THE ORGANIZATION OF LIVING MATTER

By George E. Palade

THE ROCKEFELLER INSTITUTE

Introduction.—Living systems consist of relatively few common chemical elements,[1] yet have unique properties which set them aside from the rest of matter: they are able to control their exchanges with the surrounding medium and to respond to changes therein; they can transform energy and metabolize matter; and especially they are capable of self-duplication. These remarkable properties, expressed in one form or another, have intrigued and challenged man's mind at least since the beginning of history. The answers have varied over the ages, but for a long time have retained an element of reverence and have assumed the involvement of extraordinary forces. Now, after millenia of illusions, doubts, and probing, it turns out that these unique properties are due to the way in which common chemical elements are put together in time and space. For our times, life—human life included—is the outcome of an elaborate organization based on trivial ingredients and ordinary forces. Historically speaking, this has been a drastic readjustment which is still affecting, sometimes with devastating force, whole fields of human endeavor.

The Structural Hierarchy of Living Systems.—The elaborate organization just mentioned consists of a hierarchy of structural patterns which begins with relatively simple molecules and proceeds, step by step—first to polymeric macromolecules; then to molecular or macromolecular aggregates, which often take the form of elementary structures[2] such as fibrils, membranes, and particles; then to aggregates of such structures which turn out to be cell organs; then to cells; and beyond cells to tissues, organs, and organisms.

Life with its full complement of attributes, life self-sustained and self-reproducible emerges at the cell level within the hierarchy. The sufficiency of this stage is attested by the fact that so many living forms do not go beyond it, and by the ease with which cells of metazoic origin revert to an independent state under culture *in vitro*. Below this level there are self-reproducible forms, like the viruses, but they are not self-sustained; they subsist only as cell symbionts or cell parasites. Hence our discussion can be limited to living matter at the cellular and subcellular level.

Already at this level, life depends on an extensive organization in depth, on a superimposition of patterns which amount to infinitely more order than matter usually tolerates. This thermodynamically improbable situation is achieved by continuously supplying energy to a pre-existing structural framework. It is this framework, the hierarchy of patterns already mentioned, that captures energy and matter from outside sources and channels them into a complex series of reactions whose outcome is the maintenance of the system and its eventual duplication. As far as we know, at present the framework is always inherited, never created

Editorial Note: This lecture was presented during the Second Scientific Session of the Academy's Centennial Celebration. The texts of other lectures presented during the various scientific sessions of the Centenary may be found in this issue of these Proceedings, in no. 5 (May) of volume 51, and in no. 6 (December) of volume 50.

de novo. How it originally came into being is a fascinating problem which has tempted many minds, without as yet obtaining a satisfactory answer.

The Extent of Cell Structure and Its Relation to Metabolism.—Before analyzing the structural framework of the cell, its extent should be defined and its position in relation to cell metabolism should be understood. It is usually assumed that cell structure is limited to those elements sturdy enough to resist biochemical or morphological preparative procedures. As a rule these elements consist of matter in a solid or mesomorphic state. Undoubtedly, extensive disorder is introduced by the procedures mentioned wherever cell organization relies on weaker and fewer forces. Even matter in relatively rapid transit through a cell is disposed at any given time with a certain amount of order imposed by the rates of the reactions in which it is involved. Finally water, quantitatively the major component of living systems, is by necessity extensively organized in relation to various cell structures. Hence we are dealing with a gradient of order which encompasses practically the whole cell, and of which only parts survive our attempts to analyze the whole.

As far as relations to metabolism are concerned, it should be understood that structure and metabolism are two aspects of the same set of phenomena. Matter flows continuously through a living system, it is metabolized by it and, while in transit, forms the hierarchy of patterns on which the system depends. What the biologist describes as structure are usually the more slowly revolving phases of the general metabolism. Atoms, molecules, and even larger aggregates move continuously in and out of the framework, but since their replacement is gradual, the patterns subsist while their components turn over at rates that vary throughout the hierarchy. The only exception appears to be the genome of the cell or, in other words, its set of DNA[3] molecules, which may last untouched throughout a succession of cell generations.

The Structural Framework of the Cell.—General considerations: If we consider the structural framework of the cell, as we know it today, we are faced with a rather disturbing situation: the information about it is already staggering in volume, yet far from complete and, therefore, far from being properly understood. During the last decades, however, enough progress has been made in the analysis of two different structural levels, namely those of macromolecules and of subcellular structures, to justify a tentative interpretation of the whole framework.

At the macromolecular level, the primary, secondary, and tertiary structure of many proteins, the structure of DNA and various types of RNA's, and that of other biological polymers has been elucidated to a greater or lesser extent, with remarkable and far-reaching results. One of them is the fundamental importance assumed by certain structural features of these macromolecules, primarily by their surface details, viewed in terms of tridimensional configuration as well as distribution of functional groups. Such details apparently constitute the genetic code in the DNA molecule; the template for protein synthesis at the surface of messenger RNA; the adapter that recognizes the right spot on the template for the corresponding amino acid in the case of transfer RNA; the active sites at the surface of protein molecules endowed with enzymatic properties; and so on. This level in the hierarchy of patterns turns out to be not only fundamental, but also common, as far as we know at present, for all living systems. All apparently rely on the same type of molecules and the same kind of structural details to

transmit genetic information from one generation to another, and to give expression to this information in the synthesis and activity of their specific constituents.

The other level at which substantial progress has been recorded is that of cellular and subcellular structure. Here, however, the situation is different: the corresponding patterns are no longer common to all living systems, and the functional significance of many of their features remains unknown. Yet the cellular level deserves full attention for, as already mentioned, the fundamental characteristics of life emerge only at this level, not before it.

Recent advances in the analysis of cellular organization have been achieved mainly by two relatively new technical developments: electron microscopy and cell fractionation procedures. The first technique means resolving power of the order of 10–5 Å, an improvement of ~200–400 times over light microscopy, and the possibility of carrying structural analysis down to the molecular level of organization. The second allows the isolation in mass of most subcellular structures and their subsequent chemical and functional characterization *in vitro*. By an unusually fortunate coincidence, the two developments have been contemporary; hence they have been frequently combined, and have led to an integrated, structural and functional characterization of many cell organs.

The results obtained can be put into better perspective by considering first the average dimensions and main features of the whole cellular framework. The usual animal or plant cell is a very small body: its average diameter is $\simeq 20\ \mu$, and its volume $\simeq 5{,}000\ \mu^3$. It is bounded by a thin membrane ($\simeq 8$ mμ) and its content, or protoplasm, consists of a nucleus $\simeq 5\ \mu$ in diameter ($\simeq 16\ \mu^3$ in volume) surrounded by a shell of cytoplasm. It is in this exiguous space that the most significant part of the structural hierarchy of living matter must be fitted. This extreme miniaturization is probably imposed by the slow means of communication, primarily diffusion, that operate in the protoplasm which, as a first approximation, might be considered as an aqueous sol-gel system. At closer scrutiny, however, it turns out to be highly heterogeneous and extensively compartmented. Its cytoplasmic layer contains, for instance, numerous bodies which belong to different classes among which one of the best known is that of mitochondria: spherical or rod-like bodies with an average volume of $0.8\ \mu^3$ and a population density of a few hundred per cell. This class of subcellular components can serve as a first example of the type of results obtained and progress made by the concurrent use of electron microscopy and cell fractionation procedures.

Mitochondria: Figure 1, taken from an article published in 1900 by Michaelis,[4] shows mitochondria stained in living cells with Janus green, a dye which when oxidized has a blue-green color. Michaelis' drawing shows clearly mitochondria as they appear in the light microscope in the cells of a gland, namely, the pancreas. This figure served as "official portrait" of the mitochondria until 12 years ago. Figure 2 shows an electron micrograph of a thin section through a part of a single mitochondrion in a similar cell. The salient point is the existence of a large amount of membranous material which forms more or less regularly arranged infoldings,[5] called cristae. Figure 3 shows a fragment of a single crista prepared by a different procedure which gives a negative image by embedding the specimen in an opaque matrix. It demonstrates the existence of small particles ~80–100 Å in diameter

at the surface of the cristae. These are the mitochondrial "elementary particles" recently discovered by Fernández-Morán.[6]

Shortly before their fine structure was unraveled, mitochondria were isolated in mass[7] and found to contain the enzymatic equipment the cell uses to oxidize various intermediates of the Krebs cycle,[8, 9] pyruvate and fatty acids.[9, 10] This equipment includes the terminal electron transport chain from dehydrogenases to oxygen[11] and the enzymes and factors which use the energy released during oxidation to synthesize ATP,[12] the general fuel of living systems. At that time, a correlation between concentration of oxidative enzymes and frequency of cristae was already discussed.[5, 13] During the last years, after the discovery of the "elementary particles," attempts were made to give this correlation a more precise form: recent evidence[14] suggests that the electron transport chain and its associated phosphorylating enzymes are located in the elementary particles. It is still debated whether such a particle can accommodate the entire multienzyme system involved, or only part of it[15] and in this case what particular part. But these questions are now under active investigation in many laboratories and answers will be soon forthcoming.

