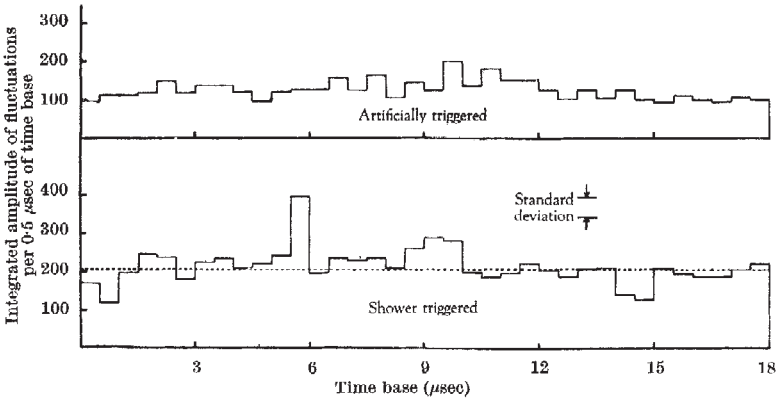


Fig. 3. Histograms showing integrated amplitudes of fluctuations for the same recordings as in Fig. 2

detector, and was then applied to the deflexion circuits of one beam of the oscillograph, the second beam carrying a 4-Mc/s sine-wave for calibration purposes. All the low-level stages of the receiver were run from an independent battery supply, and the total power was monitored on a chart recorder. As required, a small 44-Mc/s pulsed transmitter $T$, connected to a dipole $D$, could be driven from one of the counters. The sensitivity of the receiver was limited by cosmic noise, at a system noise temperature varying between 7,000° K and 14,000° K according to sidereal time.

The following results were obtained. During the recording of 1,799 showers five pulses have been observed which were bandwidth-limited in duration and which had amplitudes of 8, 10, 10, 11 and > 45 (off scale) times the root mean square noise level, corresponding to received energies of 4, 5, 3, 3 and $> 20 \times 10^{-12}$ ergs. These all had delays of between 5·4 and 6·0 μsec with respect to the start of the time-base. No bandwidth-limited pulses were observed in the intervals 0–5 or 6–20 μsec on any of the 1,799 shower recordings, and no such pulses were observed on any portion of the 20-μsec time-bases for the 1,117 artificially triggered recordings.

A statistical analysis of the recordings was made to search for smaller events. Fig. 2 shows histograms both for 1,794 shower-triggered and for 1,117 artificially triggered recordings, in which the ordinates represent frequency of occurrence of the fluctuation of greatest amplitude along the time-base, between 0 and 18 μsec. Fig. 3 shows a similar histogram in which the ordinates represent the integrated amplitudes of the fluctuations. Excluding the five large pulses mentioned earlier, the main peaks in both histograms occur in the interval 5·5 to 6·0 μsec. In Fig. 3 the peak of the histogram is four times the standard deviation of the fluctuations. The pulses were observed to have a time-delay identical with that from the test transmitter when it was driven from a single counter, after allowing for the delay in firing the counters themselves. This latter delay, which averages 0·75 μsec, and which was determined in a separate experiment, is sufficient to allow us to eliminate completely the possibility that the observed radio pulses originated in the electronics of the Geiger–Müller counter circuits.

These observations lead us to conclude that short radio pulses of duration < 0·25 μsec have been observed from extensive air showers. Further details of the work will be presented in due course, and experiments are to continue.

While the energy received in these pulses is consistent with the hypothesis of enhanced Čerenkov radiation, it appears also that pulses of comparable amplitude may be expected to arise from the separation of charges in the Earth's magnetic field. This latter radiation would be linearly polarized east–west, affording some possibility of distinguishing between these two mechanisms. We have also considered bremsstrahlung and transition radiation as possible origins of the pulses, but we believe these to be of secondary importance. We also believe that direct effects of induction by the shower particles in close proximity to the aerial elements are negligible.

We thank Sir Bernard Lovell for his enthusiasm and encouragement throughout this work, and also for making available the wide facilities at Jodrell Bank.

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# A THEORY OF MACROMOLECULAR AND CELLULAR ORIGINS

By Prof. SIDNEY W. FOX

Institute of Molecular Evolution, University of Miami, Coral Gables, Florida, and Institute for Space Biosciences, Florida State University, Tallahassee, Florida

At this point in the history of science, descriptions of life can be treated more rigorously and with less controversy than can comprehensive definitions of life. Two outstanding aspects of the description have been its bewildering variety and the overwhelming complexity of its units.

The first quality of variety is that which clearly confronted Charles Darwin, A. R. Wallace, other naturalists, all scientists, and indeed all thinking people more than a century ago. A general dismay engendered in attempting to understand such variety was dispelled by resort to the evolutionary principles which rely on progression from simplicity to complexity.

In this century, we are confronted with the problem which concerns the processes which must have been a prelude to the primitive organism from which all species, according to Darwin, descended. From present vantage points the complexities of the incompletely understood molecules of protein or nucleic acid can easily be overwhelming, as can the chemical and structural complexity of the simplest cell that we might visualize. The heart of the problem has been aptly stated at one level by Harold Blum[1] as follows:

". . . I do not see, for example, how proteins could have leapt suddenly into being. . . .

"The riddle seems to be: *How, when no life existed, did substances come into being which today are absolutely essential to living systems yet which can only be formed by those systems?* [italics Blum's]. It seems begging the question to suggest that the first protein molecules were formed by some more primitive 'non-protein living system', for it still remains to define and account for the origin of that system."

We can, again, hope to learn by honouring, however, in heuristic experiments the evolutionary principles of the stepwise development of complexity from simplicity and of the internal self-limitation of such development[2]. This philosophy is truly naturalistic in that it emulates the empirical approach which the evolutionist is required to impute to the primitive environment.

Although much of the knowledge of biochemical origins has been controversial, fairly general agreement now exists for the analytical outline of the problem of the origin of the first terrestrial organism (Table 1).

Table 1. SUBORDINATE PROBLEMS OF THE ORIGIN OF THE FIRST ORGANISM

(1) Production and proliferation of organic micromolecules from the simplest compounds.
(2) Spontaneous generation of macromolecules.
  (*a*) Protein.
  (*b*) Nucleic acid.
  (*c*) Polysaccharide.
(3) Self-organization of multimolecular systems (precellular forms).
  (*a*) Membranous properties.
  (*b*) Growth.
  (*c*) Division.
(4) Origin of metabolism.

This analysis is evolutionary in that it, in large measure, proceeds from simplicity to complexity. It also constitutes a gross outline of biochemistry. The sequence in the outline is based on a premise of the emergence of the first complex macromolecules prior to the origin of cells. Perhaps most interesting is the fact that almost entirely within the past decade an experimental model for each item in the outline has been brought forward. The problem is regarded by increasing numbers of theorists as solvable; the more favourable evaluations regard the problem in principle as more solved than unsolved.

The experimental demonstrations of the geological origin of biochemical substances indicate not necessarily what did happen, but what might have happened. The problem may now be viewed more as one that requires limiting of speculation by determining which model reactions examined in the laboratory are sequentially compatible, rather than as a problem of determining at least one way in which each key biochemical substance might have arisen spontaneously. Investigation of the variation in volcanic gases in eruption[3], for example, helps to emphasize the tremendous variety of possible spontaneous chemical reactions in the history of the Earth.

The intellectual climate and technical understanding which stimulated and permitted the experiments that have been recorded owed much of their basis to the detailed proposals of Oparin[4,5], Haldane[6], Bernal[7], Blum[1], Calvin[8], Urey[9], Wald[10], and others.

The first experiments expressly designed to explain the origin of organic compounds such as occur in living things were those of Calvin *et al.*[8], who converted carbon dioxide and water to formaldehyde and formic acid in the cyclotron. A primitive atmosphere having some carbon dioxide is in accord with the models of Rubey[11], Revelle[12] and Bernal[7]. Through the aldol condensation, one could visualize the formation of carbon–carbon bonds and a variety of organic compounds from formaldehyde. It is Calvin who deserves the credit for initiating the era of experimental chemical abiogenesis. Additional attention to experimental abiogenesis was stimulated by the Urey–Miller experiments, using electrical discharge in a 'primitive atmosphere'[13]. Miller obtained four amino-acids of the protein type and many other organic compounds[14,15].

As Miller pointed out[15], Löb[16] had earlier (1913) synthesized glycine by electric discharge in a mixture of carbon monoxide, ammonia and water. Many papers published in the era of early organic chemistry can, in fact, be re-interpreted in the more modern context of biochemical origins. Löb was actually close to such interpretation inasmuch as he proposed his synthesis as a model of reactions that could occur in Nature.

Apart from amino-acids, which could arise in many ways demonstrated in the laboratory[17], other micromolecules which have been synthesized in a presumably geological fashion are the monosaccharides[18,19] (especially deoxyribose[20]), urea[15], nitrogen bases[21,22] of nucleic acid, and the biochemically significant energy transfer substance, ATP (ref. 23). The production of guanine is noteworthy in that it arises during heating of amino-acids[24], a process which also leads to salient macromolecules.

In view of the attention which has been devoted to abiotic syntheses of amino-acids, the finding that most of the amino-acids common to protein are produced simultaneously from methane, ammonia and water at 1,000° C, in the volcanic range of temperature, may be especially pertinent[25].

