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CURRENT PROBLEMS IN RESEARCH

Organelle Systems and Biological Organization

Structural and developmental evidence leads to a new look at our concepts of biological organization.

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What are organelle systems and precisely how do they relate to the higher and lower levels of biological organization? Are traditional definitions of organelles as elements or as units of the cell structure still possible or desirable? Or, if our concept of the organelle has changed, then what difference does this make to theories in biology concerning the "living matter" and the "cell"? These are some of the critical questions under examination and at least partially answerable at the present time (*1*). Before the invention of the electron microscope such questions were naturally of a hypothetical nature, yet even now precise interpretations of living cells in terms of their constituent elements continue to present formidable difficulties to the biologist. However, in the past ten years, through contributions from electron microscopy, biochemistry, and microbial genetics, a sufficient degree of common order has emerged to permit a distinction of two supramolecular hierarchies below the cellular level of biological organization: that of the macromolecular aggregate and that of the organelle. Classical cytology, through light microscopy in its various forms, has provided the background for the contemporary outlook, and Wassermann (*2*) has reviewed the contributions of early workers in the field. It appears at present that the few concepts of subcellular particles that have arisen to date are fragmentary at best.

While from the present data we cannot expect a revolutionary overthrow of classical cell theory, its decline in some borderline and otherwise difficult areas (discussed below) appears inevitable. For an understanding of the borderline cases as well as for an understanding of the cell in the classical sense, at least two supramolecular hierarchies of structural organization appear to be necessary, and may indeed be sufficient. The first level has been nebulously termed lattice, polymer, crystal, colloid, or macromolecular aggregate by the solid-state physicist in or out of biology. The second, composed of the first and, in turn, composing the cell, has been similarly termed organelle (*3*). Organelles reside uniquely in the biosphere and have not yet been synthesized outside of it. It is particularly to the second level that I here address my attention.

Some Systems of Organelles in a Single Cell

One of the many instructive organisms for these studies has been the ciliated protozoan *Paramecium*. The apparently unique and complex cell surface of this little animal has been the subject of study and controversy for nearly 100 years. Zoology textbooks have long presented elaborate diagrams of the "fibrillar systems" of pellicle and gullet as figures worthy of study by the elementary student. The lesson has been essentially to point out how different this is from other cells: the fibers and ridges in the shape of chicken wire, the cell-mouth in the form of a woven cornucopia, and the bank of trichocysts militantly arranged behind the walls, ready to fire away at the enemy.

Today, another view, summarized in Figs. 1 and 2, prevails. The reconstruction techniques applied here are similar to those routinely used in studies of embryonic development, but in this case they are applied to single cells, by means of the electron microscope (*4*). The pellicle system of the cell surface is composed of two sets of closely packed organelles: the ciliary corpuscles and the trichocysts. In this simple repetitive-unit construction resides the physical basis for the ridge-and-wire patterns; the junction edges of the packed corpuscles give rise to those hexagonal and rhomboidal patterns on the surface. Beneath the surface and between the corpuscles, the trichocysts are located. Along the line of trichocysts, the cilia are found. Each corpuscle contains either one or two cilia; to the right of each ciliary base (or kinetosome) is a small pocket known as the parasomal sac. Only one sac is seen in a corpuscle, whether one or two cilia are present. From the anterior filaments of the kinetosome (the posterior kinetosome in cases where there are two), a kinetodesmal fiber extends anteriorly the distance of three or more corpuscles and laterally to the right, between the columns of corpuscles, and forms a bundle of fibers by overlapping (*4–6*) or spiraling around (*4*) other fibers of the same column (see Fig. 1, sections *A–A* and *C–C*). The hexagonal and rhomboidal complexes of organelles together form a portion of the cell surface known as the pellicle system, which, but for a small gap, entirely contains the liquid phase and inner envelope systems of the cell. Another system of similarly

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packed organelles, the gullet or food-intake system, closes this gap. The gullet cilia are much more closely spaced than those of the pellicle, with about 0.46 micron between the centers of the kinetosomes (see cover photograph and Fig. 3*A*). However, instead of one or two cilia per parasomal sac, there are four. Since the unit organelle of the gullet system bears four cilia and one sac, three columns of about 90 of these quadriciliated units bear the approximately 1000 cilia that nearly fill the food-intake cavity. A fourth organelle complex to complete the gullet system of organelles is the non-ciliated ribbed wall, in which the inter-rib distances are similar to the inter-ciliary distances of the other three complexes.

Comparative Anatomy of Organelles as Units and in Groups

From studies such as these, several rather remarkable relationships between the components that make up the cell soon became apparent. The first and most striking is that just below the cellular level of organization there are a number of anatomically characteristic components that are identifiable as such in nearly all cells. The second is that these components, these organelles, pack together into simple and orderly arrays. To treat of the first relationship, the organelles include cilia, mitochondria, and certain laminated and concentrically layered or vesiculated objects. We shall now center our attention on some of the roles that are played by one class of organelles, the cilia.

Cilia are built in the same way wherever they are found in the plant or animal kingdom (*7*, *8*). Of equal importance has been the discovery that many organelles, bearing little superficial resemblance to cilia, are nevertheless built upon the same ultrastructural ground plan—are as similar to one another as the isotopes of an element. A striking illustration of this second point is given in Fig. 4, in which a mammalian cilium (*9*) is seen closely to resemble the connecting fibers (*cf*) between segments of the rods (*OS*, outer segment; *IS*, inner segment) (*10*) in the retina of the eye, with their nine sets of concentrically arranged filaments. Numerous equally remarkable examples of "organellar isotopy" upon the cilium ground plan can be given. The plantlike stalk of another protozoan, the colonial ciliate *Opercularia*, is composed of bundles of cilium-like fibers (*11*); the hair cells of the organ of Corti in the cochlea or inner ear of the guinea pig are tufted with cilia and cilium-like fibers (*12*); the tip of the trichocyst is cilium-like, although a clear count of 9 or 18 perimeter filaments has not been resolved (*4*); a broomstick-like structure in the protozoan *Lophomonas* (the axostylar filament) looks superficially unlike a cilium (*13*), yet it is composed of 18 (9×2) radially arranged plates about a central fiber, and each plate has a period of about 400 angstroms, like that of the typical kinetodesmal fiber (*5*).

Thus, in one form or another this versatile organelle performs a wide variety of tasks in the lowest as well as in the most complex forms of life. In man, not only does the cilium propel the male spermatozoon; it also guides the female ovum into the oviduct, helps keep the lungs free of dust, is a receptor point in vision, hearing, and balance, and is a part of the division apparatus [the centriole (*8*, *14*)] in nearly every cell of the body.