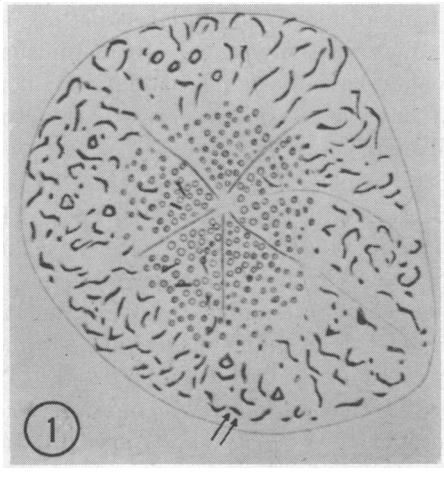


FIG. 1.—Reproduction in black and white of Fig. 6 from Michaelis' paper in *Arch. f. mikroskop. Anat. Entwicklungsmech.*, **55**, 558 (1900). Pancreatic acinus of a mouse. The arrows (added by me) indicate part of a mitochondrion comparable to the one shown in Fig. 2 of this paper.

The main function of the mitochondria is now well understood: they supply most of the energy (~90%) required for the various endergonic reactions carried out by the cell, in the form of ATP produced during oxidative phosphorylation. In addition, they are apparently involved in the energy-dependent accumulation of various ions and as such in the regulation (or homeostasis) of the internal medium of the cell.[16]

The typical organization of a mitochondrion comprises an outer membrane, an

Figures 2, 5–8, and 10–13 in this article are electron micrographs of cells fixed in 1 per cent OsO_4, either in acetate veronal or phosphate buffer (pH 7.4–7.6), and embedded in epon. The sections were stained with lead hydroxide or uranyl acetate followed by lead hydroxide. The specimen in Figure 3 (mitochondrial fraction isolated from *Neurospora crassa*) was stained negatively with K phosphotungstate. The specimens for Figures 14 and 15 were prepared by Kellenberger's procedure. Abbreviations for all figures are as follows: *cm*, cell membrane; *n*, nucleus; *cy*, cytoplasm; *m*, mitochondrion; *er*, endoplasmic reticulum; *rs*, rough-surfaced element of the endoplasmic reticulum; *ss*, smooth-surfaced element of the endoplasmic reticulum; *ar*, attached ribosome(s); *fr*, free ribosome(s).

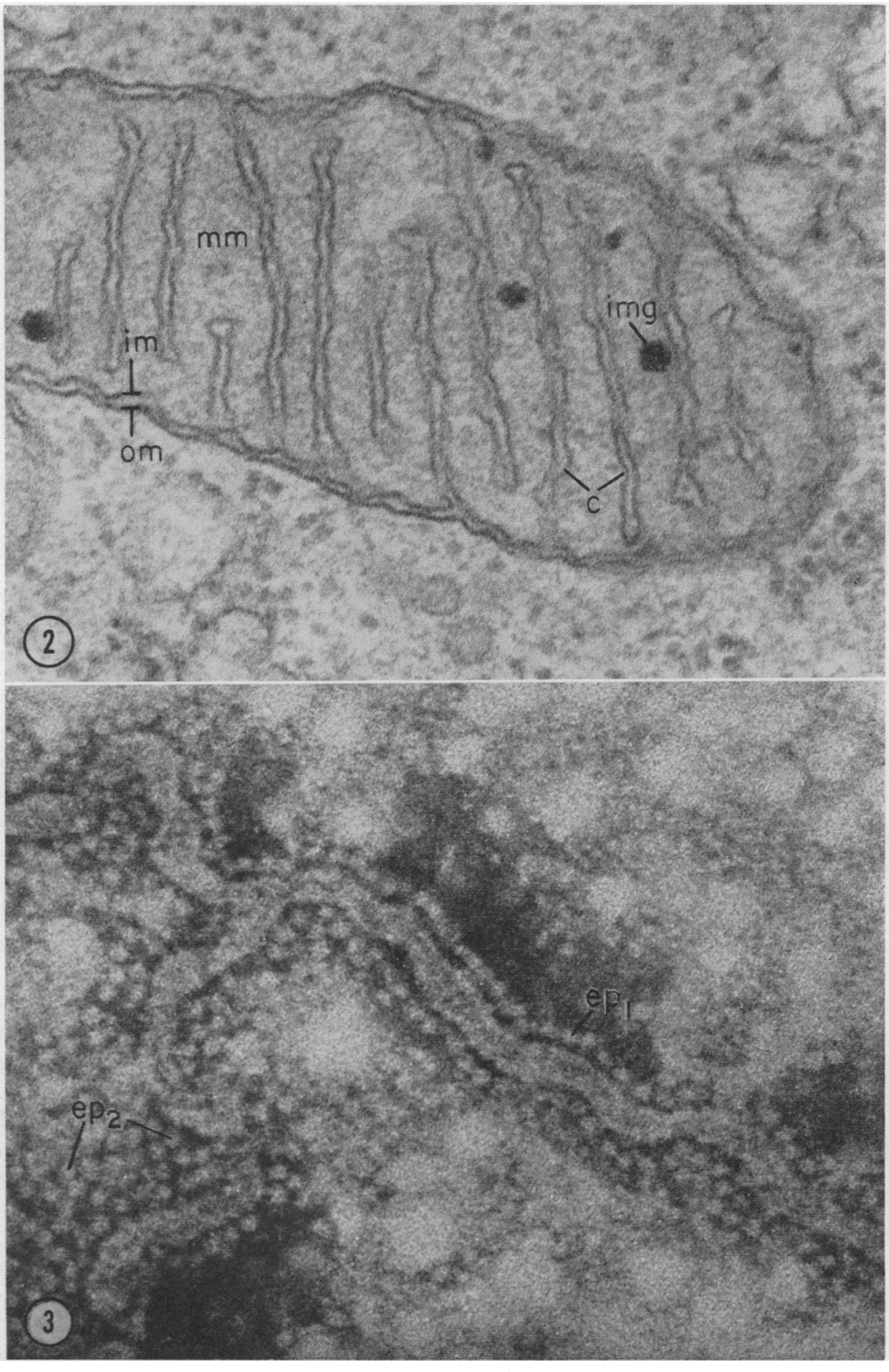


FIG. 2.—Electron micrograph of a section through part of a mitochondrion (pancreatic exocrine cell, guinea pig). *om:* outer membrane; *im:* inner membrane; *c:* crista; *mm:* mitochondrial matrix (inner chamber); *img:* intramitochondrial granule. ×100,000.

FIG. 3.—Electron micrograph of a negatively stained mitochondrial crista. Elementary particles lining a branching crista appear in side view at ep_1 and in full-face view at ep_2. Courtesy of Dr. Walther Stoeckenius, The Rockefeller Institute, New York. ×300,000.