Although amino-acids have resulted under many sets of conditions, indicating the possibility of their synthesis under primordial conditions, the synthesis from primitive gases has been investigated only recently. Dr. Harada has examined the conversion of ammonia, water and methane at volcanic temperatures to products hydrolysable to amino-acids[25]. He used vapour phase reactions of these gases through beds of silica gel, silica beach sand, a volcanic beach sand from Stromboli, and alumina gel. Except for the two kinds of silica, the results were quite different. Amino-acid formation was not observed at 800°, but it was evident at 900°. In each of many experiments, fourteen of the eighteen common amino-acids were identified. Two of the other four, histidine and tryptophan, will require special identification. Cystine and methionine could not be anticipated inasmuch as no form of sulphur was included in the reaction. This absence is part of the evidence that the amino-acids are not due to hydrolysates of microbial contaminants. No amino-acids of a non-protein variety have been found in such a reaction product. Examinations of this type have been indicated by the continuing development of the thermal theory[26]. At about the same time, the laboratories of Ponnamperuma and of Oró[1] had begun similar investigations[27].

The interest in explaining the origin of amino-acids, manifest in the fact that so many possibilities of this sort have been published[17], stems from the need for understanding the origin of protein. This emphasis comports with Blum's 1955 analysis of the overall problem of origins. The approach which has developed in our laboratory was based on a back-extrapolation. The extrapolation was from data accumulated during investigations of Darwinian evolution at the molecular level, with proteins[28].

The systematics of proteins can be examined in a number of ways[28]. One of these is through the perspective of the quantitative contents of unfractionated proteins of organisms throughout phylogeny (Fig. 1). The chart shown[29] reaffirms in a quantitative way the principle of the 'unity of biochemistry'[30].

The other emphasis derivable from this investigation is that of the relatively high proportion of two amino-acids, the dicarboxylic amino-acids, aspartic acid and glutamic acid. This fact suggested the possibility that the generation of primordial protein relied on a relatively large proportion of the dicarboxylic amino-acids, a dominance which might persist in the record of molecular evolution in cells.

Focusing on the origin of proteins at that stage in the development of the theory of abiogenesis differed from investigations of the origin of amino-acids and of other micromolecules. We had been influenced in our thinking by the chemists' typical assumption that, whereas simple compounds require simple syntheses, complex compounds, for example, proteins, require complex processes. What now seems to be a more logical premise is that Nature would not have been able to accommodate a process as intricate as, for example, a carbobenzoxy synthesis and would have used instead some internally self-organizing simple process such as an appropriate type of

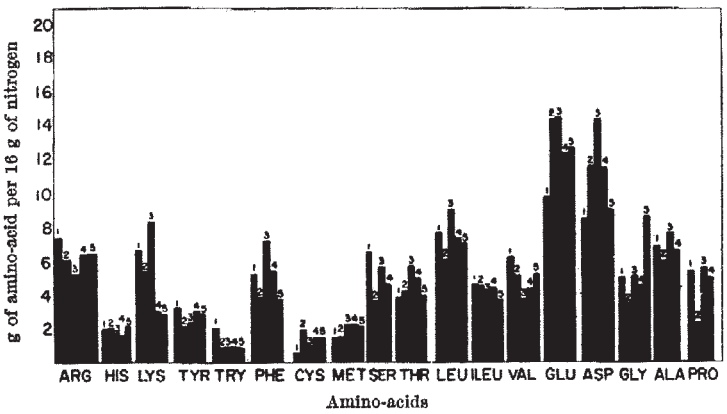



Fig. 1. Amino-acid compositions of unfractionated proteins of different organisms: (1) algae; (2) bacteria; (3) protozoa; (4) invertebrates; (5) mammals

polymerization. An additional reason for polymerizing amino-acids rather than analogues or derivatives is based on a recapitulationist premise[26,31,32]. This emphasis led to experiments different from those used as other models of formation of peptide bonds in the primitive world[33,34].

The effort to use the presumed clue furnished by the moderately high aspartic acid and glutamic acid contents was based on heating in the dry state, for thermodynamic and other reasons[35]. When heat was used, a second scientific heresy was committed. The literature reveals that heating α-amino-acids above the boiling-point of water typically yields diketopiperazines, amines and pyrolytic tars[36]. If one heats the amino-acids in the initially dry state and heeds the admonition from the evolutionary analyses, lightly coloured polymers having peptide bonds are, however, produced. These polymers can then be further purified by procedures which are standard for protein fractionation either by salting out from aqueous solution by ammonium sulphate[37] or on ion-exchange columns[38]. In order to obtain workable quantities of such polymers, at least one mole of dicarboxylic amino-acid per two moles of total neutral and basic amino-acid should be used.

An ultimate objective of these experiments was the simultaneous combination of all or nearly all the amino-acids common to contemporary protein. Comparative examinations suggest that the primordial reactions must have embraced a maximum number of types of amino-acid. This inference is consistent with the fact that 'primitive' organisms contain all the contemporary amino-acids plus diaminopimelic acid[39], and the genetic and biochemical reasoning which leads to the inference that a maximal variety of organic compounds was present in the primordial reaction[40]. Any method which would combine all the 18–20 common amino-acids could be expected easily to combine a smaller number. In addition, inclusion of all the eighteen amino-acids would provide a heteropolymer which, if it had a sufficient number of activities, might constitute (subject to definition) a synthetic general protein.

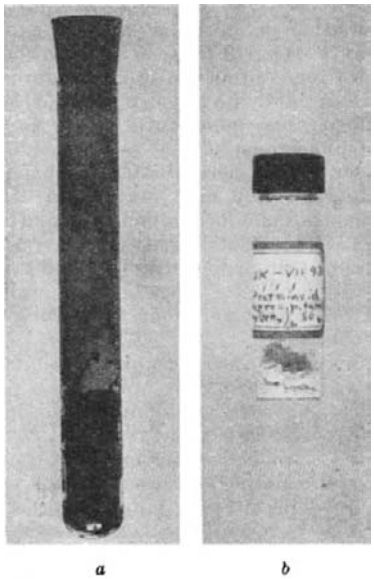
Many man-years of effort were, however, expended in systematic investigation of the thermal copolymerization of two or three amino-acids[41,42]. When the essential principle of thermal copolymerization was tested simultaneously on all the eighteen amino-acids common to protein[43], it was found to apply to those circumstances also (Fig. 2). Some of each of the amino-acids was found in the polymer. This result was somewhat surprising against the background of knowledge that nine or ten of the amino-acids have reactive side-chains. The visualization of irreversible reactions of such side-chains was easy. However, thermal proteinoids which have undergone moderate purification can be hydrolysed by mineral acid and the contained amino-acids can be recovered quantitatively[44]. The conditions of the reaction, therefore, are not brutal, except for substantial destruction of serine and threonine; this is, however, largely controllable by use of a reducing mixture of hypophosphite and polyphosphoric acid[45]. The thermal copolymerizations and many of the analyses of the products have been repeated in other laboratories. These polymers were at the outset found to have, besides a complete qualitative roster of amino-acids, many of the properties of proteins. They are referred to as proteinoids, a term which originally indicated their similarity to proteins at the same time that it disavowed a claim of identity with contemporary protein. The possibility that the identity is great enough to allow regarding proteinoid as synthetic general protein is, however, in purview.

Typical reaction conditions are 170° C for 6 h. A 1 : 1 : 1-proteinoid, an example of one type of such polymer, is made from one part of aspartic acid, one part of glutamic acid, and one part of a mixture of the sixteen other amino-acids present in that part in equimolar proportions. As has been documented, temperatures of 170° C are terrestrially quite common[46], yet the minimum temperature for polymerization within hours can be lowered to 65° by addition of appropriate phosphates to the reaction mixture. Yields of proteinoids, depending on conditions of reaction and degree of purification of product, typically range between 5 and 40 per cent. The higher yields are obtained with phosphates in the reaction mixture.

These conditions are closely similar to those reported from Schramm's laboratory and identical with ours for the polymerization of mononucleotides[47]. The fact that products as complex as proteinoids could be produced in a simple, and therefore geologically plausible, way suggested that polymers resembling polynucleotides might also be produced by simple heating. The characterization of thermal polynucleotides is, however, as yet, far less complete than that of the proteinoids.

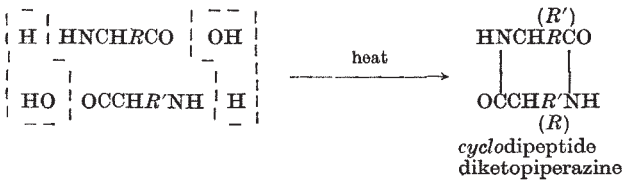
In order to understand the essential processes which occur when amino-acids are heated, some of the special chemistry deserves examination.

The neutral amino-acids tend to undergo a head-and-tail condensation of two molecules to give a *cyclo*dipeptide or diketopiperazine:



*a* *b*

Fig. 2. *a*, Amino-acids heated above the boiling-point of water; *b*, ditto, with sufficient aspartic acid and glutamic acid, followed by purification by salting out

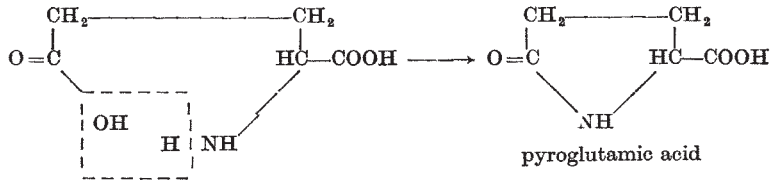



They also are decarboxylated to leave amines:

$$H_2NCHRCOOH \longrightarrow H_2NCH_2R + CO_2\uparrow$$

and more profound decomposition results in the formation of tar, as already noted.

Glutamic acid, however, undergoes an internal condensation in the single molecule to yield a lactam, pyroglutamic acid (α-pyrrolidonecarboxylic acid).




When glutamic acid and a neutral amino-acid are heated simultaneously, they behave together in a way that is different from their individual behaviours.