The ciliary corpuscles and trichocysts of the pellicle system and the cilia of the gullet are, of course, excellent examples of the packing together of organelles into orderly arrays. It is interesting to find ciliary complexes in certain renal tumors of the hamster [Fig. 3*B* (*15*)] that are very similar to the gullet complex of *Paramecium* [Fig. 3*A* (*16*)]. In each case, the packed cilia appear to compose considerable portions of the cell surface and

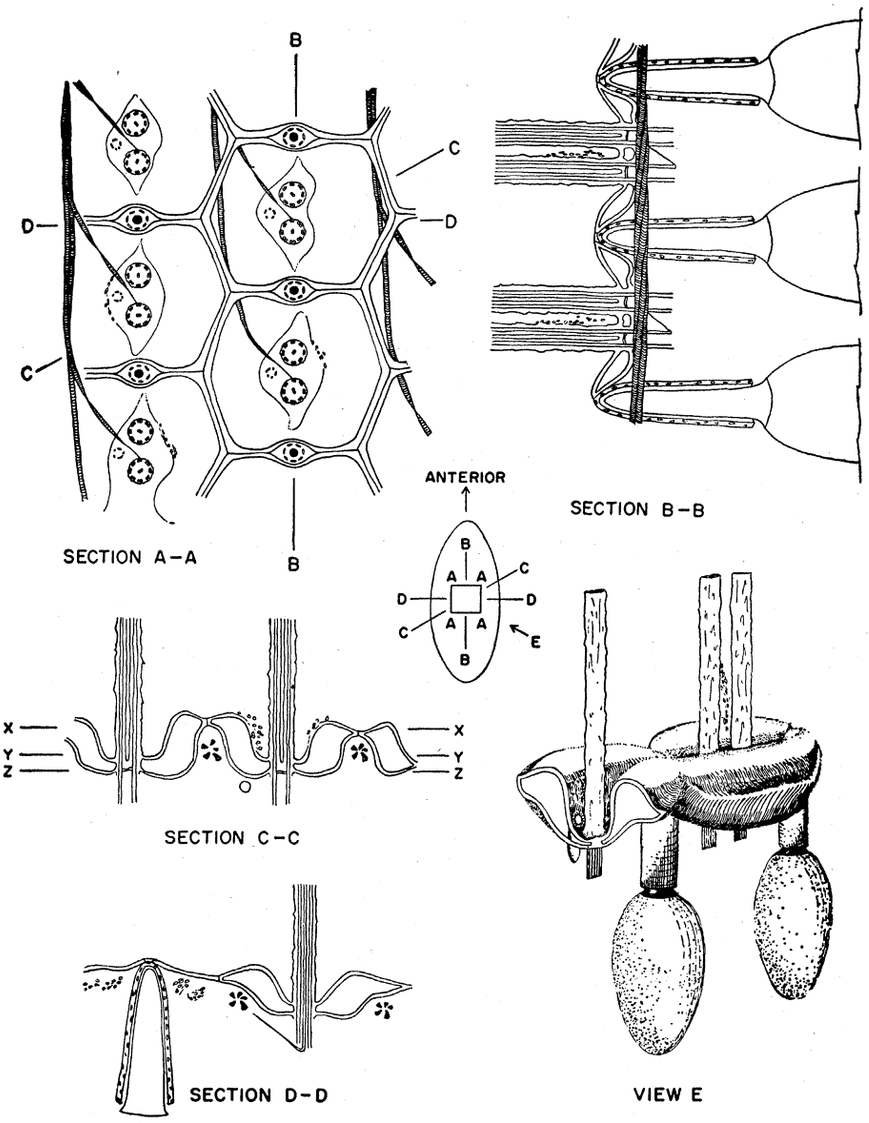

Fig. 1. Sections through plan, elevation, and profile orientations of the cell surface of *Paramecium*. The reconstruction view *E* is of two ciliary corpuscles and two trichocysts. The parasomal sac is evident on the hemisectioned forward corpuscle. Planes of sectioning are shown on the outline of the cell in the center of the figure. [From Ehret and Powers, *4*]

sometimes appear highly compacted, as in the footlike cirrus of the protozoan *Euplotes* (*17*). Whether diffuse, compact, or a composite of both patterns, sheets like these of two-dimensionally packed organelles are seen in many free-living cells to surround the cytoplasm entirely, thereby forming the outer envelope of the cell. Inner envelopes of two-dimensionally packed nonciliated units (pore complexes) (*18*) similarly compose the nuclear envelope of most cells (*19*). Arrays of three-dimensionally packed units, in some dimensions (*20*) quite like those of the pore complex, are seen in the cytoplasm and are called ergastoplasm or endoplasmic reticulum (*21*).

Similar considerations of the homologies among units and of the packing or developmental patterns of units could be, and indeed have been, made for other organelles and their complexes—particularly for the mitochondrion and structurally distinct cytoplasmic organelles (*22*). On the other hand, it is now well known that some organisms are totally devoid of such characteristic cytoplasmic organelles as *true* cilia and mitochondria—notably, the bacteria. All available evidence seems to indicate that bacteria are simple one-envelope systems, and that the larger organelles normally associated with the outer envelope of typical cells are absent. In this sense, bacteria are more like free-living nuclei than they are like typical cells. Even the most complex bacterial organelles —for example, the tubular and lamellated photosynthetic organelles of *Rhodospirillum* (*23*)—are small and simple as compared to typical mitochondria; along with chromatin, these tubular and lamellated organelles share the same "karyoplasm" and are encased by the same envelope.

As a summary to this brief exposition of the simple and orderly arrays of organelles in living systems, Fig. 5 associates graphically and extends some of the ideas just discussed. The list of terms on the right is a much abbreviated compilation of a few of the noncommittal names given to objects that we find in and around cells (compare the decision-tree in *3*). On the left are the simple space groupings appropriate to these. For example, mitochondria and multivesicular bodies (*24*) as they are found normally in cytoplasm are, like points, spatially restricted groups. As they are found in the spermium, mitochondria are chains or one-dimensional groups of points (the spiral filament, *25*). Pore complex units (*18*) exist as two-dimensional sheets of points in the nuclear envelope. The nebenkern (*26*), old nucleolus (*27*), and endoplasmic reticulum (*20, 21*) are three-dimensional lattices of mitochondrial, nucleolar, or pore-complex-like units, respectively. Of particular interest is the manner of employment of sheets as envelopes—in the case, for example, of the nuclear and outer envelopes of cells—as well as several interesting properties of the isopoly combinatorial systems of all groupings.

It may be noted, for example, that in the division of a two-envelope system, the inner envelope is usually lost at mitosis, and a cyclical transition from a two-envelope to a one-envelope system occurs. Similarly, virus propagation occurs through cyclical transitions from a one-envelope to a zero-envelope system. In each case, at organellar replication, the inner envelope system is lost. These parallels between nuclei and infected bacteria provoke the experimental question: Is a specific lysis-inducing enzyme or "lysozyme" released by metazoan nuclei just before fission to disrupt the fabric of the inner envelope, as in the case of bacterial lysis (*28*)? A rare but plausible example of a defective two-envelope