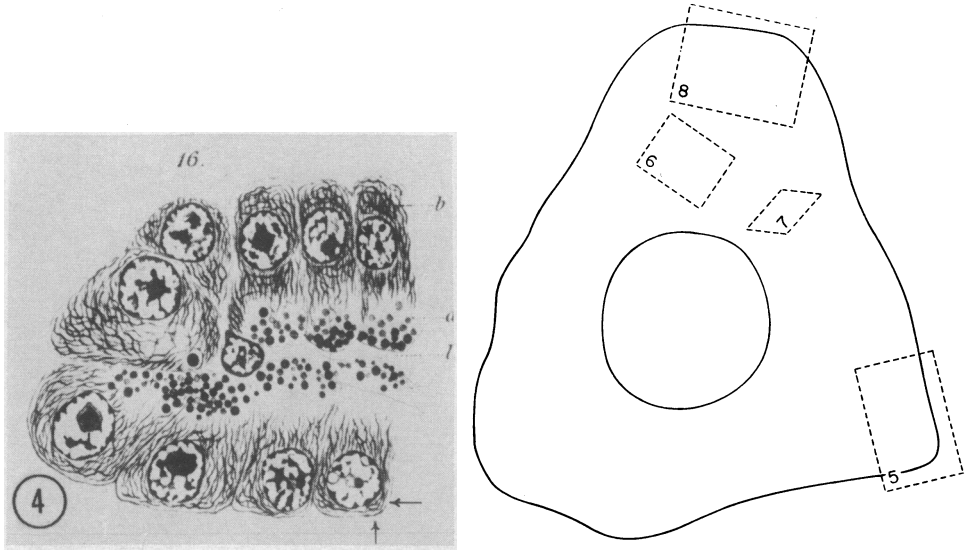


FIG. 4.—Reproduction of Fig. 16 from A. Mathews' article on "The changes in structure of the pancreas cell," *J. Morphol.*, **15** (suppl.), 171 (1899). The figure shows an acinus in the frog pancreas. The basal region is occupied by a basophilic fibrillar material. The arrows (added by me) mark a region comparable to that shown in Fig. 5. The accompanying diagram represents a pyramidal pancreatic exocrine cell (guinea pig) similar to the large cells at the bottom of the acinus in Mathews' drawing. The enclosed areas marked 5 to 8 indicate the position and the size of the fields of Figs. 5–8 within such a cell.

inner membrane whose infoldings form the cristae, and two chambers. It is the inner membrane and its cristae which are lined with elementary particles.[17] Some of the features of the mitochondrial pattern can be understood in terms of available data: the outer membrane probably controls mitochondrial permeability,[18] whereas the inner membrane provides support for the multiplicity of enzymes involved in oxidative phosphorylation. Ion accumulation occurs in the inner chamber,[19] possibly in relation to some intramitochondrial granules.[20] The functional meaning of the other features remains for the moment obscure.

Although recent and still under development, the work on mitochondria has acquired historical significance for, in addition to the elucidation of the function of these subcellular components, it has introduced a number of new concepts among the basic premises with which we are trying to understand cell organization. The first concerns the existence of functionally specialized cell organs; the second recognizes the ample use of membranous material to separate compartments within the cell and to provide a solid framework for the precise, stabile positioning of active parts: each mitochondrion is a membrane-bounded cytoplasmic compartment, which in turn is subcompartmented by an inner membrane of its own. Finally, the third concept concerns the widespread occurrence of common patterns of organization at the subcellular level: mitochondria were found to have basically the same structure from animal cells to fungi.

Endoplasmic reticulum and ribosomes: The next examples will show how these concepts have influenced further research and how in turn they have been modulated by subsequent experience.

Figure 4 illustrates the distribution of "chromidia," "ergastoplasm,"[21] or "baso-

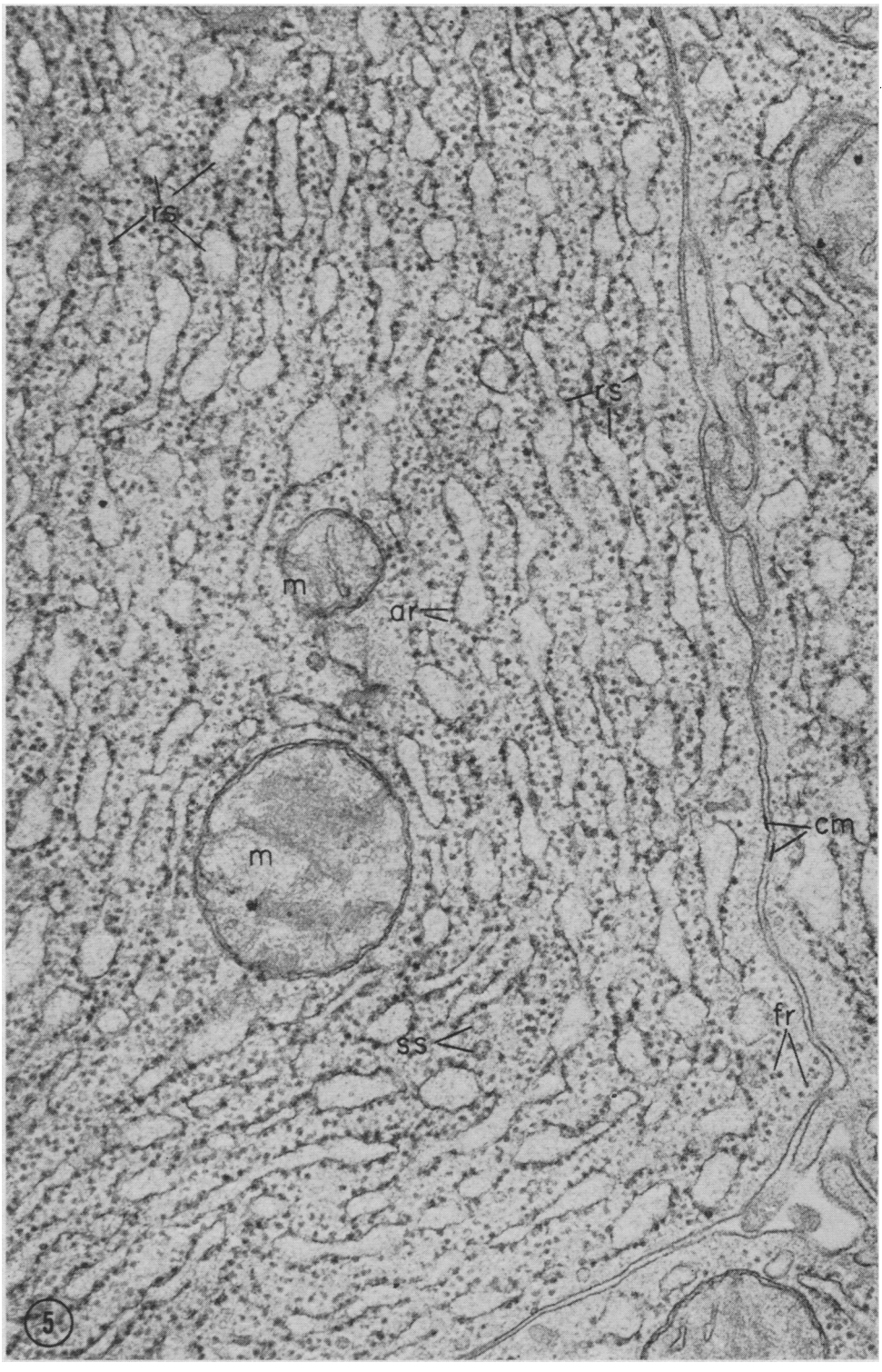


FIG. 5.—Small field in the basal region of a pancreatic exocrine cell (guinea pig). Parts of two adjacent cells appear along the right margin and in the lower right corner. ×50,000.