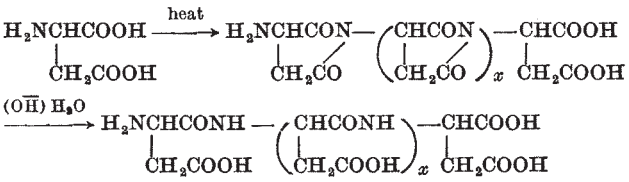
Glutamic acid $\xrightarrow{\text{heat}}$ Pyroglutamic acid

Neutral amino-acid $\xrightarrow{\text{heat}}$ Diketopiperazine, amine, tar

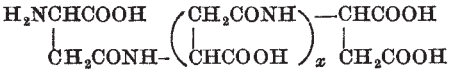
Glutamic acid + neutral amino-acid ⟶ Poly-α-amino-acids + by-products

The concerted heating yields unwanted products also, but it also yields genuine peptide-type poly-α-amino-acids, often as the major product with little discoloration or contamination by tar.

Aspartic acid participates in copolymerization in a manner which is different from that of glutamic acid. Aspartic acid yields a polymer which is a polyimide:



The residues may in part be β-linked as indicated:



for either or both the interior and N-terminal residues.

The polymerization of aspartic acid also permits bringing into polymeric combination amino-acids which would themselves undergo decomposition.

Aspartic acid $\xrightarrow{\text{heat}}$ Polyanhydroaspartic acid

Neutral amino-acid $\xrightarrow{\text{heat}}$ Diketopiperazines, amine, tar

Aspartic acid + neutral amino-acid ⟶ Copoly(anhydro-aspartic acid, neutral amino-acid) (*A*)

*A* + $O\bar{H}$ (cold $H_2O$) ⟶ Copoly (aspartic acid, neutral amino-acid)

Most of the aspartic acid residues are in the imide form in (*A*). The hydrolysed *A* is the true peptide type of polyamino-acid instead of the imide type.

The basic amino-acids do not decompose as do neutral amino-acids; they also undergo thermal copolymerization. As stated before, all the amino-acids, including both dicarboxylic amino-acids and the three basic amino-acids, and the thirteen other α-amino-acids common to protein, can be simultaneously copolymerized thermally. When the purified thermal proteinoid is hydrolysed, quantitative recovery of the amino-acids which it contains is recorded, and one must conclude that irreversible reaction of functional side-chains cannot have occurred in a major portion of the raw polymer.

The basic amino-acids, especially lysine, are moreover capable of substituting for the acidic amino-acids in thermal polymerization[48]. Lysine can also be used for simultaneous thermal copolymerization of all the amino-acids to yield a basic lysine proteinoid[49].

These results emphasize that the effect of simultaneous heating of amino-acids could not be predicted from homopolymerization or attempted homopolymerization. This difference between homopolymerization and copolymerization is consistent with the general experience of the polymer chemist. The polymer chemist, however, has not dealt with reactions involving eighteen monomers simultaneously. Accordingly, no precedent existed for the results of the present investigation. One may on this basis more easily understand a frequently observed first reaction of many chemists that such polymerizations can scarcely be reproducible or controllable, and that the polymers produced must be random in Nature.

Fig. 3 helps to answer this question. In Fig. 3 are seen three chromatograms, from the automatic amino-acid analyser, of three separate syntheses of proteinoids. These are three 2 : 2 : 1-proteinoids which differ in their synthesis and purification in one respect only. In *b*, the proportion of phenylalanine was the usual figure. In the experiment of *a*, all phenylalanine was omitted. In the experiment of *c*, the proportion of phenylalanine in the reaction mixture was 3·5 times that in *b*. Comparison of *a*, *b* and *c* reveals that the proportion of each amino-acid other than that of phenylalanine is quite constant. The thermal condensations of amino-acids are thus highly reproducible, as observed in many ways in other experiments. The proportion of phenylalanine is higher in the product from the reaction mixture containing a higher proportion of phenylalanine. Other experiments have shown that the proportion of an individual amino-acid in an acid proteinoid is regularly relatable to its proportion in the reaction mixture. The thermal polymerizations are thus quite controllable as well as reproducible.

The pair of proteinoids which differ by the absence of one amino-acid offer opportunities for investigation in protein nutrition. Some of the early nutritional biochemists such as Abderhalden and Mendel sought proteins lacking individual amino-acids so that they might examine the effect of individual amino-acids in protein nutrition. In this search they were doomed to failure because of the absence of such proteins in Nature. Instead, much of what we know we owe to W. C. Rose, who obtained the desired type of information by feeding first rats, then humans, with mixtures of pure amino-acids with systematic omissions. As Rose pointed out, such answers are imperfect in that caloric imbalances attend the feeding of amino-acids instead of protein[50]. In true protein nutrition, furthermore, amino-acids are gradually released from peptide bonds. In this respect the thermal proteinoids can function as synthetic proteins from which single amino-acids can systematically be omitted qualitatively and quantitatively. The question of nutritional availability of amino-acids from thermal polyamino-acids has been affirmatively answered for bacteria[37], and for rats by Krampitz and Knappen[51].

The question of molecular weights has been examined by end-group assay and by some analyses in the ultracentrifuge. Mean molecular weights of 3,000–10,000 have been recorded[37] for the acid proteinoids. These values may be compared with 6,000 for a small protein molecule, insulin. Since insulin has two end-groups, the figure for comparison is 3,000. Most proteins are considerably larger than 6,000, however; the molecular weights of the

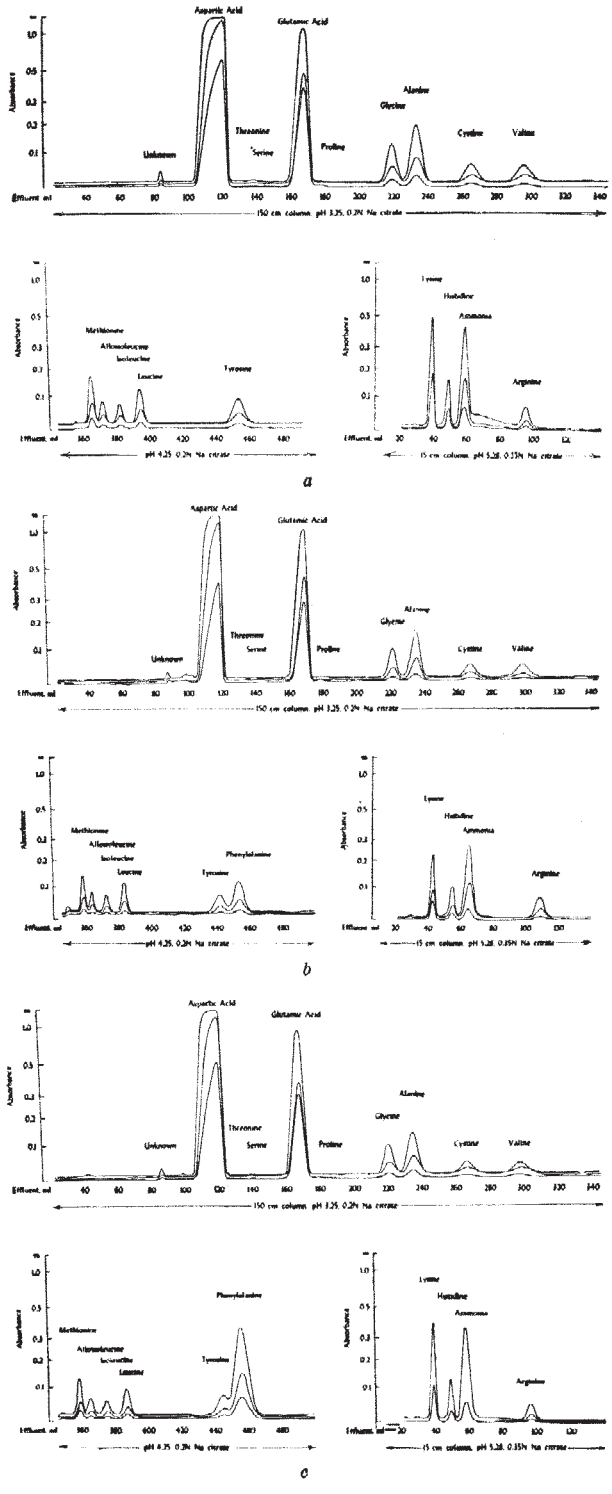



Fig. 3. Chromatograms of hydrolysates of proteinoids, *a*, 2 : 2 : 1-proteinoid reaction mixture lacked phenylalanine; *b*, standard 2 : 2 : 1-proteinoid; *c*, same as *b* with 3·5 times as much phenylalanine in the reaction mixture

proteinoids are thus in the lower end of the range of molecular weights of proteins. Cross-linking within the proteinoids is limited[19].

A rigorous comparison of proteinoids with proteins cannot be carried out, because, like life itself, proteins are not rigorously defined nor rigorously definable. The variable nature of proteins precludes the possibility of a rigorous definition of those materials. Two descriptive definitions of proteins as a group can, however, be cited[52,53]. The requirements of these definitions are met by the proteinoids as a class. The first of these requirements are the qualitative content of amino-acids and the molecular weights. In determining to what degree the complexities of protein molecules are mimicked by thermal proteinoids, many characteristics have been examined. These are outlined in Table 2. Missing from proteinoids as yet are the properties of antigenicity and helicity. They have not been assiduously sought in the proteinoids, however, nor are they universal properties of proteins. These properties are also found in synthetic polyamino-acids produced from Leuchs anhydrides[54].

