darkness, room light (fluorescent), and UV-radiation (Ultraviolet Products SC-1). The dark reaction and the second case showed no spheres after 24 h whereas the irradiated sample displayed spheres after 15 min.

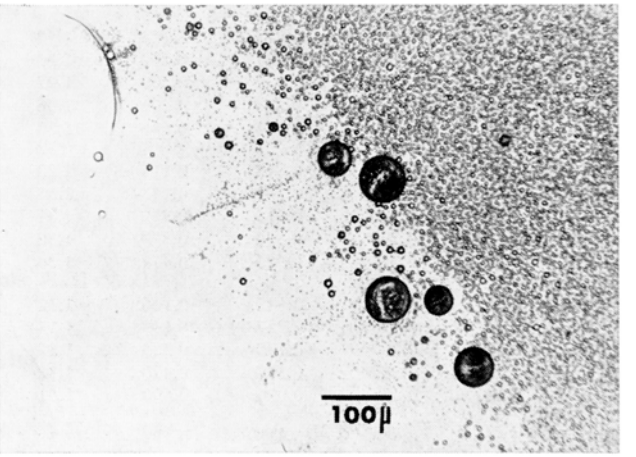

Fig. 1. Multitude of small spheres from a 7 ml 37% formaldehyde, 3 g NH_4SCN mixture. The dark vacuolated spheres were formed by rehydration of the drying drop.

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In order to see how the phenomena varied with concentration of starting constituents, we used equivolume mixtures of 0.05*M* NH_4SCN with 0.5, 1.5, 3.0, 6.0*M* formaldehyde solutions and subjected them to UV-radiation (up to 24 h). The microscopic phenomena were essentially the same in all cases.

To study the possibility of localizing catalytic activity into the spheres, an aqueous solution was prepared containing 3.33*M* formaldehyde, 0.5*M* NH_4SCN, and 0.5*M* $ZnCl_2$. (Previous results in the pyrocondensation of amino acids to spheres have indicated that zinc incorporation can lead to a localized 'ATPase'-like activity[7].) The solution was irradiated by a rack of germicidal lamps for 1.5 h. After drying, various amounts of the resultant solid (compound A) were added to 1 ml aliquots of 0.002*M* ATP and stirred for 17 h at room temperature. Insoluble material was removed and analysis of the supernatant by the molybdate test for free phosphate[8] and the luciferase test for ATP[9] revealed that a small but definite amount of hydrolysis could be related to the amount of compound A. Compound A was further studied by elemental analysis and IR-spectroscopy (KBr). It was found to consist of C-32.00%, H-4.41% and residue (possibly $ZnCl_2$) 10.53%. Since formaldehyde is 36% C and NH_4SCN 14% C, assuming 10.53% inorganic residue, it may be seen that compound A is apparently a combination of both reagents. Its IR-spectrum revealed at least 2 peaks not found in the starting reactants, 1580 and 1035 cm^{-1}. These may be due to imine and primary alcohol groups, respectively.

These experiments have demonstrated how simple compounds likely to have been on the primitive earth can lead to the formation of cell-like structures which could have served as matrices for subsequent stages of biogenetic evolution. The spheres, capable of interacting with the environment (since they absorb dyes, exude vacoules, and incorporate smaller spheres) are thus possible precursors to a higher level of organization. However, no suggestion is made that these spherules are in fact 'alive'. Rather, these experiments demonstrate one means whereby a delimited, localized environment could have arisen bearing a number of the characteristics from which a biodynamic system might evolve.

It is also worth noting that our experiments represent only one of several means by which the transition from molecules to structures could have been fulfilled. Coacervates and cell-like structures have already been shown to form from paraformaldehyde[10], pyrocondensation products of amino acids[11], nucleosides[12], proteins[13], and nucleic acids[14]. Preliminary results indicate that a combination of amino acids, thiocyanate, and UV can also produce microspheres[12]. These experiments demonstrate that given any one of a large variety of possible primordial conditions, the production of protocells seems to have been a likely event[15].

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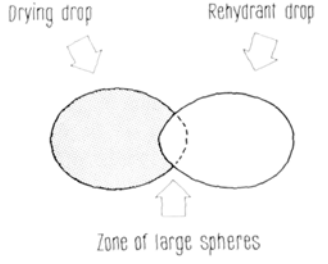

Fig. 2. Rehydrant drop placed so that it overlaps the drying mixture.