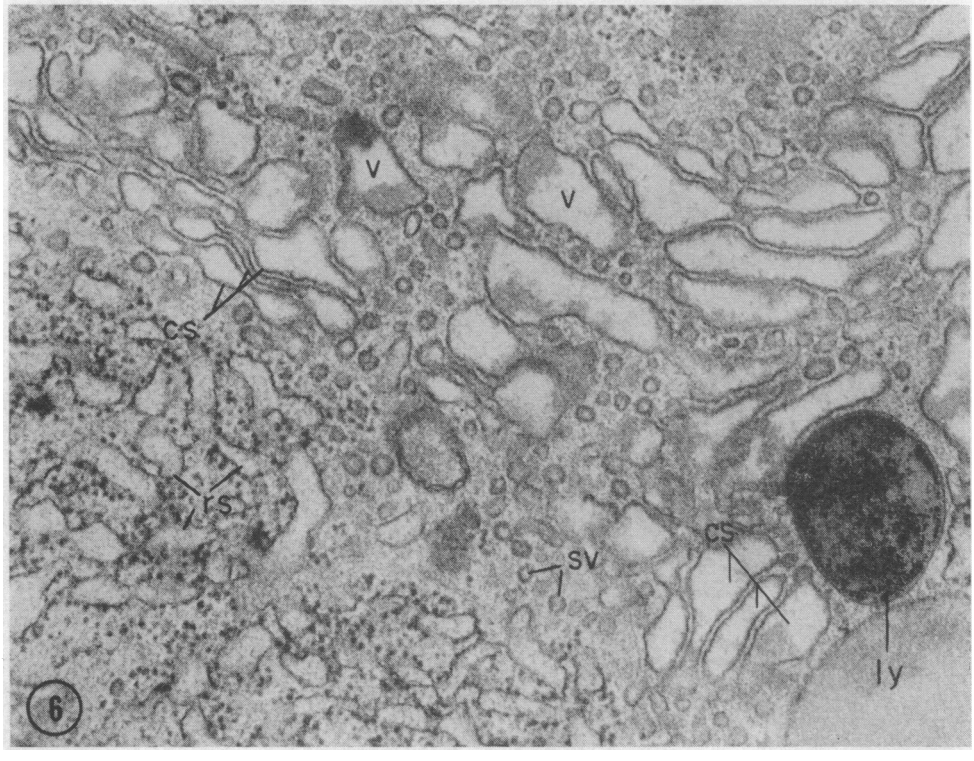


FIG. 6.—Small field in the Golgi region of a pancreatic exocrine cell (guinea pig). Rough-surfaced elements of the endoplasmic reticulum occupy the lower left corner and smooth-surfaced elements of the Golgi complex the rest of the field. The complex consists of small vesicles (*sv*), stacked cisternae (*cs*) (distended in this case to a varied extent), and large vacuoles (*v*). The body marked *ly* is a lysosome. ×50,000.

phil substance" in a glandular cell (the exocrine cell of the pancreas), as seen by an American cytologist, A. Mathews,[22] around 1900. Electron micrographs of certain regions [i.e., basal, centrospheric, and apical (see diagram accompanying Fig. 4)] of the same type of cell, which differ from one another in the extent of their "basophilia," i.e., their RNA content, are shown in Figures 5–8. The intensely basophilic basal region is packed with membrane-bounded tubules, vesicles, and cisternae (Fig. 5) which belong to the intracellular membranous system of the endoplasmic reticulum.[23] Most of these elements are rough surfaced, i.e., bear attached dense particles—the extensively studied ribosomes—on the outer surface of their limiting membranes. In addition, the intervening cytoplasm contains a sizable population of free ribosomes.[24]

The centrosphere region contains the Golgi complex,[25] a characteristic accumulation of vesicles, vacuoles, and stacked cisternae, which are smooth surfaced, i.e., free of attached ribosomes (Fig. 6). At the periphery of this complex, elements partly covered and partly free of ribosomes join the rough- to the smooth-surfaced channels of the system (Fig. 7). Finally, in the apical region (Fig. 8) large secretion granules, bounded by a smooth-surfaced membrane, occur interspersed with ribosome-bearing elements of the endoplasmic reticulum.

It is known at present that these membrane-bounded spaces are extensively

interconnected within the cell; that they probably stretch as a network from the nucleus, which is surrounded by a perinuclear cisterna,[26] to the cell membrane; and that they open at the cell surface, but that these openings as well as the communications from one part of the reticulum to another are intermittent. The cell apparently controls, at the level of the Golgi complex and of the cell membrane, the time, the volume, and the direction of flow of the content of the system.[27]

As far as we know at present, the content consists either of matter ingested in bulk from the surroundings, or substances produced by the cell to be discharged into the external medium, or both. Intermittent connections enable the cell to handle in small packets (or quanta) its products of secretion; to concentrate them

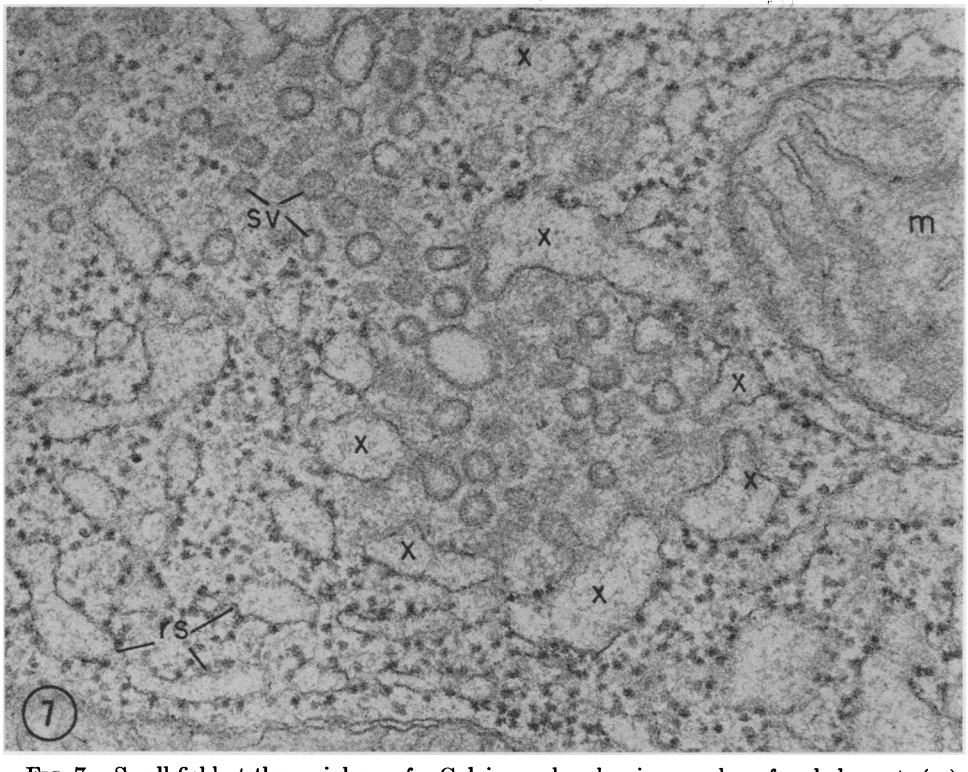


FIG. 7.—Small field at the periphery of a Golgi complex showing rough-surfaced elements (*rs*), smooth-surfaced vesicles (*sv*), and elements of intermediary appearance: part rough and part smooth. The cisternal space of the latter is marked with an *x*. ×70,000.

for storage; and to quantitate their discharge. In the case of ingested matter, temporary isolation in a vacuole or compartment appears to be a prerequisite for concentration and digestion. The cell discharges into such a vacuole hydrolytic enzymes apparently stored in small granules or vesicles called lysosomes,[28] and thus the vacuole becomes a site of intracellular digestion. The import of matter in mass is ubiquitous among animal cells and can be considered a consequence of their heterotrophy: the fact that they depend on exogenous organic compounds to compensate their biosynthetic deficiencies. The development of the endoplasmic reticulum seems to be connected, at least in part, with such activities, but

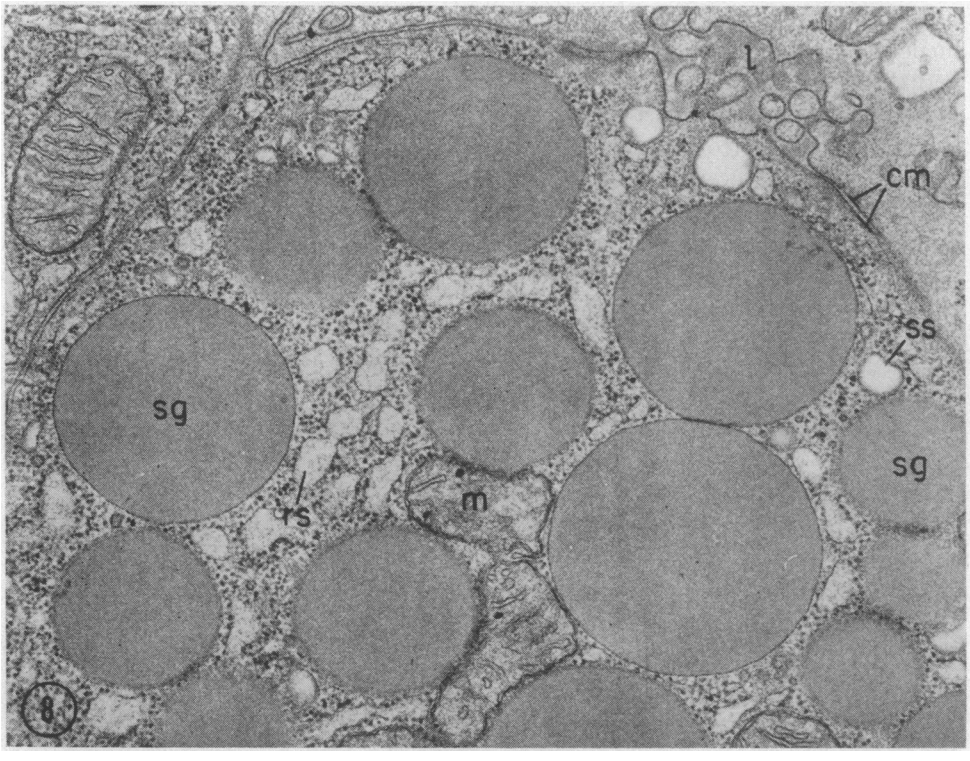


FIG. 8.—Small field in the apical region of pancreatic exocrine cell. A glandular lumen appears at *l* and parts of three adjacent cells can be seen along the upper margin of the figure. The field is occupied mainly by secretion granules, two of which are marked *sg*. ×30,000.

once acquired the system has been adapted to other parallel or alternative functions such as secretion.