Table 2. Properties and Interpretations Associated with Thermal Polyamino-acids and Proteins

Composition, qualitative
Molecular weight range
Colour tests
Inclusion of non-amino-acid groups
Solubility ranges
Salting-in and salting-out properties
Precipitability by protein reagents
Composition, quantitative
Intramolecular bonds
- Peptide (imide)
  - Biuret
  - *I.R.* maxima (1,550, 1,650, 3,080, 3,300 cm$^{-1}$) (1,720, 1,780 cm$^{-1}$ for imide)
  - Release of amino-acids by HCl hydrolysis
  - Proteolysability
- Disulphide
  - By oxidizability to cysteic acid

Non-random arrangements of amino-acid residues
- Terminal residues and total composition
- Sequences in fragments

Susceptibility to proteolytic enzymes
Nutritive quality
Catalytic activity
Morphogenicity

The property of non-randomness is investigated most meaningfully in an unfractionated polymer. While we are working towards determining an entire sequence, this kind of work needs to be done on homogeneous preparations. The sequence of residues in a homogeneous preparation cannot provide information on variation in sequence or composition such as had been determined in the experiments just described. The fact of non-random arrangements (repeatedly indicated by comparisons of total compositions, N-terminal and C-terminal compositions) betokens a measure of order in the synthesis. This order must be determined by the amino-acids themselves since almost no other material is present. Such a finding is of evolutionary significance when viewed against the

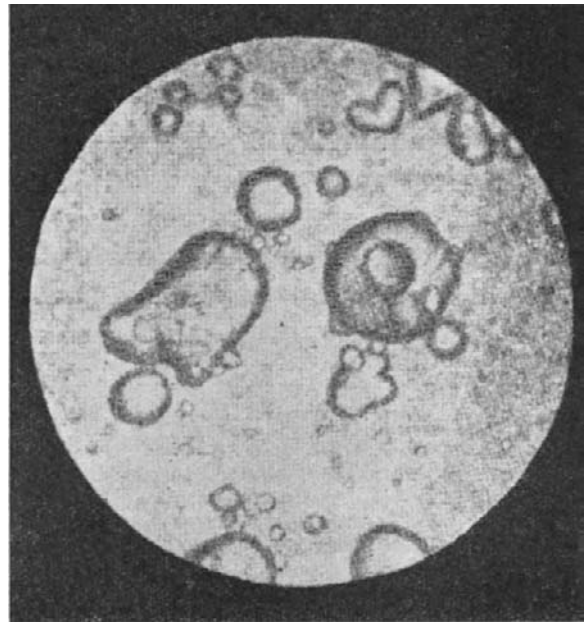


Fig. 4. Coacervate droplets from gelatin, gum arabic and ribonucleic acid (after Oparin). (× 240)

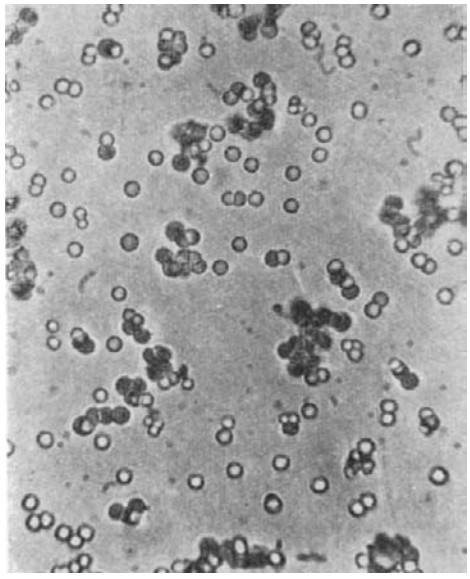


Fig. 5. Proteinoid microspheres prepared by allowing hot solution of proteinoid to cool. These units are approximately 2$\mu$ in diameter

background of recognizable order in organisms. The thermal proteinoids, as a model of abiotic polymer, demonstrate how the beginning of order could have developed at a molecular level in a pre-biological period. Since no genic nucleic acid is present in the reaction, the degree of ordering that is found is ascribed to the internal influences of the amino-acids themselves. Such an effect can be understood in terms of the specific shapes and charge distribution of each of the eighteen types of amino-acid. The concept that order could have been partly specified by the primitive protein itself is substantiated by investigations of Pattee[55], based on computer theory. The principle of internal self-direction is fundamental to general evolutionary theory[2].

The conceptual possibility that pre-biological protein molecules might have arisen before pre-biological nucleic acids can be more seriously entertained in the light of the demonstration of internal self-control of sequence. Three properties which, in pre-vital protein, would be of first significance to organisms that might evolve therefrom are: service as proteinaceous food, catalytic activity and any tendency to form microstructures having boundaries with membranous properties. The first of these activities, in proteinoids, has been mentioned.

Catalytic activity in proteinoids was first observed for the unnatural substrate, *p*-nitrophenyl acetate[49]. Rohlfing[56] has shown that the relatively high activity observed is due to the simultaneous presence in the proteinoid of histidine residues and of aspartimide linkages. As the imide linkage is progressively opened (by mild treatment), the catalytic activity is also progressively decreased. The imide type of polyaspartic acid, without histidine, is devoid of activity.

A second type of catalytic activity which has been observed is found in proteinoids which have been suitably reacted with zinc. Inorganic salts of zinc are known to catalyse the hydrolysis of ATP at 80° (ref. 57). When zinc hydroxide gel is reacted with acid proteinoid, the resultant zinc-proteinoid is active in the hydrolysis of ATP at 40°. This effect, in conjunction with organized units, is later described more fully. Activity by combination with zinc can be viewed as an experimental model of a first step in the conceptual evolution of a metal-enzyme[58].

A third type of catalytic activity is that which has been shown to enhance the decomposition of glucose to glucuronic acid, carbon dioxide, and other products[59]. Although this is a weak activity, no more than weak activity was needed in the first proteins. Furthermore, among the numerous activities now being catalogued for the proteinoids, some are considerably stronger. The essential feature is the occurrence in macromolecules of significant catalytic activity such as might launch a primordial, biochemically active, cell.

To this point, one can in any event visualize, on a basis of sequentially compatible reactions demonstrated in the laboratory, the thermal conversion of amino-acids to a primitive kind of protein.

In a coherent theory of origins, the manner in which primitive protein might modulate to primitive cells is one of the next questions.

The kind of experiment to which the biochemist, A. I. Oparin, has devoted major effort is that of the multimolecular systems, or, more interpretatively, models of precellular organization. Oparin is properly credited with promulgating a natural origin of biochemical systems through organic chemistry[4,5] at a time when naturalistic explanations were not available. Equal credit is deserved by Oparin for his emphasis on the need to understand self-organizing phenomena yielding the first cells. George Wald[10], and others, have also added explicitly to the theoretical underpinnings.

Oparin's experiments have used as a model of precellular organization the coacervate droplet introduced by the colloid chemist, Bungenberg de Jong[60]. Some of these are illustrated in Fig. 4. They can be seen to be microscopic, and not uniform. Oparin has concentrated enzymes and substrates within coacervate droplets. The presence of organization allows reactions to proceed in a concentrated fashion, apart from the environment. The value of packaged reactions in a unit which has separated from the pre-environment is that such a unit could then evolve metabolically along with the development of internally associated reactions.

Oparin has pointed out one defect of this model—the lack of stability. The coacervate droplet made from gelatin and gum arabic cannot withstand gentle centrifugation or some concentrations of salts without breaking down into two liquids. As Oparin has stated, the coacervate droplet would in an evolutionary way have to develop stability before it could function as a valid model of the late stages of precellular organization[61]. Another defect as a precellular model is the reliance for structural purposes on protein of recent origin such as gelatin.

From the thermal polyamino-acids, including thermal proteinoid, an alternative model emerges (Fig. 5). This model differs crucially from typical coacervate droplets and from other cell models[62], in that the structural material is not a polymer from contemporary organisms. The material is instead a protein-like polymer which arose from simpler units, amino-acids, which can be formed and combined, as has been shown, by a kind of experimental geosynthesis. Another significant difference resides in the fact that the formed units have stability comparable to that of many contemporary cells. They withstand centrifugation in a clinical centrifuge. As first shown by Dr. Richard S. Young[63], they are stable enough to be sectioned, allowing electron microscopy[64], as represented in some of the photographs in this article.

The manner in which the proteinoid microspheres arise is yet simpler than that by which the polymer emerges. Water or various aqueous solutions are added to the hot polymer mixture, the hot clear solution is decanted and, following a few minutes of cooling, vast numbers of individual microspheres are seen to separate. It is possible also to use cold purified proteinoid by heating the suspension produced when water or aqueous solution is added to the solid. In some preparations, the propensity to form microspheres is so great that the units appear on mere contact of cold water with cold proteinoid.

