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Membrane, Action, and Oscillatory Potentials in Simulated Protocells

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Electrical membrane potentials, oscillations, and action potentials are observed in proteinoid microspheres impaled with (3 *M* KCl) microelectrodes. Although effects are of greater magnitude when the vesicles contain glycerol and natural or synthetic lecithin, the results in the purely synthetic thermal protein structures are substantial, attaining 20 mV amplitude in some cases. The results add the property of electrical potential to the other known properties of proteinoid microspheres, in their role as models for protocells.

Artificial cells [1] assembled from thermal proteins (synthetic copolyamino acids) have been found to have many of the properties of evolved cells [2, 3]. The tendency of these vesicles to form junctions and especially to participate in "intercellular" activities [4] have prompted a search for bioelectric phenomena. Membrane, action, and oscillatory potentials have recently been reported for vesicles composed of thermal protein, glycerol, and vegetable lecithin [5].

We report here that the same kind of behavior is observed when synthetic lecithin is included instead of natural lecithin, all components thus being synthetic. We report further that the same kinds of electrical behavior are observed when the lecithin and glycerol are omitted entirely; electrical effects are thus manifestations of the selective permeability of the polyamino acid assembled as a membrane in the presence of water.

These findings open the possibility for investigating the relationship between copolyamino acid constitution and electrical readout patterns. The structure and function of artificial cells are amenable to controlled experimental manipulation, due to the synthetic nature of their components. A minimum prescription for cellular excitability is thus found to be a spherical membrane assembled from thermal copolyamino acids in the presence of water and potassium ions.

An additional significance, when one interprets the model to be a sufficiently accurate representation of terrestrial protocells [6], is that the first cells on Earth had bioelectrical properties in addition to the other functions that have been catalogued [1–4].

The total results also help to assign the functions of the membrane to the individual components. The qualities of specificity and excitability appear to be rooted in the protein component (in these experiments thermal protein) whereas the efficient barrier function of modern cellular membranes is ascribed to phospholipid. This kind of division of properties has been emphasized earlier by Nachmansohn [7, 8].

Although spherule-forming proteinoids manifest significant lipid-like characteristics of their own, the conductivity of the proteinoid-only vesicles is most often greater than that of the proteinoid-lecithin vesicles. Preliminary evaluation of the static and dynamic voltage and impedance characteristics of the two membrane types indicate that the difference is one of quantity rather than quality of conductive channels.

In judging the contribution of thermal proteins to electrical behavior in vesicles, the fact that measurable amounts of chromophores such as pteridines and flavins are formed as byproducts of the thermal condensation of amino acids [9, 10] should be taken into account. In some proteinoid vesicles electrical activity, thought previously to be spontaneous, has been found to be due to photosensitiv-

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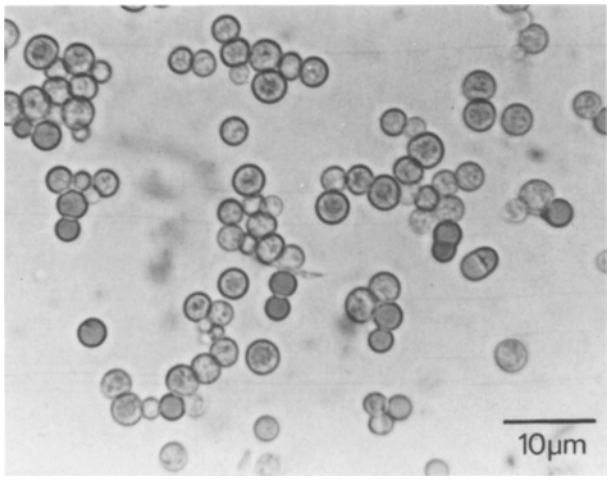

Fig. 1. Microspheres composed of copoly(asp, glu) complexed with copoly(asp, glu, arg, his)

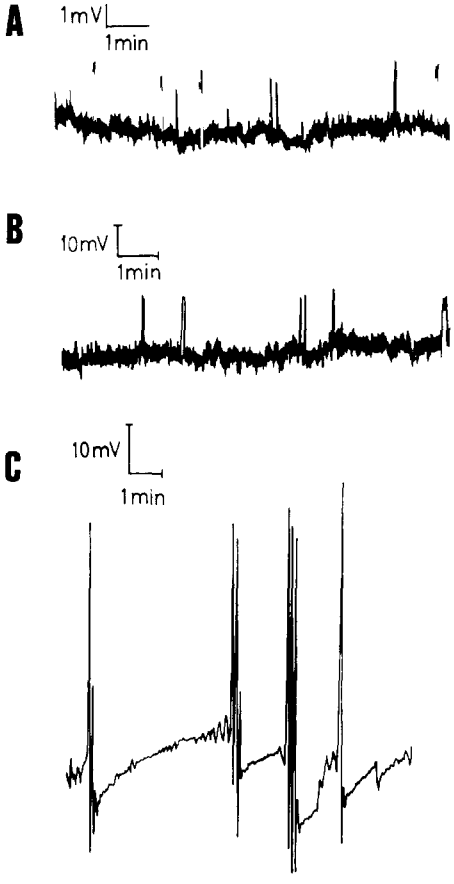

Fig. 2. Tracings of spiking in microspheres assembled from (A) copoly(asp, glu), (B) copoly(asp, glu) complexed with copoly(asp, glu, arg, his), and (C) JGII-58 proteinoid and vegetable lecithin. For each observation, controls in the solution without impalement yielded a horizontal pattern. Proteinoid JGII-58 was a 2:2:1-proteinoid [2] lacking histidine made by J. Grote

ity. One may thus consider that such sensitivity is associated with the presence of chromophores.