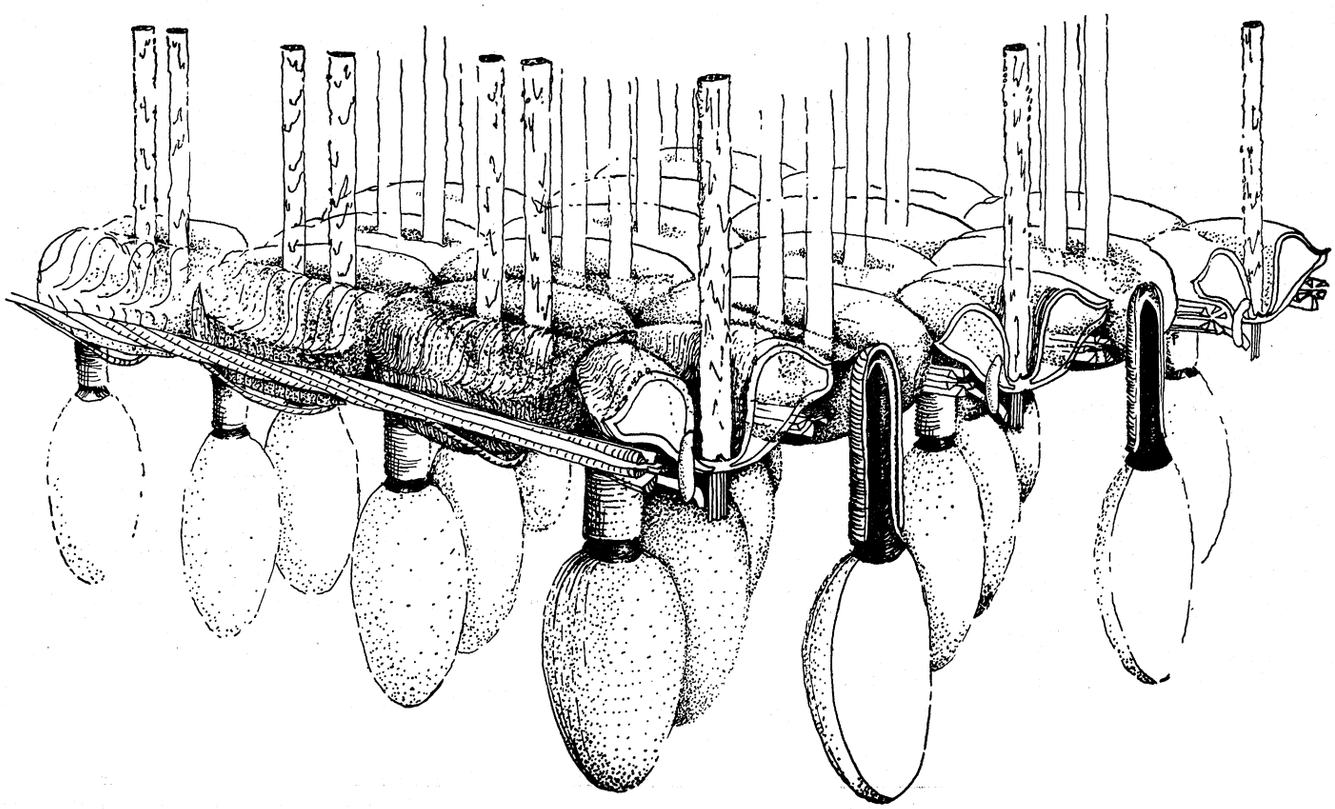

Fig. 2. Reconstruction of a region of pellicle surface composed of 18 corpuscles and attendant trichocysts. Bundles of kinetodesmal fibers link the ciliary bases and confer polarity on the whole structure. [From Ehret and Powers, *4*]

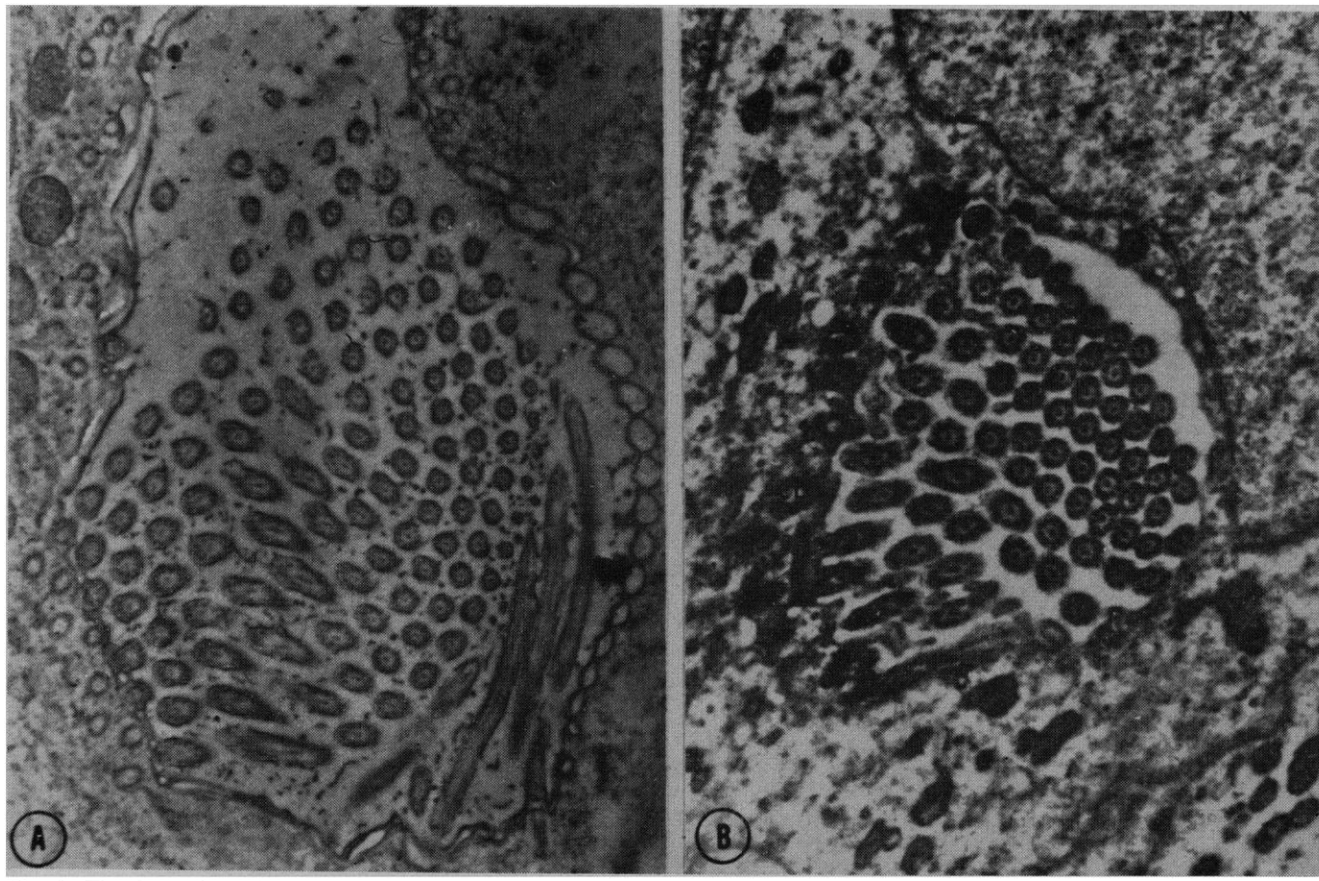

Fig. 3. The formation of two-dimensional sheets by organelle complexes in the gullet of *Paramecium* (*A*, from Ehret and Powers, *16*) and in a renal tumor cell of the hamster (*B*, from Mannweiler and Bernhard, *15*).