The size is microscopic, those in Fig. 5 being slightly less than 2$\mu$ in diameter. This is then the size and shape

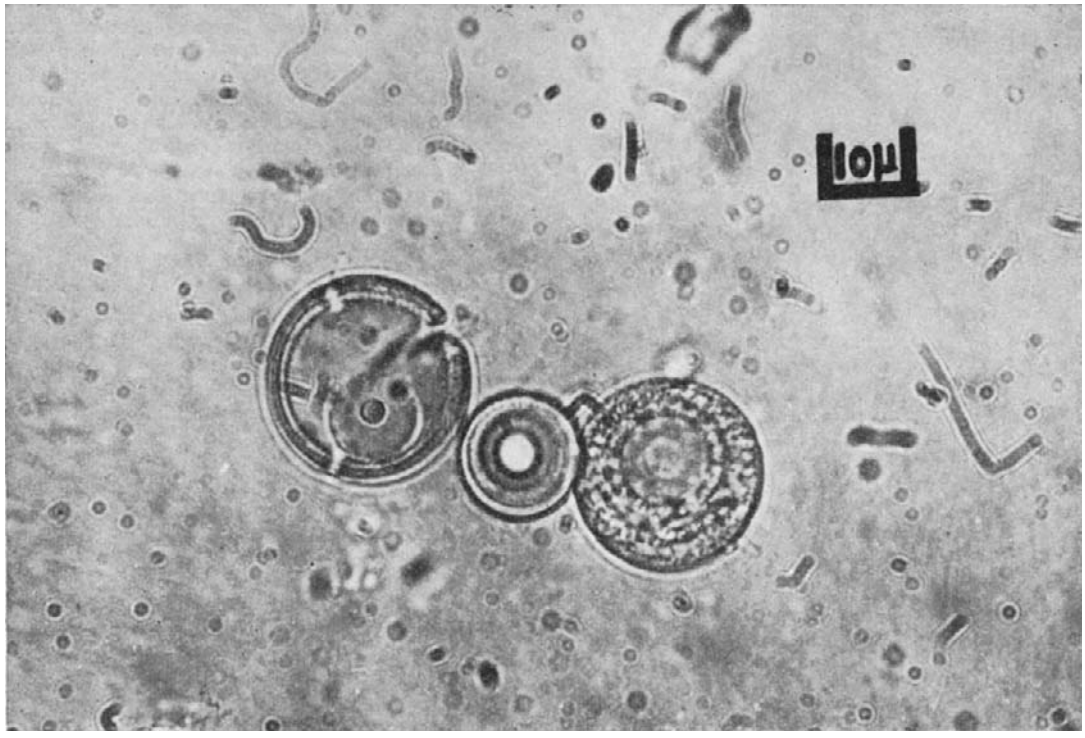


Fig. 6. Optical micrograph of microspheres. Fine details of structure may be noted

of the coccoid bacteria, which have been regarded by Kluyver and van Niel, and Lipmann[65], as the most primitive of the bacteria.

As can be seen in Fig. 5, the spherules can be produced in a uniform size. This picture, however, demonstrates some of the variations in shape. The uniformity which is obtainable permits quantitative experiments. When transferred to solutions hypertonic or hypotonic to those in which they were made, the microspheres shrink or swell correspondingly[66]. This kind of behaviour is manifest at concentrations of salt much higher than those necessary for the demonstration of osmotic effects in true cells. What is seen, however, may be the basis for a kind of primitive osmometer[67].

Another figure of interest is the simulated budding. In addition, 10 mg of proteinoid yields more than $10^7$ microspheres. In the context of natural experiments, the appearance of this numerousness emphasizes, in addition to the number of experiments in vast numbers of individual locales, a very large number of experimental individuals per experiment. A single terrestrial experiment on a geological scale would be much larger than what can occur with 10 mg of thermal polymer.

Fig. 6 shows some larger microspheres, in which more anatomical detail is apparent than in Fig. 5. What looks like concentric shells, or a kind of double layer, may also be seen. One cannot be sure of this observation inasmuch as it is the result of optical microscopy. Apparent also are some filamentous particles. These are not lint or other result of unclean microscopy. They are associations of microspheres from a second population of smaller units, which are barely visible. These latter are quite uniform in size; their diameter is the same as that of the filaments. The filaments, on close examination, are seen to be segmented associations of the small microspheres. These structures, like the 'buds' and microspheres, are provocative in the context of biomorphology. Only the microspheres have so far been extensively investigated.

Knowledge of the composition of the proteinoid has permitted using them as a model unit in investigation of cytochemical staining. In 1924, Stearn and Stearn attributed the Gram stain of bacteria to the acceptance of the stain by structural protein of the bacterial cell[68]. Because of the protein-like nature of the material composing the microspheres, the latter were tested for their acceptance of the Gram stain. The possibility that they could be used also for explaining the difference between Gram-negativeness and Gram-positiveness also suggested itself. Of the many theories of this difference, the recent concept that the difference depends on the proportion of basic biopolymer invited attention[69].

The first finding was that, consistent with the stipulation of Stearn and Stearn, the typical microspheres of proteinoid accept the Gram stain; they are Gram-negative. To test the possibility that basic polymer might influence the sign of the stain, the lysine proteinoid

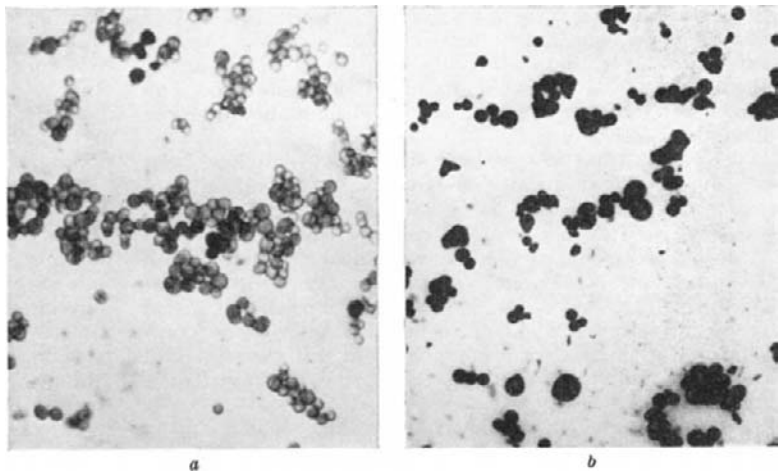
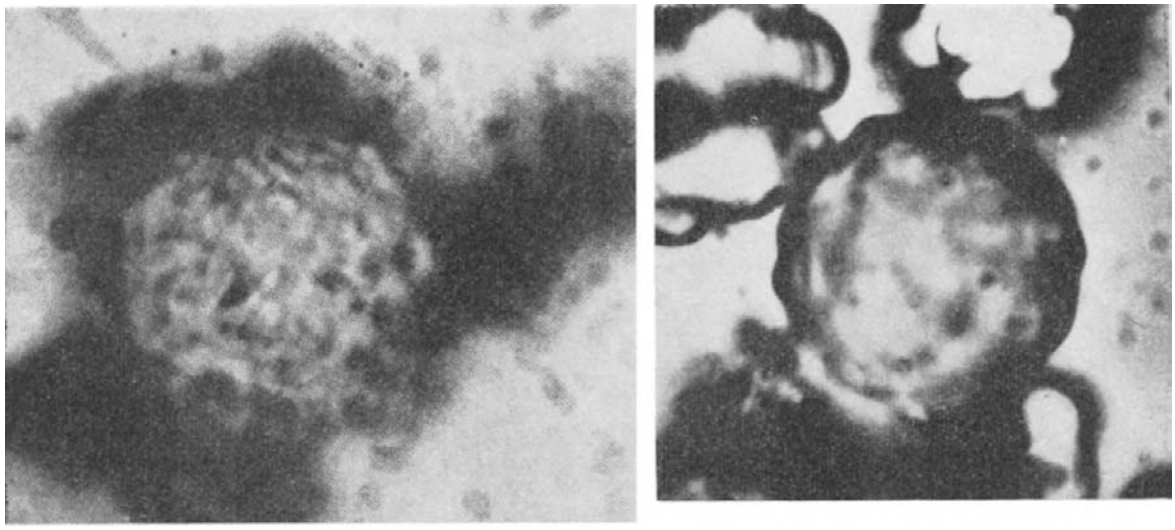
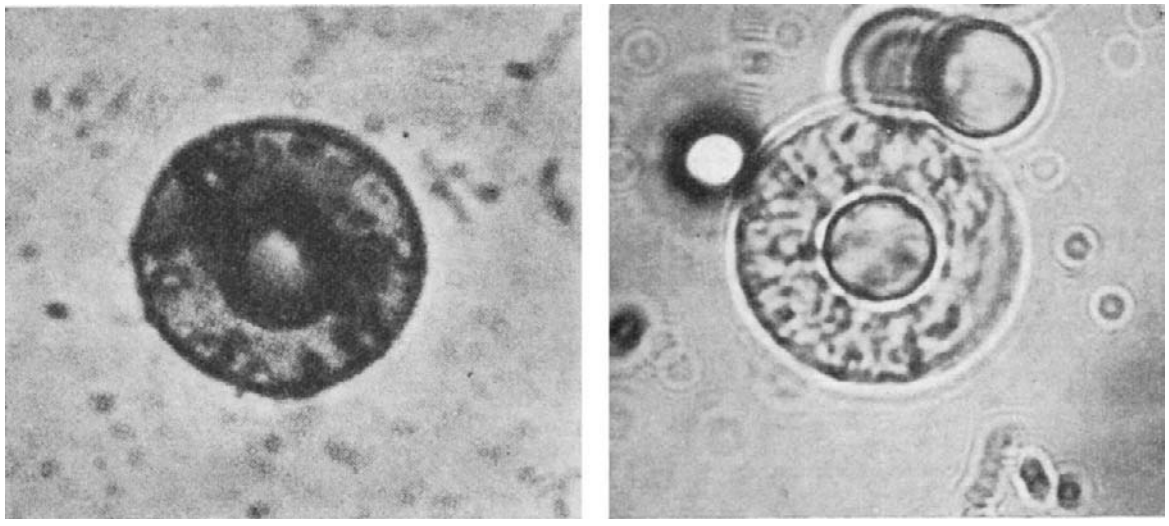