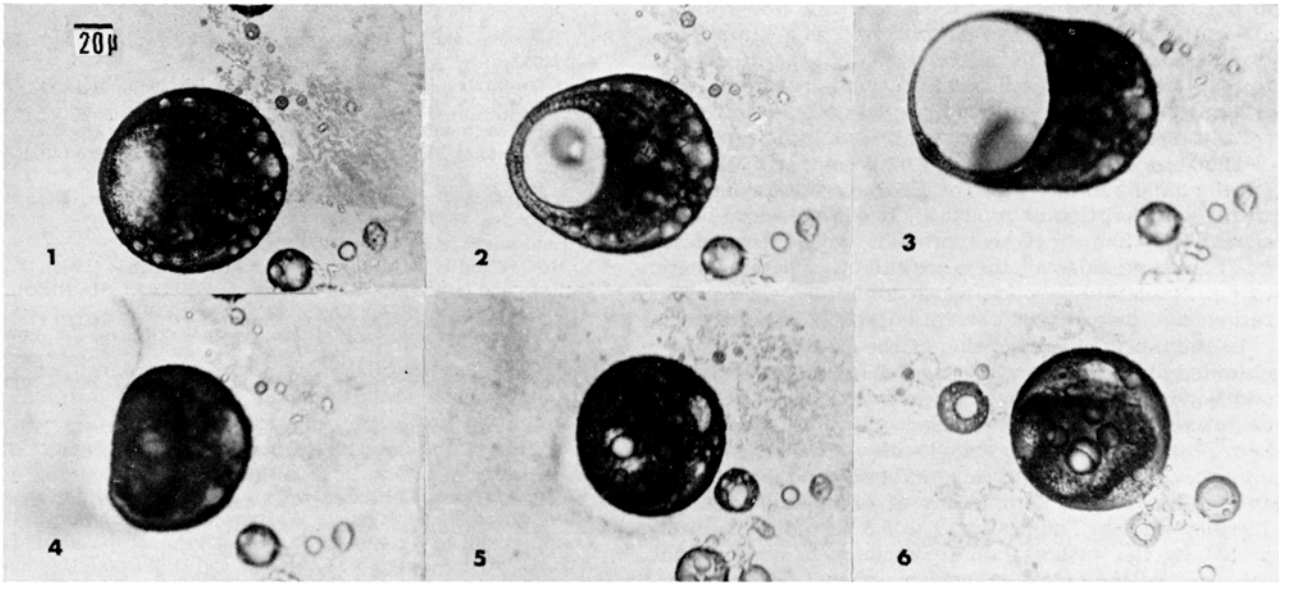

Fig. 3. Consecutive stages in dynamic interactions of the spheres. (1-3) A vacuole forcing the sphere to enlarge. It is exuded and the sphere returns to its circular shape. (4-6) Mass at lower right being incorporated into the sphere. Time duration is about 30 sec.

Résumé. Le sulfocyanate d'ammonium et l'aldéhyde formique, formés probablement au cours de l'évolution géochimique, fournissent les structures ressemblant à des cellules. La formation des ces structures, sous des conditions variables de concentration, est catalysée par les irradations UV. Ces formes protocellulaires expulsent leurs contenus vacuolaires et absorbent des matières colorantes. La transition de l'évolution géochimique aux protocellules semble être une étape admissible selon le résultat de nos expériences.

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Electron Spin Resonance Studies of Catalase and some of the Catalase Compounds

A magnetic study of the catalase and some of the catalase compounds has been made by THEORELL et al.[1–3]. According to these investigations, the iron of catalase, of catalase azide compound and of catalase fluoride is in ionic bond. Only the catalase-cyanide compound is a covalent complex; however, in the case of liver catalase only 3 hemin irons enter this covalent bond, while the fourth iron atom remains in an ionic bond. Compared with measurement of magnetic susceptibility, electron spin resonance (ESR) measurements permit a closer insight into the electron structure of hemoproteids[4–12].

This report presents the results of ESR studies of bovine liver catalase. The preparation of crystalline catalase was made according to the method described by SCHNUCHEL[13] with subsequent purification by gel filtration on Sephadex G 100 at pH 6.9. For ESR measurements $1–2 \times 10^{-4}$ valar catalase solutions were used. The measurements were carried out at a temperature of 77 °Kelvin.

The ESR spectrum of catalase (Figure 1) is an axial-symmetrical spectrum with the g-factors $g_{\perp} \approx 6.3$ and $g_{\parallel} \approx 1.92$ as found with the isolated prosthetic group of hemoglobin, the chlor hemin[14]. The additionally occurring absorption at $g = 4.2$ is due to an iron that is not bound to porphyrin – similar to what EHRENBERG[7] found in ESR measurements of myoglobin, MORITA and MASON[8] in peroxydase, YONETANI, SCHLEYER and EHRENBERG[12] in the cytochrome c peroxydase and BEINERT and SANDS[15] in the DPNH cytochrome c reductase. The iron with an absorption at $g = 4.2$ is to be conceived as a contamination of the catalase; it can be partially separated from the catalase protein by the gel filtration purification, that is the strength of absorption at $g = 4.2$ decreases after the gel filtration without altering the rest of the spectrum.

The absorption peak at $g_{\parallel} \approx 1.92$ is comparitively high for the axial symmetry of the ESR spectrum compared with the absorption of g-vertical. It can therefore be assumed that like other hemoproteids, myeloperoxydase[7] and plant peroxidases[8], there are still other paramagnetic cations, preferably copper, which contain catalase preparations and increase the absorption at $g = 2$.

In the repeated preparation of the catalase we always obtained, within the absorption band of $g \approx 6.3$, an additional peak which has been retained also in the ionic complexes of catalase. Such peaks at $g \approx 5.3$ have also been observed by EHRENBERG[7] with the alkaline form of myoglobin and with myeloperoxidase, and by MORITA and MASON[8] with peroxidases of horse-radish and of Japanese radish. The peak at $g \approx 5.3$ has not been interpreted by the authors; we are inclined to assume that this peak in the catalase suggests either 2 different possibilities of binding of the prosthetic group to the protein or that there is no longer an axial symmetry of the molecule as supposed by WATARI et al.[16,17] for the ferrihemoglobin M_{OSAKA}.

In ESR measurement catalase fluoride behaves very similarly to the catalase. So the 2 compounds – like methemoglobin fluoride[9] and the fluoride compound of

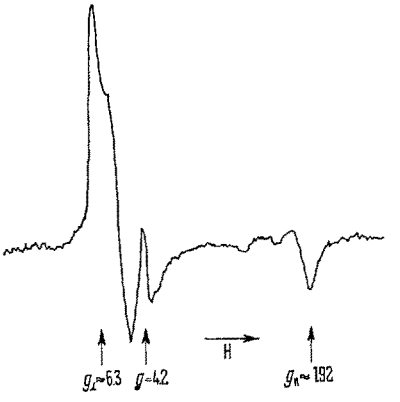

Fig. 1. First derivative of electron spin resonance absorption spectrum from catalase (pH = 6.9).

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