The proteinoids for these experiments have been made in the usual manner of heating mixtures of amino acids containing sufficient trifunctional amino acid (at least a few percent) under a blanket of nitrogen gas [2]. One kind of microsphere studied is that composed of thermal copoly(asp, glu) complexed with copoly(asp, glu, arg, his). The latter are of special interest in that such microspheres are stable at relatively high pH, and would have withstood the conditions of a primitive alkaline ocean [11]. They are depicted in Fig. 1. Their electrical behavior is traced in Fig. 2B.

Figure 2 displays also the electrical pattern from microspheres from copoly(asp, glu) alone and from a preparation of acidic proteinoid complexed with vegetable lecithin. The comparison illustrates extremes in amplitude of response. The spiking activity in microsphere (A) of copoly(asp, glu) is approximately 2 mV above background. That from (B), the mixed polymer microsphere, is approximately 20 mV above background. The vesicles (C) containing lecithin and polymer JGII-58 display 60–70 mV above background.

The quantitative similarity in amplitude of spikes within trains A or B is noteworthy. As a general observation, the amplitude and patterns of spiking is more regular in the phospholipid-free preparations. The ways in which the patterns may be correlated with the polyamino acid constitution are accordingly being studied.

The repeatability of the results has been evaluated in multiple (average = 5) preparations of microspheres with numerous spherules impaled in each preparation. In addition to the proteinoid polymers already mentioned, similar electrical manifestations were found in spherules made of the following: copoly(asp, glu, lys), copoly(arg, his, lys, leu), copoly(lys, leu), or copoly(asp, glu, his). Both the proteinoids [12] and the microspheres appear to be indefinitely stable. The latter can be dehydrated and rehydrated; they still manifest electrical activity.

The membrane potentials and electric discharges observed, especially in the membranes of mixed basic-acidic proteinoid, indicate a tighter membrane than would be otherwise suggested. Other evidence for the membrane nature of the boundary include the electronmicrographic evidence of unit infrastructure [2], osmotic properties [13], selective permeability [13], and the resealable and black films found by Baumann [14]. Moreover, the double layer observed in photomicrographs [2] may explain the occurrence of two successive elec-

tric responses, often of opposite sign, which are recorded when the microelectrode impales the microspheres.

Proteinoid membranes function to some degree like lipid membranes due to apolar sidechains [15], but provide a less efficient barrier than the phospholipid in modern cells. In a larger view, the principal properties sometimes postulated for the first cell: beginnings of metabolism, ordered macromolecules, and membrane function, are found. The membrane function in the simulated protocells is now more strongly supported and the existence of ordered polyamino acids not having required nucleic acids has been explained as due to self-sequencing of amino aids [2, 16, 17]. The main requirements of membranicity, metabolic activity, molecular order, and specificity needed for emergence of a first cell would in this analysis thus have been met by proteinoid microspheres [1, 2]. A need for unlikely assembly of chemically diverse functional components would thus have been obviated.

Membrane potentials in evolved cells have been suggested as being maintained by and arising from, cellular metabolism [8]; the view that cellular metabolism is not necessarily a basis has also been argued (cf. [18, 19]). The continuing observation of potentials in microspheres from which metabolic substates are absent is consistent with the view that the potentials are due to macromolecular configurations in the membrane and channeling; the activity does not necessarily require metabolism. The contribution of phospholipid to excitable membranes appears to be characteristic in general; however, the fundamental membrane functions of electrical and some metabolic activities [2] are found to be present in proteinoid microspheres lacking phospholipid (cf. [7, 8]). This view has been supported by results such as those contained herein.

The microspheres stable at high pH (Fig. 1) are of interest for additional reasons. As a structure composed of oppositely charged proteinoids, it is the kind compared after artificial fossilization [20] with artificially fossilized algae and found to be indistinguishable from some of the latter. The microspheres containing lysine-rich proteinoid are also the kind that have been shown to be capable of making an offspring generation of (nonthermal) peptides from amino acids and ATP or pyrophosphate [21] in the presence of water. This type of microsphere arises from dilute solutions of each kind of proteinoid, does not require proteinoid-saturated solution [11], is stable at pHs above 7.0 [11],-and can also make oligonucleotides [16].

Since the production of thermal proteins and microspheres can be controlled over a wide compositional range, and since excitability is a state dependent upon phase-separated proteins as Szent-Györgyi [22] has explained, this function is open to investigation in the models being studied. The experiments demonstrate that phase-separated protein structures provide membranes active in the presence of water [23].

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