Fig. 7. *a*, Gram-negative microspheres; *b*, Gram-positive microspheres



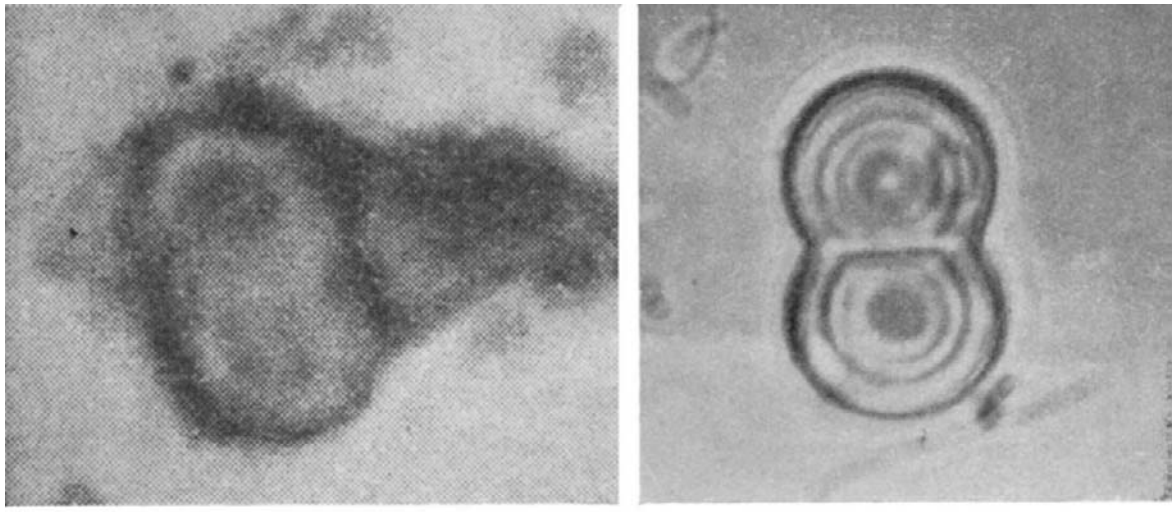
*a* *b*

Fig. 8. *a*, 'Life-like' microparticle from Orgueil meteorite (Claus, G., Nagy, B., and Europa, D. L., *Ann. N.Y. Acad. Sci.*, **108**, 580; 1963); *b*, microparticle of thermal proteinoid from this laboratory



*a* *b*

Fig. 9. *a*, As in Fig. 8*a*; *b*, as in Fig. 8*b*



*a* *b*

Fig. 10. *a*, 'Life-like' form undergoing 'cell division', from Orgueil meteorite. (Claus, G., and Nagy, B., *Nature*, **192**, 594; 1961); *b*, as in Fig. 8*b*, *p*H has been raised moderately. All microparticles are roughly in same range of size

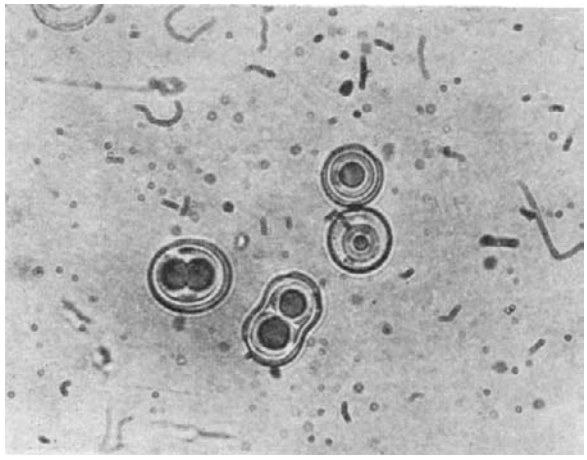


Fig. 11. Twinned proteinoid microspheres observed in suspension in which *p*H has been raised

mentioned earlier was used. Lysine proteinoid does not form microspheres in the manner that the acid proteinoid does. Mixture of lysine proteinoid and acid proteinoid containing less than 60 per cent of the former polymer will form microspheres which contain the latter. If the proportion of lysine proteinoid in the mixture exceeds 35 per cent, the microspheres stain Gram-positive (Fig. 7). Although this use of the model does not rule out other explanations, further consideration of the Gram stain must take such results into account, particularly since the results in the model are not subject to confusion by a number of other metabolites. The solubility of the Gram-negative microspheres in dilute alkali and the virtual insolubility of the Gram-positive type furthermore parallel quite exactly the solubilities of the Gram-negative and Gram-positive bacteria[69], respectively.

The morphologies of the various microparticles, inadvertently obtained, bear remarkable resemblances to almost all the meteoritic 'formed elements'[70,71] which have been published. Comparisons of three of these are shown in Figs. 8–10.

In Fig. 10 can be seen a formed element undergoing 'cell division' while it is being constricted at the middle, as pointed out by Claus and Nagy. On the right is a microparticle from a laboratory experiment. The same features are brought out more clearly. In this case, the microspheres originally formed have been treated under the coverglass with a few drops of buffer of *p*H 5·5–6·5, the original *p*H being approximately 3·0. Evidently some redistribution of charge is responsible for the re-arrangement of structure.

These comparisons pose the possibility that the 'formed elements' were at no time alive, rather that they were natural physicochemical experiments which terminated before life emerged[72]. If so, they are of profound interest in a context of molecular evolution.

These similarities also are consistent with the inference that the laboratory experiments are closely akin to natural experiments. The thermal gradients and moisture which are present in the carbonaceous chondrites during their trips into the Earth's atmosphere[73] would provide the necessary conditions. Of related interest is the inference by Kaplan *et al.*[74] that the types of carbon compounds which occur in organisms may be formed in meteorites.

One of the clearest pictures showing microspheres simulating cells in cleavage is that of Fig. 11. This picture has been mistaken often enough by biologists to be a field of dividing bacteria that the question has properly been asked if the view is not indeed one of contaminating bacteria in the process of division. That the particles in such views are synthetic is shown by the fact that they arise in periods of less than 2 h, and the fact that they can be redissolved by warming the microscope slide. In this picture may also be seen the small microspheres, and the filamentous associations thereof. A particularly provocative question in this case is the one of whether the particles which appear to have cleaved are the products of fusion or fission.

This question has been answered for some years as a process of fission. The first documented experiment is presented in the frames from a time-lapse sequence reproduced in Fig. 12. The change between the second and third frames in the mount clearly shows the formation of a septum and of two centres. This kind of cleavage simulates the type of division observed in the septate cocci[75].

This time-lapse sequence also illustrates other phenomena. When the initial solution of proteinoid is heated for many minutes instead of the usual few seconds, the particles are not uniform; hence, the large microsphere near the centre of the frames. The sequence of photomicrographs shows the progressive disappearance of the centre of the larger microsphere. In many experiments this centre has disappeared entirely but the boundary

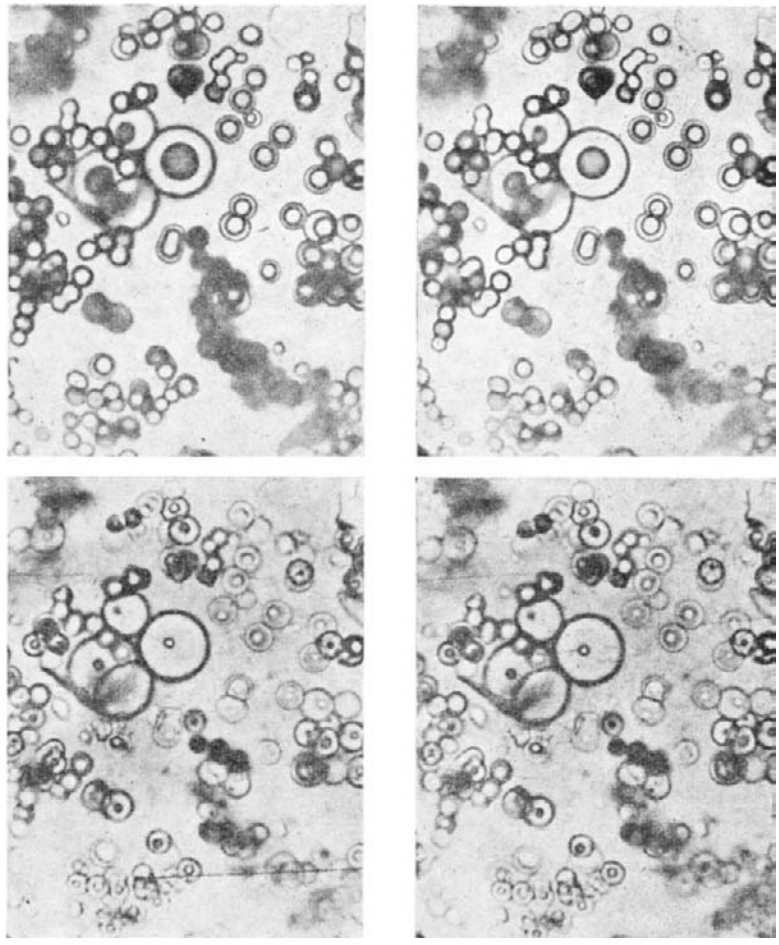


Fig. 12. Frames from a time-lapse series showing cleavage

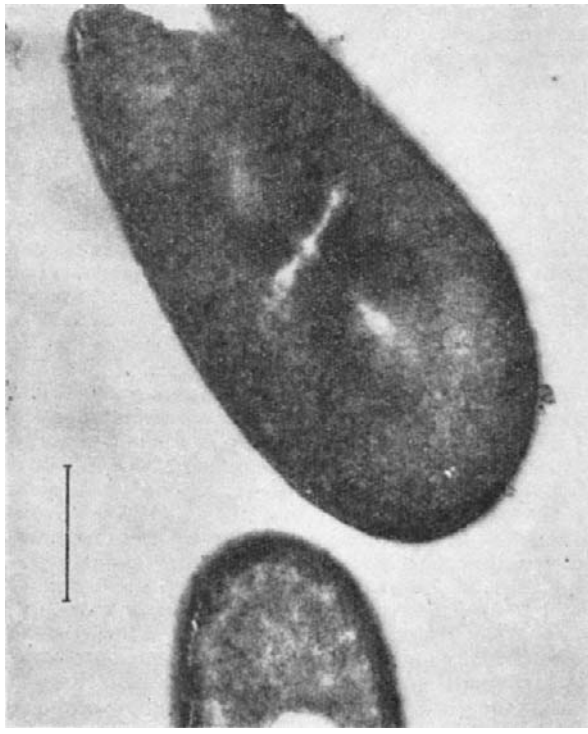


Fig. 13. Electron micrograph of proteinoid microsphere which has been stained with osmic acid, and embedded in a methacrylate block which was cured and sectioned

remains. Such a phenomenon poses a provocative chemical question of the difference between the visually unchanged boundary and the interior of the unit. Analyses have as yet shown no difference in amino-acid composition.