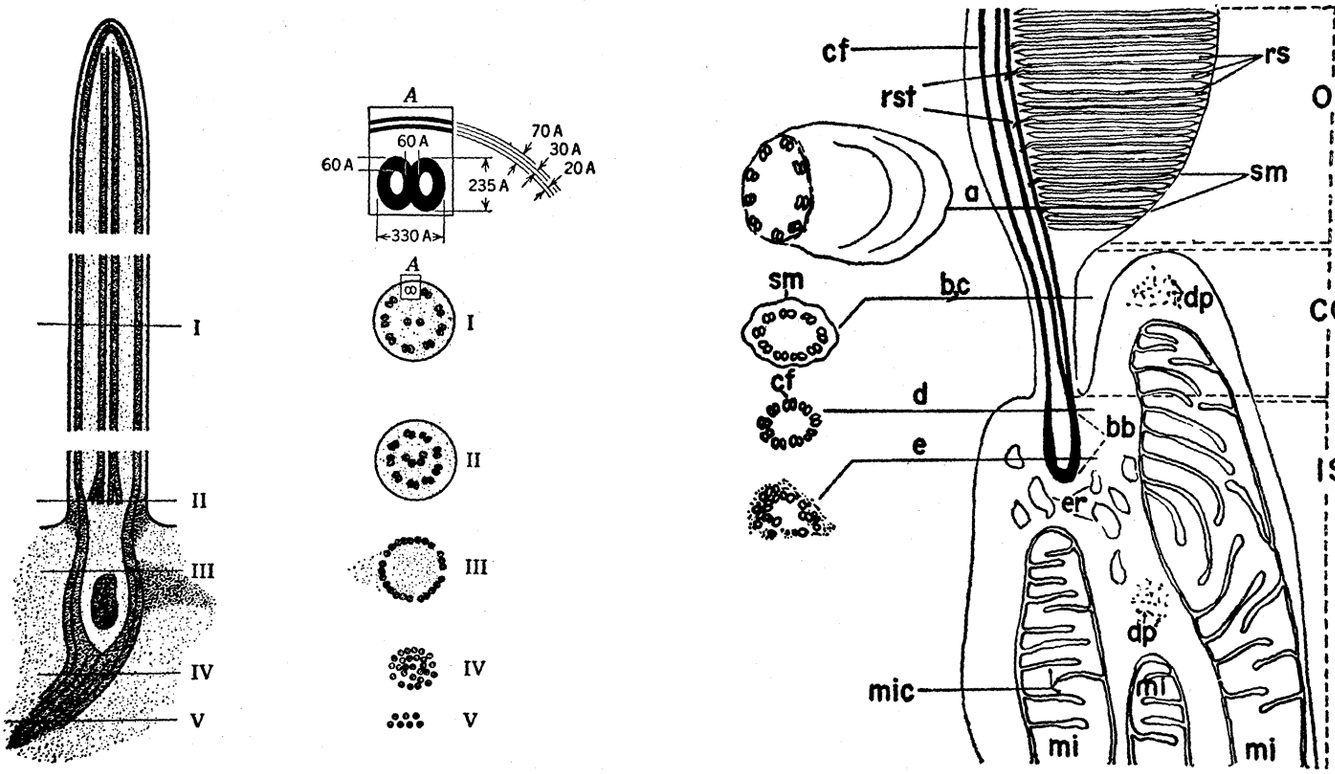

Fig. 4. An illustration of organellar isotopy, showing the cilium of mammalian tracheal mucosa on the left (from Rhodin and Dalhamn, *9*) and the connecting fiber (*cf*) of the retinal rod on the right (from DeRobertis, *10*).

system is also on record, in the case of the mammary tumor virus. Bernhard (*29*), for example, has shown that such a virus, wrapped in an inner envelope picked up earlier in its passage through the nuclear membrane of the host cell, can then acquire an outer envelope in its passage through the cell membrane. It is logically satisfying to find even the "trivial" or "improbable" mathematical possibilities nevertheless represented.

Definitions, Formulations, Perspectives

What is an organelle? The term itself suggests a miniature organ and has been casually applied to many cell parts without much regard for homology or comparative topology. A rigorous definition at this time might prove to be unduly restrictive and has been difficult to achieve for even such well-established units as atoms and molecules. Some characteristic properties are, however, worth considering. In the first place, we may classify organelles according to structure: mitochondria contain tubules, plates (lamellae), or ridges (cristae); cilia contain nine oriented sets of filaments, and this number itself may be regarded as a space-filling constant. Less effective are functional classifications according to action, enzymatic capacity, or cytochemistry, since *some* organelles lack these attributes. For example, Ephrussi's petite mutant of the yeast cell still contains structurally typical mitochondria, while lacking respiratory enzymes (*30*). Therefore, while synthetic functions may be performed upon organelles at the chemical level (*3*), the primary role of organelles is best described as a niche-filling or space-filling one. In this way, organelles are structurally supportive (for example, ciliary corpuscle or pore complex), or kinetically active (cilium or flagellum), or mechanically translational (cochlear hair). It appears probable that they have additional purely physical functions, such as transmission, diffraction, oscillation, and reception of electromagnetic energy (*31*).

If we think of the organelle as an ordered laminate of heterogeneous macromolecular aggregates, we may find its prototype best exemplified by the plant and bacterial viruses, with their protein wrappers and nucleic acid cores (*32*). A structurally similar laminate of lipopolysaccharide and protein is the receptor site unit of the bacterial cell wall for the bacterial virus T-5 (*28*). Analogous laminates of other macromolecular aggregates such as lipids and protein were proposed as the ultrastructural basis of membranes, even before the invention of the electron microscope, especially from results with polarized light and x-ray diffraction methods. Along these lines, the pioneering studies of Frey-Wyssling (*33*), Seifriz *et al.* (*34*), Davson and Danielli (*35*), F. O. Schmitt (*36*), and many others have been substantiated and extended (*31, 37*), and the principal continuing emphasis in electron microscopy is quite justifiably at this lower level of the macromolecular aggregates. However, it is equally necessary at the organelle level to characterize the consequences of multiplicities and groupings of organelles in the formation of the large space-filling and macrokinetic devices of the cell.

The way in which these components may be used in quantitative formulations of cell structures is shown in Fig. 6. In this treatment, each of the organelles of the surface is regarded as an isotope of a "type cilium" and is symbolically represented by roman letters and appropriate superscripts. In the case of the ciliary corpuscles, the superscripts refer to the number of cilia per unit; the units c^1, c^2, and c^4, despite differences in ciliary count,