One is tempted to assume that originally the smooth-surfaced part of the endoplasmic reticulum was primarily connected with the import of matter in bulk, while the rough-surfaced part was concerned with the production of hydrolytic enzymes, the connection between the two parts being established by a lysosome, i.e., a small storage vacuole filled with hydrolytic enzymes. In a crude analogy the smooth part of the system could be regarded as the functional equivalent of a metazoan's intestine, and the rough part as the equivalent of a digestive gland.

A salient consequence of the existence of the endoplasmic reticulum is the partition of the cellular space into three main compartments (Fig. 9). One surrounds the network, is continuous at any time, and represents the cytoplasmic space proper bounded on one side by the cell membrane and on the other by the membrane of the endoplasmic reticulum. The other is enclosed by the limiting membrane of this system; it is usually referred to as the intracisternal space; it is occupied by ingested material or secretory products and represents a buffer compartment between the internal and external medium of the cell. As already mentioned, the cell can operate this space as a continuum or as a succession of temporarily isolated, secondary compartments. The third major compartment is bounded by the nuclear envelope and is occupied by the nucleus.

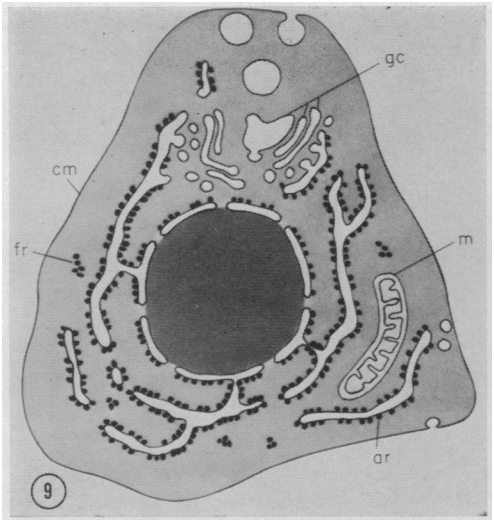
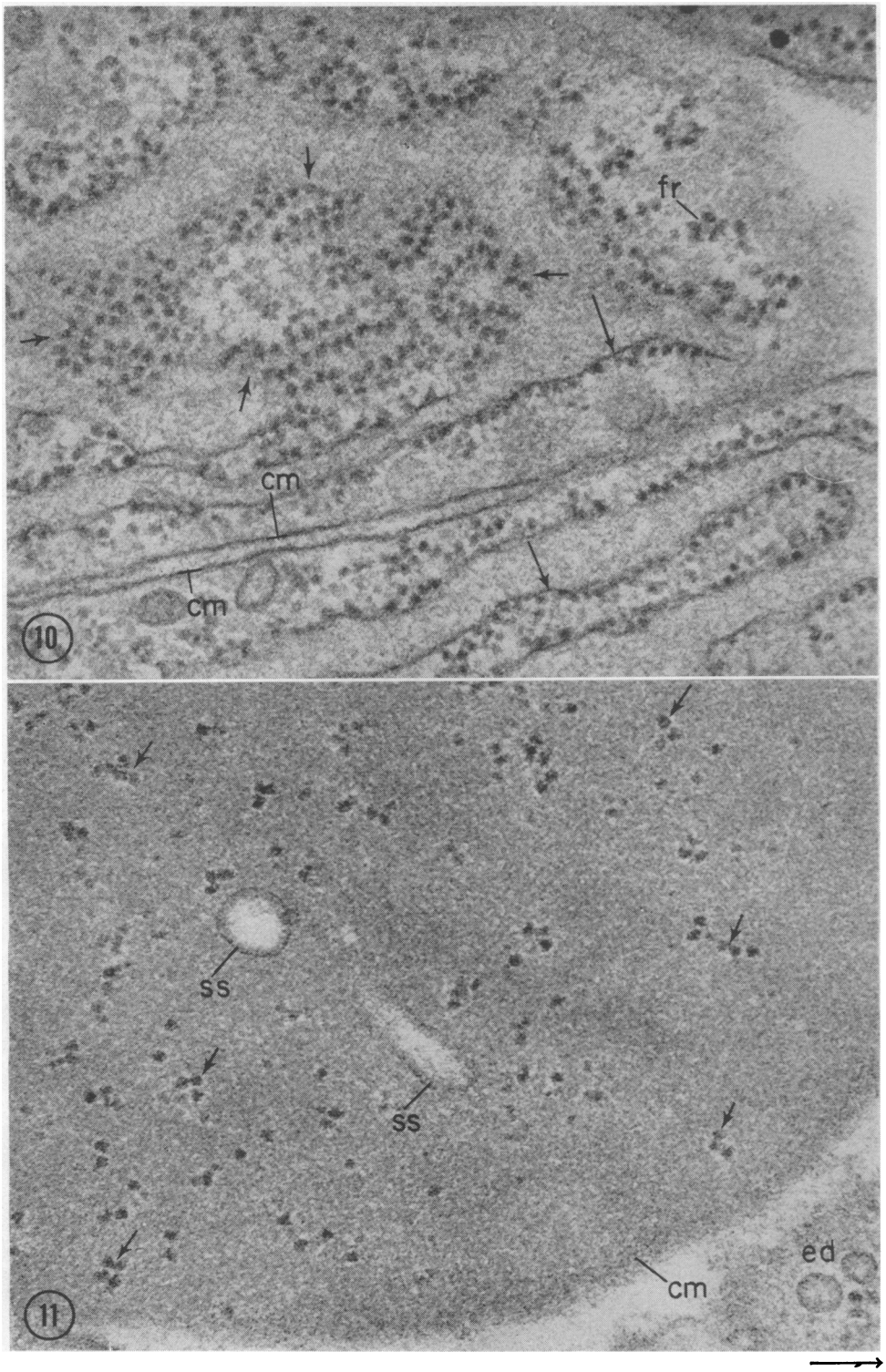


FIG. 9.—Diagram showing the three main compartments of a cell: *Light grey*, intracisternal space divided in this case into a series of secondary compartments; *grey*, cytoplasmic matrix which contains as a rule a series of subcompartments (like the mitochondrion marked *m*); *deep grey*, nucleus. The external medium of the cell appears in light grey like the content of the intracisternal space. This does not mean that they are identical.

The ribosomes populate the cytoplasmic space proper and occur either free in the cytoplasmic matrix, or attached to the membrane of the endoplasmic reticulum. Usually they are disposed in clusters when free[29] (Fig. 10), or in rows when attached[24] (Fig. 11). This tendency to form more or less characteristic groupings *in situ* may be related to the current hypothesis derived from work with isolated ribosomes, that the active form of this subcellular component is a polyribosome (or polysome) rather than an isolated particle.[30] The total ribosomal population varies with the protein output of the cell; free particles predominate when proteins are synthesized for intracellular use, and attached particles when proteins are produced for export,[24] as in the glandular cell of the previous example. The attachment of the particles to the membrane of the endoplasmic reticulum is related to the already discussed functions of this system.

As in the case of mitochondria, the mass isolation of microsomes[7] (i.e., fragments of the endoplasmic reticulum) and ribosomes[24, 31] has opened a remarkably fertile field of research which has already established that the ribosomes consist of about equal amounts of RNA and protein, have a particle weight of $\sim 4 \times 10^6$, are comprised of two unequal subunits, and represent the basic piece of cellular equipment for protein synthesis.[31] The work on ribosomes has strengthened the view that there is well-defined functional specialization among subcellular components, but at the same time has brought forward, more forcefully than before, the concept of necessary integration among various cell organs or subcellular components.