This behaviour is part of the evidence indicating that the kind of selectivity necessary for a primitive membrane is a property of these units[75]. The boundary appears to remain unchanged through the experiment, yet the interior, made up also of proteinoid, passes out through that boundary. The absence of material in the interior while the boundary persists is corroborated by some of the electron micrographs.

The first of the electron micrographs (Fig. 13) shows a single microsphere which has been stained with osmic acid, trapped in methacrylate which was cured, and sliced by microtome[76]. The sections in this treatment tend to be 800 Å thick. Although some bacteria reveal more structure than this to the electron microscope, some do not. A section of *Bacillus cereus* micrographed and reproduced in the treatise on *The Bacteria* is comparable[77].

Fig. 14 shows five microspheres caught in a suspension[76]. In this case, the *p*H has been raised. Four of these illustrate various stages in the outward diffusion of the polymer from the interior. Several of them show very clearly a boundary.

Fig. 15 depicts more microspheres subjected to raised *p*H. Two fields are placed side by side here to show a progression in one phenomenon. This is the double layer, analogous to the double membrane of true cells[78–83]. This phenomenon had not been sought. If, however, a model of cellular origins had some validity, unsought features should occasionally appear. Recent thinking has emphasized the presence of phospholipid between the protein layers of a double membrane[84]. In this case, comparable chemical character might be attributed to the hydrocarbon side-chains of the amino-acid residues in the proteinoid. The double layer has been known to retain its structure after extraction of the lipid[79], and recently Green *et al.*[85] have reported that the layering within mitochondria is undisturbed when the lipids are extracted from the mitochondria by aqueous acetone; they have identified 'structural protein'. This kind of emphasis is consistent with the finding of double layering from proteinoid alone. These results and those with the Gram stain suggest that the emphasis in the introduction on experimenting with the conceptually simplest natural experiments can be extended. Such simple experiments can help to control investigations of complex phenomena in living cells when fewer than all the associated natural materials or processes in the living cell can be identified as pertinent.

Attempts to produce metabolizing microspheres have focused on introducing catalytically active cations, a conceptual possibility in view of the chemical structure of the proteinoids. Investigations have narrowed to zinc, which has been known to catalyse the hydrolysis of ATP in aqueous solution at 80° (ref. 57). Following many trials, zinc was introduced by reacting zinc hydroxide gel with proteinoid in suitable ratios in hot aqueous solution for 4 min. The hot clear solution deposits microspheres containing typically 1–3 per cent of zinc. The first two aqueous wash liquids show some activity, but the third through fifth washes reveal scarcely more activity than is found in the ATP spontaneous hydrolysis control, signifying the removal of zinc adhering to the microspheres (Fig. 16). The zinc-containing microspheres retain, however, substantial ATP-splitting activity. Cellular phosphatases often contain magnesium, but some of them contain zinc[86]. The level of activity in the microspheres is undoubtedly much weaker than that in a natural adenosine-triphosphatase. As Calvin has pointed out theoretically for iron-containing enzymes, weak activity would have been sufficient in the early stages of life[58]; evolution would have selected the more powerful enzymes that we find in contemporary cells.

With ATP-splitting activity localized in this fashion, visualization is possible of how a thermal synthesis of primitive protein might have modulated to the first steps towards a primitive ATP-dependent cellular synthesis of protein. Regardless of how much like the proteins the thermal proteinoids may be, the mechanisms of synthesis are energetically different. If thermal synthesis led to

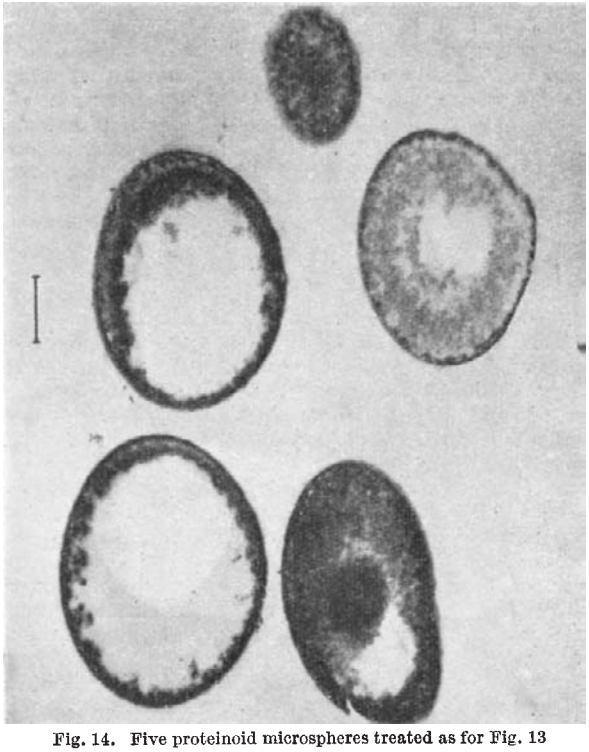


Fig. 14. Five proteinoid microspheres treated as for Fig. 13

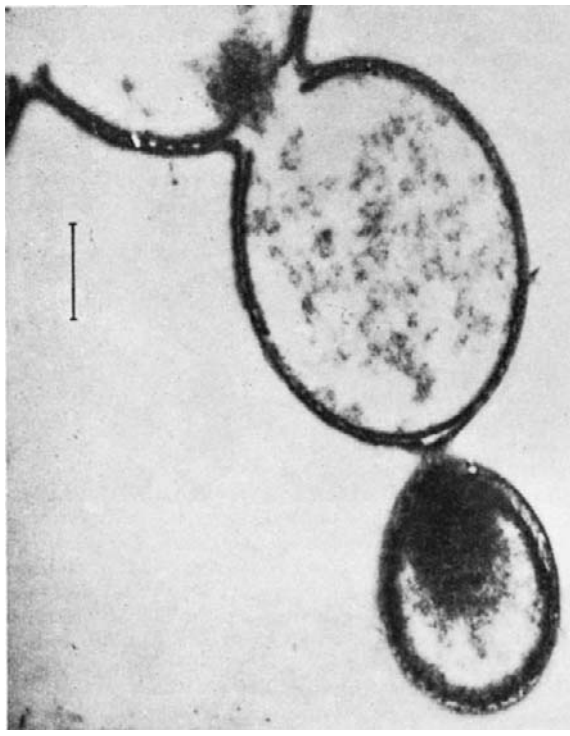


Fig. 15. Double layers in sections of proteinoid microspheres. This electron micrograph confirms optical evidence of Fig. 6

pre-protein, some modulation to a cellular type of synthesis was necessary.

The simulation of so many pre-biochemical steps by heat calls to mind the fact that the organic chemist in his laboratory usually finds heat to be most convenient for carrying out his reactions. Fundamentally, a principal reason is that many organic reactions are energized by volatilization of product water. The same explanation pertains to spontaneous geosynthesis. What then are suitable thermal locales on the Earth?

Many suitable locales for spontaneous thermal geosynthesis can be proposed at present[46,87] and geological theory suggests that this number may have been even greater in earlier times[88]. One can visualize that amino-acids might form directly from intermediates produced in vapour phase reaction in a volcanic region, according to the conditions specified by Dr. Harada's experiments. Enough water would have to enter the scene to promote hydrolysis of the amino-acid intermediates. Frequent hydrolysis and evaporation require no conceptual strain if one thinks of the frequency of rain in volcanic regions, as elsewhere. A deposit of amino-acids in a region of 100–200° would promptly yield pre-protein-like polymers to the extent that aspartic acid[67] and glutamic acid were present. A second possibility is that amino-acids might be formed in the atmosphere and then rain in the dry state on to hot volcanic areas. A third possibility is one of an oceanic soup of amino-acids of any concentration. When some of this would splash on to seaside lava beds (many volcanoes are near the sea) the water would evaporate very quickly if the amount splashed were small. The solid residue after evaporation would then consist of dry amino-acids. A fourth possibility is one in which the amino-acids in any of these ways, or in any other, came into contact with warm or hot phosphate beds[89]. Lesser temperatures could suffice for polymerization. The necessity of a proper balance of amino-acids continues to apply, however.

Any or all of these circumstances should have operated for the geosynthesis of pre-protein; probably other conditions were suitable as well. So far, other modes of producing peptide bonds in the laboratory under quasi-geochemical conditions[34,90] have shown predominantly glycine in the polymer; glycine is one amino-acid which has long been known to undergo homopolymerization readily[36]. Reliance on recovery of small amounts of other amino-acids following hydrolysis does not support a statement that heteropeptides were formed, inasmuch as hydrolysable diketopiperazines form readily[35,36]. Quasi-geochemical methods of producing in water polyamino-acids with substantial proportions of many amino-acids which are surely bound as peptides may, however, yet be found. The first demonstration of how peptide bonds may have formed yields polymers remarkably like proteins (Table 2).