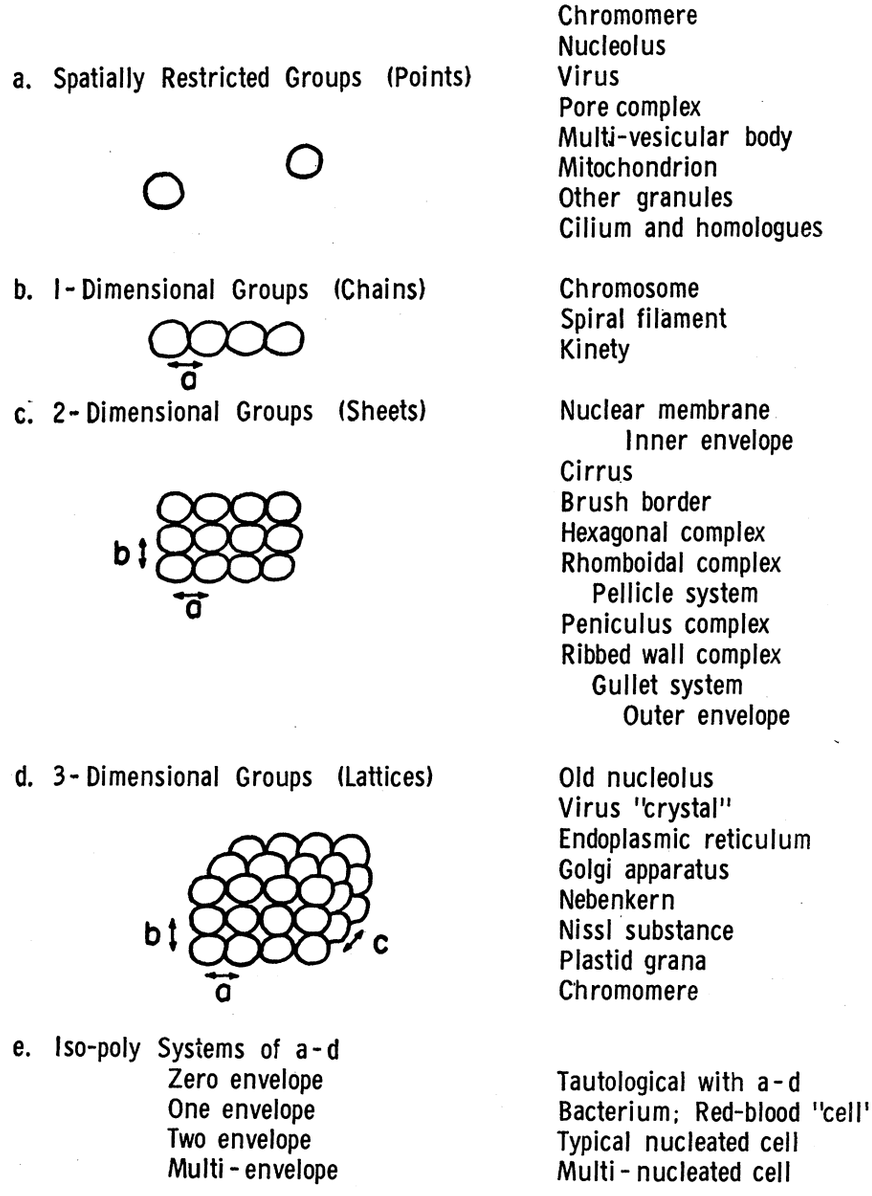

Fig. 5. The organelles, alone and in bunches: the formally simplest packing groups of organelles and some of their cytological and etymological consequences.

possess single sacs, occupy closely comparable areas of the surface space, and appear to arise in each case from single C^0 units. The forces that hold these units to one another are represented by valence bond lines. Ultrasonic disruption (*5*), as well as compression-decompression studies (*38*), suggest that the weakest of these bonds is that binding trichocyst to corpuscle, and the strongest, that binding corpuscle to corpuscle by way of the kinetodesmal fibers. The central diagram of the hexagonal complex $(c^1t)_{14,000}$ of the pellicle system of the cell surface shows the essential relationships between bonds and the approximately 28,000 units involved. For simplicity, the curved lines representing bonds between corpuscles by way of the kinetodesmal fibers are shown to influence only longitudinally adjacent corpuscles; in reality each corpuscle is bound by such forces to three or more posteriorly and anteriorly located corpuscles. The relationship of *t*'s to *c*'s is shown only in profile view. The force diagram of the disposition of trichocysts along the surface suggests that even if no other function is served by them, they must at least contribute to the structural strength of the pellicle system by serving as footings and trusses between the hexagons and the viscous gel of the cytoplasm. *Paramecium* has thus solved, in an ingenious fashion not yet patented by the construction engineer, the problem of trusswork among the hexagons of a nonrigid geodesic dome (*39*). The superficial resemblance between such a diagram and the cytologist's pictorial equivalent (Fig. 1) arises only out of topological necessity. In this way it also resembles the conventional formulations of molecules in terms of their constituent elements. From such systems formulations it should be possible for an anonymous investigator, given the properties of the units and their bonds, to specify the properties of the whole figure, including its "appearance."

Such formulations are justified because they can describe biological organizations not only for the exposed surfaces of *Paramecium* but also for analogous configurations in the nuclear envelope, in the organ of Corti, and in the Hamster renal tumor. Furthermore, most of the genetic mappings of the past 50 years are once-removed transformations of similar structural formulations. This perspective also clarifies the dual physical basis for genetic maps. The first, the subject of viral genetics, is at the molecular level and is associated with the transposition and alteration of extremely short sequences of nucleotides (*40*). The second, the subject of the bulk of classical genetics, is at the organelle level and is associated, accordingly, with much larger "chromomeric" transpositions. Each is of importance, and the primitive organisms that lack the second are deprived of a whole range of recombinational potential acquired by higher organisms through their chromosomes. Genetics is, therefore, basically a structural science concerned with the linear ordering of particles at two levels, and whether he admits it or not, the geneticist is a morphologist at heart.

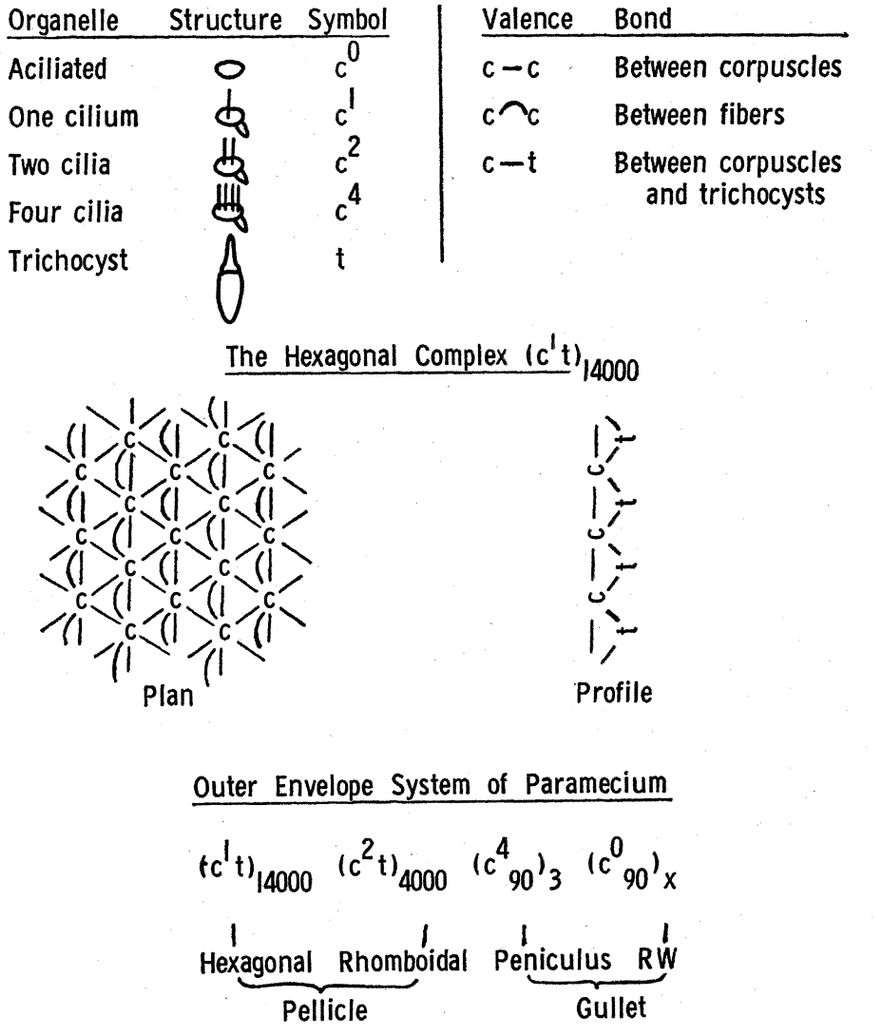

Fig. 6. Systems formulations in terms of organelles: the representation of cytological structures in a single cell by means of equations for, and graphs of, its constituent organelles.