The ribosomes apparently receive the template of the protein to be synthesized from the nucleus, more exactly from one or more cistrons in a certain chromosome in the form of a messenger-RNA molecule[32]; they accept amino acids activated by enzymes located in the cytoplasmic matrix[33] and brought to the ribosomes by transfer (soluble) RNA molecules[34]; they use a polymerase to link these amino acids by peptide bonds[35]; and finally discharge the ensuing protein either directly into the cytoplasmic matrix or across the membrane of the endoplasmic reticulum into the cisternal space, depending on final destination of the product. The energy needed for all these operations is supplied mostly by the mitochondria.


fr
cm
cm
10
ss
ss
cm
ed
11

In this case, the salient aspect is the extent of integration, which is superimposed on functional specialization, and apparently involves practically all important cell organs. This implies elaborate coordination which in part may have a structural basis. The fact that the extent of supplementation, by "cell sap" components, needed for full activity varies considerably among ribosomal preparations[36] suggests that the "soluble" enzymes and carriers involved in the preliminary steps of protein synthesis are organized in functional complexes centered on ribosomes or polysomes. Presumably, most of these structures are destroyed by our preparation procedures.

Cytoplasmic matrix: In addition to ribosomes and ancillary apparatus, the cytoplasmic matrix contains, in unknown degrees of organization, most of the enzymes of the intermediary metabolism of the cell, including those involved in preliminary or alternative pathways of ATP synthesis, such as anaerobic glycolysis[37] and phosphogluconate oxidation.[38] It also accommodates the metabolic reserves of the cell, in the form of polysaccharide (glycogen) granules[39] or lipide droplets, and its aqueous phase contains the main, but not the only cellular pool of soluble precursors. The matrix also houses a population of contractile protein molecules, varied in size and degree of organization, which is responsible for most cell movements.

Nucleus: At this point we can leave the cytoplasm, although the inventory of local structural patterns is still far from being exhausted,[40] and move into the nucleus (Fig. 12) as if tracing back the path followed by messenger RNA's. They probably reach the cytoplasm through the numerous pores which interrupt the nuclear envelope,[25] but within the nucleus their pathway is practically unknown except for the starting point which is assumed to be on a chromosome, or more precisely, on a DNA molecule. Although the nucleus was the first cell component to be isolated in mass almost a century ago,[41] and although reasonably good preparations of whole nuclei can be obtained,[42, 43] the isolation of subnuclear components, such as chromatin, chromosomes, and nucleoli, has been less successful.[43] Even the morphological analysis of the nucleus is much less advanced than that of the cytoplasm. The enzymes involved in the replication of the genetic code (DNA) appear to be located, at least in part, in the nucleus,[44] together with those which carry through the transcription of the code into messenger RNA's.[45] Moreover, there are indications that all cytoplasmic RNA's, including ribosomal and soluble RNA's, are synthesized in the nucleus,[46] but the evidence in case is still questioned. Finally, the possible role of certain nuclear proteins, the histones, in the repression or activation of genes, begins to be analyzed.[47] Yet the components directly involved in all these operations probably represent a small fraction of the mass of the nucleus. The rest is still in the shadows. There is, however, sub-

FIG. 10.Parts— of two adjacent fibroblasts (rat myocardium). Two distended, branching, rough-surfaced cisternae appear in the field. Their limiting membrane is cut normally in some places (long arrows) and obliquely in others. A grazing section, giving a full-face view of the membrane, appears between short arrows. Note the patterns (rows, double rows, and spirals) formed by the attached ribosomes on this membrane patch and in other places where the membrane is cut obliquely. Note also the clusters formed by the free ribosomes. ×85,000.

FIG. 11.—Part of a young red blood cell (rat reticulocyte). All ribosomes are free and many of them appear in small clusters or short rows (arrows). The endoplasmic reticulum is poorly developed and consists only of smooth-surfaced elements. The cell membrane obliquely cut appears as a broad, poorly defined band. *ed* marks an adjacent endothelial cell. ×85,000.

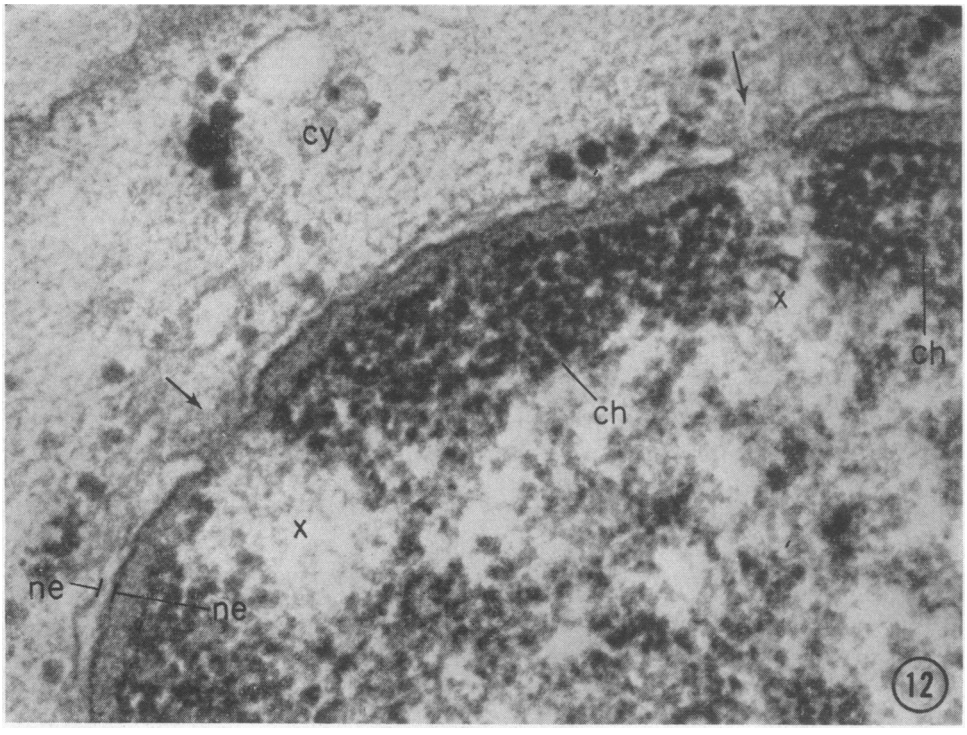


FIG. 12.—Part of the nucleus of a smooth-muscle cell (frog skin artery). The two membranes of the nuclear envelope are marked *ne*, and two nuclear pores are indicated by arrows. The dense masses of "granular" texture (*ch*) are probably chromosomes of this resting nucleus. The lighter material that fills the "channels" (*x*) leading to the nuclear pores is mostly protein. A band of dense, relatively homogeneous material separates, in this case, the chromosomes from the nuclear envelope. ×120,000.

stantial evidence that at least in some cell types the nucleus comprises a protein-synthesizing system similar to that of the cytoplasm.[48] This finding brings forward what appears to be still another principle of cell organization, namely the partial decentralization (in terms of cell compartments) of certain basic processes such as protein synthesis. Recent evidence suggests that autonomous protein-synthesizing systems operate not only in the nucleus but also in chloroplasts[49] and mitochondria,[50] possibly supported in part by a local autonomous DNA code.[51, 61] The situation has been regarded as vestigial symbiosis[52]; it could be just as well viewed as intracellular differentiation.