The geological conversion of such polymers to microspheres functioning as pre-cellular forms would be similarly simple and, according to the experiments, bound to occur in innumerable places on innumerable occasions. The requirements for the geosynthesis would merely be the intrusion of water and the inexorable tendency of such polymers to form cell-like units, as already demonstrated in the laboratory. Inasmuch as the sequence of reactions from primordial gas to microspheres under water is shown to occur in only hours, the organic material composing, or trapped in, the microspheres would be protected from destructive action of heat or other radiation by the overlying layers of water. When one recognizes the breadth of conditions under which the reactions occur and the breadth of terrestrial occurrence of these conditions, one must infer a truly tremendous number of natural experiments. Superposed on this picture is a huge number of microspheres in each experiment, with obviously some variation in individuals.

In biological evolution, selection is believed to be the major process[91]. The possibility of an internal limitation in the step of diversification preceding selection does not receive much serious attention[91]. An effect of this sort is, however, indicated for the molecular evolution which is modelled by the chemical experiments. When organic compounds are produced by imparting various types of energy to various mixtures of simple compounds of carbon, hydrogen, oxygen and nitrogen, the compounds formed tend to be predominantly amino-acids. This preference can be understood on the basis that amino-acids are inner salts; as inner salts they are relatively stable and are thus favoured[87]. Just as amino-acids are favoured over other organic compounds, certain amino-

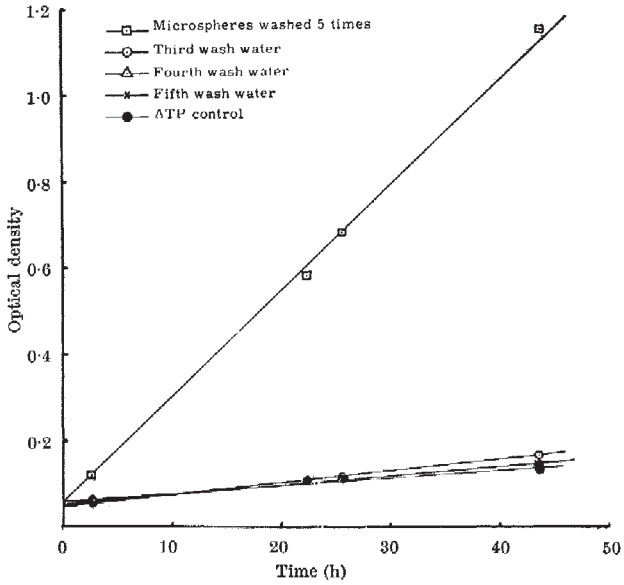



Fig. 16. ATP-splitting activity of washed zinc-containing microspheres from Fox, Joseph and Wiggert, *Origins of Prebiological Systems* (Academic Press, 1965)

acids result more easily and more often than others. Certain polyamino-acids would also be favoured over others for purely chemical reasons. If one believes that chemical processes operate in cells, we can anticipate that self-limiting diversification[2], as well as selection, operates in bio-evolution. The picture, then, would be one of self-limitation in diversification spanning the pre-biological and the biological. In the biological era alone, selection would operate in addition.

The thermal model has developed from a pluralistic philosophy which uses contemporary biology for clues and as a framework of testing and uses experimental 'geosynthesis' primarily as a laboratory technique. A pluralistic philosophy of this sort has seemed necessary inasmuch as we cannot extrapolate back to a prebiotic system in a manner that is in the usual sense testable. This follows, of course, from our ignorance of what the prebiotic system or systems were. Complete knowledge of contemporary biochemical systems may prove to be useless, or even inhibitory or misleading for the purpose of understanding or synthesizing a prebiotic system. On the other hand, a sufficiently disciplined theory of pre-biological chemistry must prove eventually to be fundamental to biological chemistry.

Once research seems to have found its way on to the track which produces relatively simple previtral systems, further experiments can be directed at introducing in a stepwise manner additional characteristics of biocells in a naturalistic and empirical (albeit now guided by the experimental evolutionist) manner.

On the whole, research of this sort generates new questions faster than it does answers; but some answers are forthcoming. The possibility of understanding how materials as complex as proteins or nucleic acids might have arisen in a manner that is sufficiently simple to have occurred spontaneously on the primitive Earth is demonstrated by experiments.

The protein-cell-protein dilemma expressed by Blum and by others[92,93] is resolved by these experiments, as are other dilemmas. Protein of properties adequate to forming primitive protocells could have arisen without a living system to produce them; all that was needed was the necessary primordial gases forming amino-acids in suitable proportions, and heat. This kind of protein not only could form; our investigations in the field[46], in the laboratory, and in the library indicate the occurrence of multitudinous geological possibilities. In this view, one can more easily visualize a large number of natural experiments of which one or more would happen to include the biochemical apparatus necessary for continuing replication. When that occurred, a first primitive cell would have emerged, and the entire parade of Darwinian evolution could begin.

A similar kind of dilemma is that posed by the need to visualize genes as arising before cells[94,95]. The demonstrated self-ordering properties of geosynthetically derivable macromolecules are such that we can now more easily visualize the origin of the cell before or simultaneous with the emergence of primordial nucleic acids[75,96]. We might refer to these synthetic polyamino-acids as morphomolecules to emphasize the degree to which the visible structure of the particles is a manifestation of the nature of the molecules[10,75,97].

The significance of any step in the thermal model is a function of the significance of the total model and *vice versa*. The larger theory is such as to permit us to visualize, through the discipline of experiments, how primordial gases could be spontaneously converted to amino-acids, these in turn to protein, and this latter to a membranous microparticle having many of the properties of cells, including the tendency to divide and to metabolize. If the pond is deferred, the visualization is then a detailed demonstration of Charles Darwin's now well-known statement[67]:

"It is often said that all the conditions for the first production of a living organism are now present, which could ever have been present. But if (and oh! what a big if!) we could conceive in some warm little pond, with all sorts of ammonia and phosphoric salts, light, heat, electricity, and carbon present, that a proteine compound was chemically formed ready to undergo still more complex changes . . .".

Any remaining discontinuity between non-life and life should, according to present understanding, be regarded as not yet understood rather than as hopelessly incomprehensible. We may even adopt the position that, while we credit Darwin with that insight into pre-biological molecular evolution, we owe him more for presenting the principle of biological evolution, inasmuch as the later processes will in the long run prove to have been composed of more intricate and subtle sets of transformations.

This theory is derived from the efforts and critical discussions of numerous associates. The work was supported by grants from the U.S. National Aeronautics and Space Administration (*NSG*–173–62), the U.S. Public Health Service (*C*–3971), the National Science Foundation, the General Foods Corp., Eli Lilly and Co., and the Rockefeller Foundation.

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# CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS, SPAIN

## TWENTY-FIFTH ANNIVERSARY CELEBRATIONS

IN 1939, soon after the end of the Civil War, the Spanish Government set up the Consejo Superior de Investigaciones Cientificas for the purpose of fostering, directing and co-ordinating research in both the sciences and the humanities. During the week beginning on October 18, 1964, the Consejo celebrated its twenty-fifth anniversary by arranging a solemn opening session, scientific meetings, and certain social events; and it invited a number of foreign guests to attend.

The Consejo is divided into eight "patronatos", grouped together to form three main sections: the Division of Humanities, the Division of Sciences, and the Patronato of Scientific and Technological Research which deals with applied science. It is responsible for a large number of institutes, many, but by no means all, of which are in Madrid.

It is at first sight somewhat surprising that it was necessary to create a new organization, and one which, though under the Ministry of National Education, enjoys a considerable degree of independence, to foster the type of research which in most countries is regarded as appropriate to universities. Spanish tradition, however, in contrast, for example, with that of Germany, has hitherto regarded the universities primarily as places of professional training rather than as centres of learning and research; and if the necessary rapid progress were to be made some organization outside them was necessary. The progress made by the Consejo in its twenty-five years, the quality of the research which it is organizing, the influence which it is beginning to have on the outlook of the universities, and the impetus which it is giving to Spanish technological development all demonstrate the wisdom of the original decision to set it up and the competence with which its activities have been developed.

The celebrations began with a solemn inauguration on October 20 in the presence of the Head of State, at which messages of congratulations from international and foreign organizations were delivered. Thereafter, except for unified social events, the proceedings were broken up into five colloquia. That organized by the Division of Humanities dealt with problems of Spanish art and history, and with linguistic questions, and had a very largely Spanish attendance. That organized by the Patronato of Scientific and Technological Research on the theme "Research and Industry" was mostly conducted in Spanish, but included papers by D. R. Vieweg (Germany) on staffing problems, by D. L. Jacqué (France) on programming of research, by D. R. R. Adams (United States) on the measurements of the profitability of research, and by D. E. Martindale (Great Britain) on scientific information. The eleven introductory papers led to much discussion, and some sessions were very lively; notably that in which the question was discussed whether university professors ought to be expected to do research.

The remaining three colloquia were organized by the Division of Sciences, and took on a more fully international character, though naturally with very considerable Spanish attendance and participation. Two were in the biological field, on current problems in biology and on the contributions of ecological and agricultural research in the world fight against hunger. The third dealt with the physical chemistry of processes on solid surfaces.

The colloquium on "Current Problems in Biology" covered a wide range of subjects; some of the contributors discussed in detail a small facet of a biological problem, and others attempted a wider conspectus of a particular field. The opening colloquium on the organization of biological investigation was largely directed to problems of organization and financing of biological research in Spain. The more general conferences were inaugurated by Dr. R. Stanier (United States), who dis-