Statics versus Dynamics

If the ultimate justification for this kind of approach is a better understanding of the dynamically active living organism (*41*), how can this understanding be derived from the frozen-points static concept described above? First, let us recognize that all particle concepts suffer from illusions of the reality of a static point, and the molecular-submolecular even more than the supramolecular concepts discussed here. With the long interfission times of cells, the actual disposition of organelles may remain architecturally unchanged and essentially as shown in Fig. 2 and Fig. 6 for hours or days; yet at the molecular level, within the very substance of these organelles the interacting particles of the metabolic cycles have relatively infinitesimal lifetimes. But as fission approaches, drastic changes occur in the organelle groupings of the nucleus and cytoplasm of the cell. The mechanisms

of deoxyribonucleic acid and of chromosomal duplication have been under extensive study recently, and I will mention them only briefly here. Suffice it to say, for comparative purposes, that in each of the several interpretations that have been applied to the data (*42*) the essential genic and chromosomal material does not appear to grow and split in two (division) but, rather, appears to be synthesized and ordered *de novo* on a template provided by the old (replication).

How do extranuclear organelles compare? Our own approach to the problem has been to isolate and observe single cells just before cell division. The most interesting changes have occurred by the time the fission furrow is even faintly visible, and most published accounts of gullet "budding" in *Paramecium* are actually descriptions of these stages in displacement of already well established new organelle systems from the old ones. But these are late stages. The first signs of the new gullet appear at the pellicle surface in the region of the vestibulum, many microns removed from the old gullet. An alignment into three columns of about 90 granules per column occurs. From each granule, four cilia sprout, and the cell is now equipped with three ribbons of cilia, still essentially at the surface and more specifically influencing the swimming than the feeding of the organism. Invagination and posterior displacement occur in much slower succession than do the preceding stages, a fission furrow forms, and the cell divides. The fission furrow is also filled with granules, from which one or two cilia appear to sprout.

Most of these events are established only through phase-contrast microscopy, and the full description of details observed with electron microscopy is in progress. Daughter cells resemble one another and their common parent not as mirror images but directly; this appears to be the consequence not of a template-replica mechanism but, instead, of a developmental process in which the new organelle systems are ordered by less specifically shaped but sufficient environmental forces. In each daughter cell nearly half of the old organelle systems are preserved intact and without gross change—like the old section of a building to which a new wing has been added. To date, no satisfactory evidence of the division of ciliary bases (kinetosomes) has appeared (but see *6*, *43*, *44*), and the alternative hypothesis that these organelles arise neither by division nor by replication but *de novo* (by biosynthesis and development from suborganellar-level pools) is strongly favored by the *Paramecium* studies. The alternatives remain to be resolved empirically but are best understood in the first place within the framework (Fig. 7, discussed below) of the choices open to the units that compose the organism at each appropriate level of complexity.

SET		SUBSETS
MOLECULE (M) (glycine; adenine; ATP)		Atoms and non-atoms: $A_1 A_2 A_n P_{1-n}$
MACROMOLECULAR AGGREGATE (C) (ribonuclease; DNA)		Molecules and non-M's: $M_1 M_2 M_n A_{1-n}$
ORGANELLE (O) (virus; chromomere; cilium)		Macromolecular aggregates and non-C's: $C_1 C_2 C_n M_{1-n}$
ENVELOPE SYSTEM		
TWO ENVELOPE (S'') (epithelial cell)	ONE ENVELOPE (S') (bacterium)	Organelles and non-O's: $O_1 O_2 O_n C_{1-n}$
TISSUE (T'') (epithelial tissue)	(T') (bacterial colony)	Envelope systems and non-S's: $S_1 S_2 S_n O_{1-n}$

Fig. 7. Relationships between components and systems of organelles: some molecular and supramolecular sets of the bios.

From Macromolecules to Envelope Systems

Quite recently a vigorous concern for relational schemes and levels of biological organization has been shown by a number of usually empirically oriented biologists (*44–46*). A crux of their problem is invariably resident in our inadequate notions of the ultrastructure of living systems. In Fig. 7 I have tried to relate such components to one another in the simplest possible hierarchal fashion. Despite the simplicity of this scheme, the physical basis for each of the relationships discussed above is represented here, the gap between the molecular and cellular levels is realistically bridged, and some interesting properties of the products of components are implied. (Below the molecular level, and above the tissue level, particles and atoms, and organs and organisms, might also appear as component elements of the bios.) Each set is composed not only of its immediately inferior subsets but also of more remotedly inferior components. Thus, free radicals or negatively charged particles may be subset components of a particular macromolecular aggregate or molecule. The scheme thereby allows for a wide variety of hybrid sets that fit no simple specification *M*, *C*, or *O*. The composition of a tendon, for example, might better be considered a *C–M* structure [of the protein collagen in a relatively diffuse molecular matrix of mucopolysaccharide molecules (*46*)] than an organelle, which is a laminated aggregate of *C*'s. Since the term *cell* is more appropriately applied to a uninucleated two-envelope system, the more general class "envelope systems" is recognized as the component that makes up tissues. Two classes of tissues are therefore recognized, those derived from one-envelope systems and those derived from two-envelope systems. It is the latter line, with its manifold possibilities for recombination of components, that provides the stem-line for the higher forms of plant and animal life. (It is interesting to note that sanitation and fermentation biologists have noted the equivalent of *S'-T'* "organs" in their vats and sludges.)

Above the *O* level, examples can be cited for sets possessing the divisional property: the two split members of the set are isomorphic or homeomorphic to one another and to the unsliced parent set. Below the *C* level it is not possible to divide and preserve homeomorphy of the parent and the fission elements. A practical consequence in metazoan biology is that identical twins

resemble one another not because they or their tissues or cells are replicas of one another but because of a remote event subsequent to gene sequence replications in their common zygote. All superior systems were synthesized uniquely and developed epigenetically *de novo,* as in the equally nonreplicative case of ice-crystal patterns on a cold windowpane or of gullet development at another surface. From this example and from microbial data it appears that at only one point on the scheme does replication enter the picture from necessity rather than fancy: at the *C* level, in the inheritable sequences of viral or chromosomal nucleotides.

When Is an Envelope System Not a Cell?