Cell membrane: The entire cell is separated from its external medium by a thin (~80 Å) membrane which appears to be a stratified structure comprised of a bimolecular layer of polar lipids covered on both sides by protein films.[53] It controls the exchanges between the aqueous content of the cell and its equally aqueous surroundings first by interposing a practically impermeable barrier, the bimolecular lipid leaflet, and then by modifying this barrier through a variety of means envisaged at present as pores, carriers, and energy-driven pumps, all operating on individual molecules or ions.[54] The result is selective permeability which has basic features common to the generality of cells, but details characteristic to each cell type. The cell membrane is probably a mosaic of functional units whose

heterogeneity is compounded by the fact that local differentiations affecting stratification, thickness,[55] and enzyme activities (presumably connected with active transport) may appear on parts of the membrane exposed to different media.[56]

The cell apparently controls not only exchanges between its external and internal medium but also exchanges among the various compartments in which its own internal medium is subdivided. The membranes limiting such compartments (endoplasmic reticulum, mitochondria, etc.) are known to be rich in polar lipids,[7] generally have a layered structure comparable to that of the cell membrane,[57] show complex enzymatic activities,[58] and at least in some cases are known to be semipermeable.[18, 59]

Evolution and Differentiation at the Cellular Level.—Since the structural framework described is that of an animal cell in an advanced state of differentiation, one can ask to what extent the prototype of the framework, if such exists, is affected by this condition. The answer is given by the following examples, which represent a precipitous journey against the double current of differentiation and evolution towards more simple, supposedly earlier forms of the framework.

An undifferentiated animal cell consists of the usual combination of structural patterns. The differences are only quantitative: it has a less well-developed endoplasmic reticulum and a larger population of clustered, free ribosomes than its differentiated counterpart.[24]

An algal cell has a nucleus, mitochondria, an endoplasmic reticulum, Golgi complexes, and ribosomes[60] built along the same patterns as in animal cells (Fig. 13). In addition, algal and plant cells in general contain an important organ specialized in photosynthesis the chloroplast, which, like the mitochondrion, has a large amount of internal membranous material but shows more elaborate secondary compartmentation.[61] The organization of a fungus cell (hypha)[62] (Fig. 14) is even closer to that of an undifferentiated animal cell. In a bacterial cell, however, the situation changes (Fig. 15). The cell volume is about 500 times smaller than that of an animal cell. The only easily recognizable components are the cell membrane and the ribosomes, which have been extensively studied and found to be comparable in structure and function to those of animal cells. The rest is definitely different. A central irregular zone in the protoplasm is occupied by fine fibrils, probably DNA molecules.[63, 64] This nuclear zone is sometimes in contact with membrane impocketings, called mesosomes,[65] which are presumably modest precursors of mitochondria.[66] The cell has no recognizable endoplasmic reticulum, no perinuclear cisterna, hence no well-defined nucleus. Moreover, bacterial cells have no recognizable mitochondria, although it is known that they are effective in oxidative phosphorylation and that this activity is associated with a membrane fraction, presumably derived from the cell membrane[67] and possibly from mesosomes.

It seems, therefore, that in this case the rule "same function—same structure," which clearly applies for ribosomes, is no longer respected. Yet if the existence and functional role of elementary particles be satisfactorily established in animal mitochondria, a new explanation becomes possible. A combination of elementary respiratory and phosphorylating particles may exist in all living systems from bacterial to animal cells, and the differences encountered may concern only a secondary superimposed structure, namely the membranous framework to which

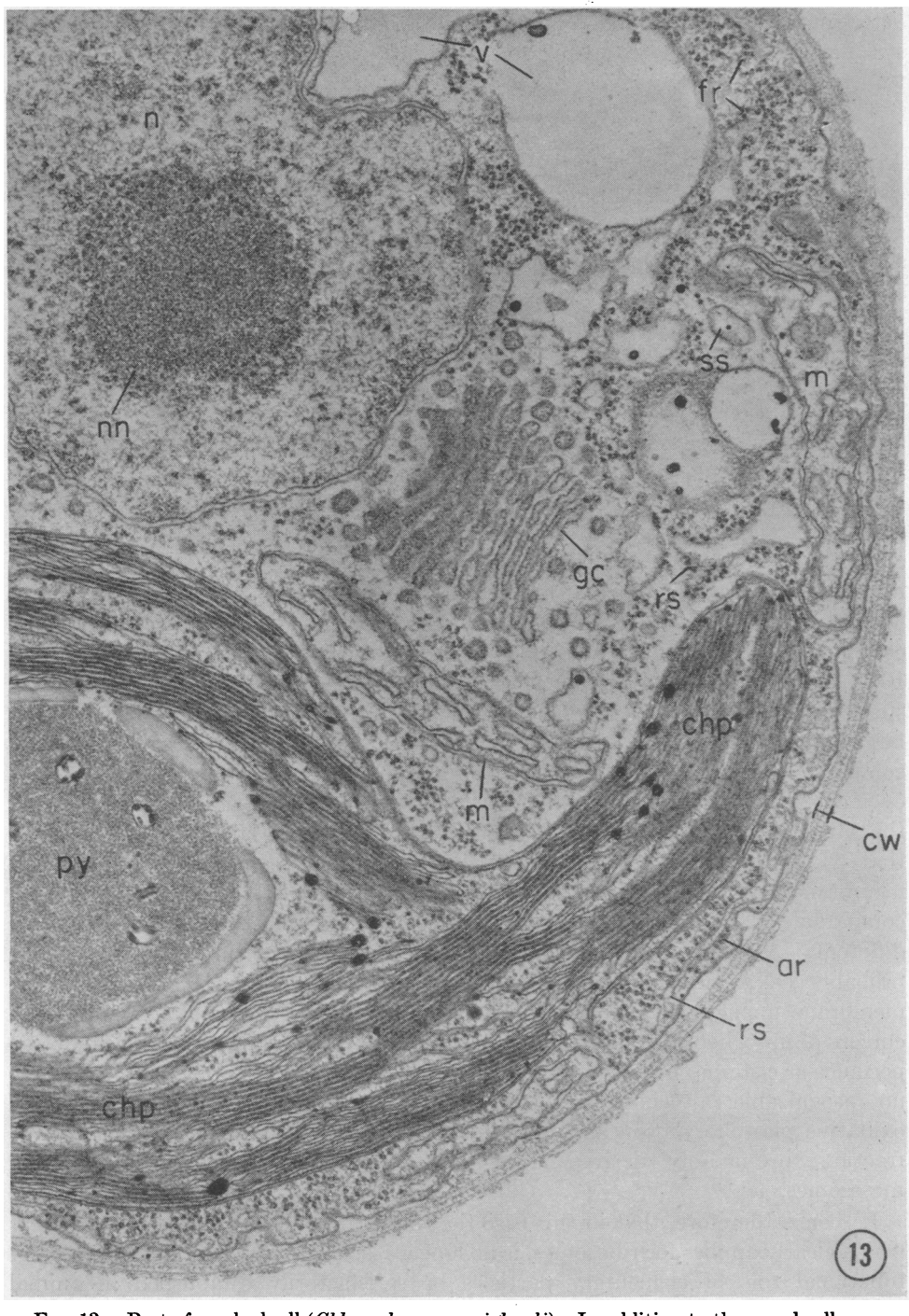


FIG. 13.—Part of an algal cell (*Chlamydomonas reinhardi*). In addition to the usual cell organs, i.e., nucleus (*n*) with a large nucleolus (*nn*), mitochondria (*m*), Golgi complex (*gc*), rough- (*rs*) and smooth-surfaced (*ss*) elements of the endoplasmic reticulum, attached- (*ar*) and free (*fr*) ribosomes, the cell contains a large chloroplast (*chp*) with a pyrenoid (*py*). Vacuoles are marked *v* and the cell wall *cw*. ×25,000.