One of the most interesting distinctions highlighted here is the distinction existing between single- and multiple-envelope systems, in which the object conventionally and restrictively termed "cell" is regarded as a special case of the latter grouping and bacteria are regarded as prime examples of the former. Are bacteria then not cells? Some people insist that bacteria are cells because the cell is the basic unit of life and, as everyone knows, bacteria are alive! Others say, looking back to Hooke, that they are cells because they are shaped like cells, which are tiny rooms inhabited by monks. Still others say they are cells because they have deoxyribonucleic acid, ribonucleic acid, and a quite respectable genome. Needless to say, each argument is either circular or insufficient in itself to categorize uniquely the organizational stratum that is appropriate to a bacterium. More cogent reasons would be the presence of a typical nucleus within a typical cytoplasm. What is typical? The contemporary experts agree unanimously that ultrastructurally the *bacterial* flagellum is not a typical flagellum [lacking the cilium-like filamentous order (*47*)]; that the *bacterial* mitochondrion is not typical [being orders of magnitude smaller, though not necessarily lacking some form of lamellated or tubular ultrastructures not unique to cytoplasm (*48*)]; and that the *bacterial* nucleus is not typical [lacking the characteristic nuclear envelope (*49*)]. When we speak of a "bacterial this-or-that" we deliberately so modify our object as to remove it from the framework of rigorous structural homology with other objects not so modified (whereas set theoretic terms point up the likenesses as well as the differences among categories). Because atoms have nuclei, as do ethnic social groupings, it would be preposterous to presume rigorous structural homologies to be present between them and cellular nuclei, even though they all may share in common interesting topological properties. It is, therefore, quite an unfortunate, though an understandable, etymological turn of events that two distinctly different biological objects share nearly the same name, to the point of confusing all but the nonexperts in the field.

The homely term *cell* will and should continue to be used both colloquially and specifically by the biologist. However, in restrictive matters, when properties of the units are claimed or sought (such as "nuclei" and "mitotic-spindles"), then his units should be unambiguously defined, preferably in operational systems-theory terminology.

Transcendency of Levels

It would be premature to declare, "the proof is in, all bacteria are one-envelope systems and are therefore homologous to the archaic 'nucleus.'" The point that I make is this: the properties of the bacterial species, as well as of those actinomycetes adequately studied to date, seem to indicate that they fit the one-envelope level of organization better than any other. Is it not conceivable that some species are multi-enveloped or even zero-enveloped? Indeed, is not a transcendency of levels formally plausible, so that within a life cycle many levels might be manifested by a single organism? Perhaps the filterable forms of bacteria, including the so-called L-forms and pleuropneumonia and pleuropneumonia-like organisms, are a manifestation of such transcendency from the zero- (virus-like, filterable elementary bodies) envelope level to the one-envelope level of organization. Certainly this capacity for transcendency in levels superior to the cellular is one of the splendid evolutionary innovations of the metazoa, and its counterpart ought to be sought elsewhere. Most important, a formal realization of the general phenomenon is provocative: What are the limiting rules of the game, within what boundaries may the phenomenon operate? The answers are beyond most biologists' present competence, but the questions may sharpen us to the task.

Conclusion

Within this reference-frame of understanding, the cell ceases to occupy a central location as a fundamental unit of life. It appears, instead, as a special case among the single- and multiple-envelope systems that comprise all forms of life. The typical interphase cell is a two-envelope system whose inner envelope contains an intact genome resident upon the linearly packed chromomeres, and whose outer envelope encases the distinctive cytoplasmic organelles. However, other mathematically plausible possibilities are also found. The inner envelope is frequently represented many times, as in multi-nucleate protozoa and mammalian muscle. The inner envelope is sometimes lost or absent, as in mammalian red cells and certain mutant protozoa and typical cells in metaphase. Examples of organisms whose "outer" envelope is absent are the bacteria and actinomycetes (*50*). Being one-envelope, "inner-envelope" systems, these *protobiotes* are drastically distinguished not only from plants and animals but also from cells. Finally, certain deficient two-envelope systems are recognizable in the form of some of the mammalian viruses, and zero-envelope systems, in the form of the bacterial viruses—neither of which is capable of reproduction without the intact genome of another inner-envelope system.

The synthetic biologist is at nearly the same stage in the development of his science that the synthetic chemist had reached in Dalton's day, and no one has as yet constructed organelles from their constituent macromolecular aggregates. However, some promising attempts have been made at virus development in vitro, and the transplantation of entire inner-envelope systems into different outer envelopes has become routine in some laboratories (*51*). It is not unreasonable to expect to see certain of the simpler cell organelles synthesized in many laboratories within the next few years; their assembly into single- or multiple-envelope systems will indeed present a more difficult, but exciting, challenge.

In one sense, despite the combined efforts of hundreds of microscopes, thousands of investigators, and countless research papers, we have barely scratched the surface of the biotic landscape, and there is much work ahead. In another sense, we have some cause to rejoice, as did the tunnel-diggers

who joined hands in the middle of the mountain when the first rays of light broke through. Electron microscopy has succeeded in forming a thin but actual join between the molecular and the cellular levels of biological organization. In so doing, it has united form with function and has established the foundations of a mathematically rigorous morphology (*52*).

References and Notes

1. This work was performed under the auspices of the U.S. Atomic Energy Commission.
2. F. Wassermann, introduction to the symposium "The impact of electron microscopy on biology," at the Chicago meeting of the AAAS, December 1959.
3. C. F. Ehret, in *Symposium on Information Theory in Biology*, H. Yockey, R. Platzman, H. Quastler, Eds. (Pergamon, London, 1958), p. 218.
4. C. F. Ehret and E. L. Powers, *Intern. Rev. Cytol.* **8**, 97 (1959).
5. C. B. Metz, D. R. Pitelka, J. A. Westfall, *Biol. Bull.* **104**, 408 (1953).
6. A. Sedar and K. R. Porter, *J. Biophys. Biochem. Cytol.* **1**, 583 (1955).
7. D. W. Fawcett and K. R. Porter, *J. Morphol.* **94**, 221 (1954); W. T. Astbury, *Symposia Soc. Exptl. Biol.* **9**, 1 (1955).
8. C. Rouiller and E. Fauré-Fremiet, *J. Ultrastructure Research* **1**, 289 (1958).
9. J. Rhodin and T. Dalhamn, *Z. Zellforsch. u. mikroskop. Anat.* **44**, 345 (1956).
10. E. DeRobertis, *J. Biophys. Biochem. Cytol.* **2**, 319 (195 → K. Tokuyasu and E. Yamada, *ibid.* **6**, 225 (1959).
11. C. Rouiller, E. Fauré-Fremiet, M. Gauchery, *Exptl. Cell. Research* **11**, 527 (1956).
12. H. Engström and J. Wersäll, *Exptl. Cell. Research Suppl.* **5**, 460 (1958).
13. H. W. Beams, T. N. Tahmisian, E. Anderson, B. Wright, *J. Biophys. Biochem. Cytol.*, in press.
14. E. deHarven and W. Bernhard, *Z. Zellforsch. u. mikroskop. Anat.* **45**, 378 (1956); M. Bessis and J. Breton-Gorius, *Bull. microscop. appl.* **7**, 54 (1957); S. Amano, *Cytologia (Tokyo)* **22**, 193 (1957).
15. K. Mannweiler and W. Bernhard, *J. Ultrastructure Research* **1**, 158 (1958).
16. C. F. Ehret and E. L. Powers, *J. Protozool.* **4**, 55 (1957).
17. L. E. Roth, *J. Biophys. Biochem. Cytol.* **2**, suppl., 235 (1956).
18. M. Watson, *ibid.* **6**, 147 (1959).
19. L. Rebhun, *ibid.* **2**, 93 (1956); S. Wischnitzer, *J. Ultrastructure Research* **1**, 201 (195 → G. D. Pappas, *J. Biophys. Biochem. Cytol.* **2**, 431 (1956).
20. W. Bernhard, *Exptl. Cell Research Suppl.* **6**, 17 (1958).
21. K. R. Porter, *J. Histochem. and Cytochem.* **2**, 346 (1954).
22. E. L. Powers, C. F. Ehret, L. E. Roth, O. T. Minick, *J. Biophys. Biochem. Cytol.* **2**, suppl., 341 (195 → A. J. Hodge, *ibid.* **2**, 597 (195 → C. Rouiller and W. Bernhard, *ibid.* **2**, 355 (1956); K. E. Wohlfarth-Bottermann, *Protoplasma* **49**, 231 (1958); H. Fernández-Morán, *Exptl. Cell. Research Suppl.* **5**, 586 (1958); C. Oberling, *Intern. Rev. Cytol.* **8**, 1 (1959); J. André, *J. Ultrastructure Research* **2**, 288 (1959); A. M. Jezequel, *ibid.* **3**, 210 (1959).
23. D. D. Hickman and A. W. Frenkel, *J. Biophys. Biochem. Cytol.* **6**, 277 (1959).
24. J. R. Sotelo and K. Porter, *ibid.* **5**, 327 (1959).
25. D. Fawcett, *Intern. Rev. Cytol.* **7**, 195 (1958).
26. T. N. Tahmisian, E. L. Powers, R. L. Devine, *J. Biophys. Biochem. Cytol.* **2**, suppl., 325 (1956).
27. C. F. Ehret and E. L. Powers, *Exptl. Cell Research* **9**, 241 (1955); N. K. Das and M. Alfert, *Anat. Record* **134**, 548 (1959).
28. W. Weidel in *Virus* (Univ. of Michigan Press, Ann Arbor, 1959).
29. W. Bernhard, *Cancer Research* **18**, 491 (1958).
30. B. Ephrussi, personal communication.
31. H. Fernández-Morán, *Revs. Modern Phys.* **31**, 319 (1959).
32. M. F. Perutz, *Research (London)* **12**, 326 (1959).
33. A. Frey-Wyssling, in *Macromolecules in Cell Structure* (Harvard Univ. Press, Cambridge, 1957).
34. W. Seifriz, Ed., *A Symposium on the Structure of Protoplasm* (Iowa State College Press, Ames, 1942).
35. H. Davson and J. F. Danielli, *The Permeability of Natural Membranes* (Cambridge Univ. Press, New York, 1952).
36. F. O. Schmitt, *Physiol. Revs.* **19**, 270 (1939).
37. ———, *Revs. Modern Phys.* **31**, 455 (1959); F. S. Sjöstrand, *ibid* **31**, 301 (1959).
38. C. F. Ehret, unpublished observations.
39. R. B. Fuller, *Consulting Engr.* **13**, 90 (1959).
40. S. Benzer, *Proc. Natl. Acad. Sci. U.S.* **45**, 1607 (1959).
41. P. Weiss, *Revs. Modern Phys.* **31**, 11 (1959).
42. C. Levinthal, *Proc. Natl. Acad. Sci. U.S.* **42**, 394 (195 → J. H. Taylor, P. S. Woods, W. L. Hughes, *ibid.* **43**, 122 (195 → M. Meselson and F. W. Stahl, *ibid.* **44**, 671 (1958); R. Williams, *Revs. Modern Phys.* **31**, 233 (1959).
43. E. Chatton, A. Lwoff, M. Lwoff, *Compt. rend.* **188**, 1190 (1929); A. Lwoff, in *Problems of Morphogenesis in Ciliates* (Wiley, New York, 1950).
44. A. Lwoff, *J. Gen. Microbiol.* **17**, 239 (1957).
45. T. M. Sonneborn, *Nature* **175**, 1100 (1955); F. O. Schmitt, *Revs. Modern Phys.* **31**, 5 (1959); R. Zirkle, *ibid.* **31**, 269 (1959); S. E. Luria, in F. M. Burnet and W. M. Stanley, Eds., *The Viruses* (Academic Press, New York, 1959); B. Strehler, *Quart. Rev. Biol.* **34**, 117 (1959); S. Wright, *Science* **130**, 959 (1959).
46. F. Wassermann, *Ergeb. Anat. u. Entwicklungsgeschichte* **35**, 240 (1956).
47. B. A. D. Stocker in E. I. C. Spooner and B. A. D. Stocker, Eds., "Bacterial flagella: morphology, constitution and inheritance," *Proc. Soc. Gen. Microbiol., 6th Symposium, London* (1956).
48. S. Mudd, *Bacteriol. Revs.* **20**, 268 (1956).
49. E. Kellenberger and A. Ryter, *Schweiz. Z. allgem. Pathol. u. Bakteriol.* **18**, 1122 (1955); P. Giesbrecht, *Naturwissenschaften* **45**, 473 (1958); S. Spiegelman, A. I. Aronson, P. C. Fitz-James, *J. Bacteriol.* **75**, 102 (1958); G. B. Chapman, *ibid.* **78**, 96 (1959); E. Kellenberger, in "The physical state of the bacterial nucleus," *Proc. Soc. Gen. Microbiol., 10th Symposium, London*, in press.
50. R. T. Moore and G. B. Chapman, *J. Bacteriol.* **78**, 878 (1959).
51. R. Briggs and T. J. King, *J. Morphol.* **100**, 269 (1957).

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Trends in Polarography

J. Heyrovsky

For some 38 years I have carried on electrochemical research with the dropping-mercury electrode because of its exquisite properties as electrode material. Its physical conditions of dropping as well as the chemical changes that occur during the passage of the electric current are well defined, and the phenomena displayed at the dropping-mercury electrode proceed with strict reproducibility. Owing to the latter property the processes of the electrode can be exactly expressed mathematically. According to the registering apparatus, called a "polarograph," which automatically draws curves characteristic of the electrode processes, the electrochemical studies with the dropping-mercury electrode and the analytical method developed on the basis of these investigations have been called "polarography."

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Apparatus

The capillary electrode is normally a tube 8 centimeters long and 5 to 6 millimeters wide with an inner bore of 0.05 to 0.1 millimeter (Fig. 1), from which the drops of mercury fall off every 3 to 6 seconds, according to the height of the mercury reservoir, which is about 40 centimeters above the tip of the capillary.

In order that the current passing through this electrode may be entirely governed by the composition of the solution surrounding it, the second electrode has to be indifferent, unpolarizable, and of a constant potential; most suitably, it is the layer of mercury at the bottom of the electrolytic vessel (Fig. 2).

To apply the external voltage to the cell we use a potentiometric arrangement, shown in Fig. 2. From a 2- or 4-volt lead accumulator, an increasing voltage E is branched off, and the corresponding current is determined by