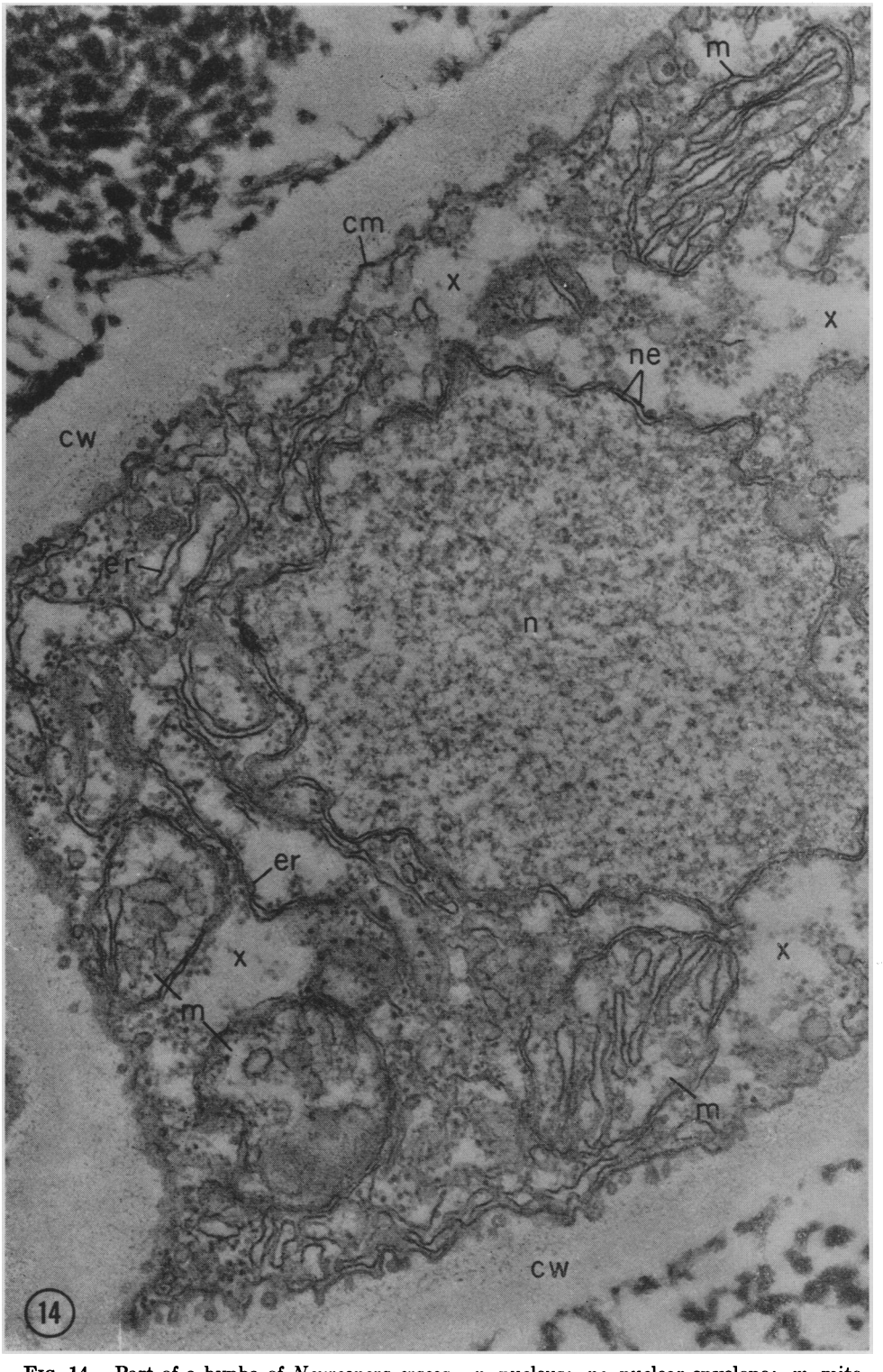
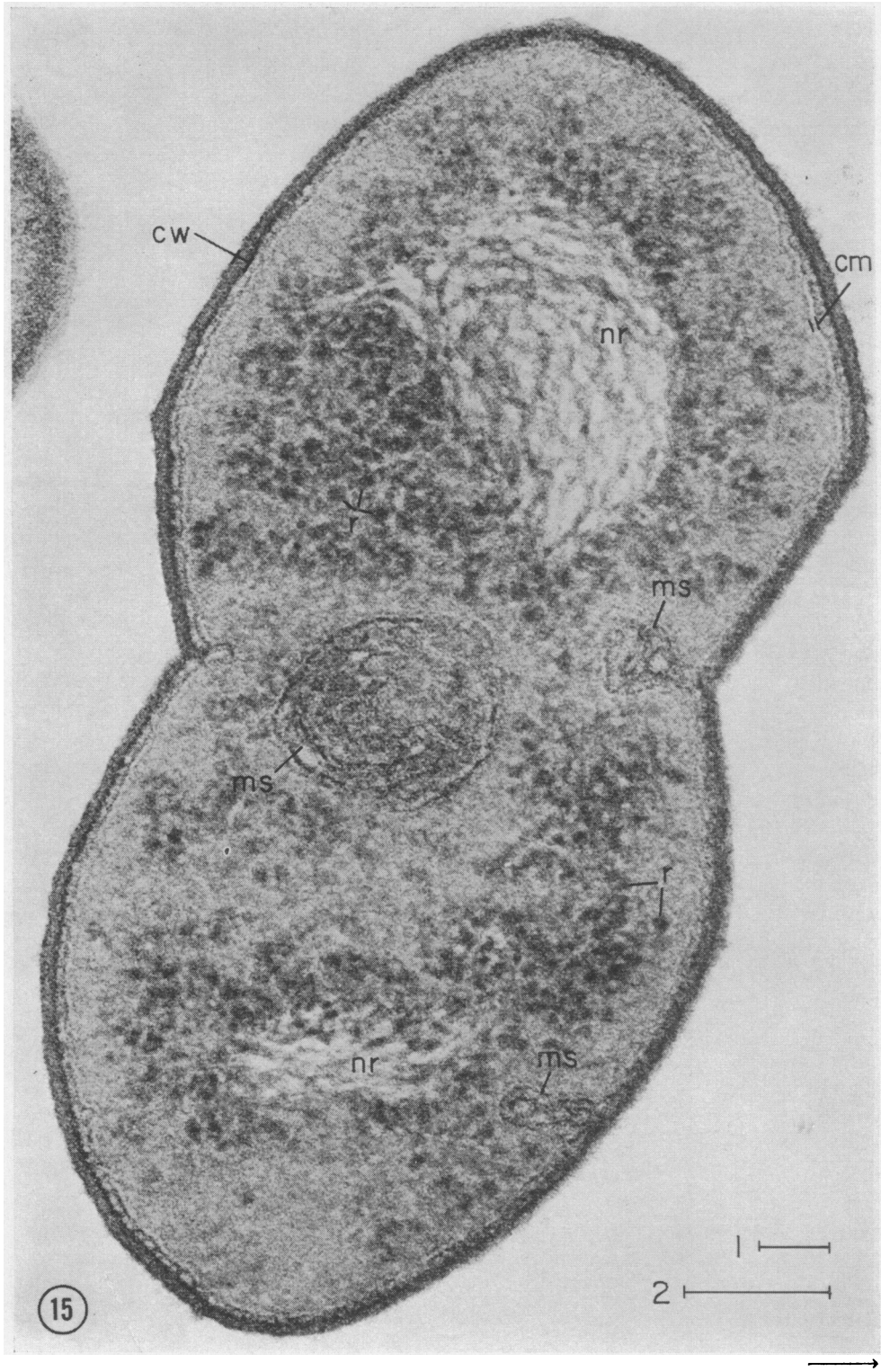


FIG. 14.—Part of a hypha of *Neurospora crassa*. *n*, nucleus; *ne*, nuclear envelope; *m*, mitochondria; *er*, elements of the endoplasmic reticulum, most of them smooth surfaced; *cm*, cell membrane; *cw*, cell wall. Most of the ribosomes of the cell are free in the cytoplasmic matrix. The light material in regions marked *x* is polysaccharide. Courtesy of Dr. D. J. L. Luck, The Rockefeller Institute. ×65,000.


cw
cm
nr
r
ms
ms
r
nr
ms
1
2
15

the particles are attached or in which they are embedded. Of course it remains to be seen first whether "elementary particles" can be identified at the surface of the cell membrane or of the mesosomes in bacterial cells. A similar situation may apply to the variety of extant photosynthesizing structures. An elementary unit, the "quantasome," has been recently described as being present in or on chloroplast membranes.[68] Here again the discrepancy between similar function but dissimilar structure could be explained by the existence of a common elementary particle attached to a membranous framework of increasing degrees of elaboration from photosynthesizing bacteria to blue-green algae and green plants.

Although it is known that the genetic code consists of DNA in bacterial as well as animal cells, and although chromosomes are supposed to exist in all cells, morphological studies have clearly shown that the bacterial DNA strand and the animal cell chromosome do not represent equivalent levels of organization. Here again the existence of a common elementary particle close in dimensions and chemistry to the bacterial nuclear fibrils and the addition of a series of secondary complications introduced by the folding and packing of such structures could explain the difference. Bacterial flagella and animal-cell cilia may be related in a similar manner.

The main conclusion of this survey is that there are already far-reaching distinctions among the units we call cells. Recent work has stressed the extensive and striking similarity of many subcellular patterns from fungi to mammals; it has also revealed that the structure of bacteria has little in common with that of animal or plant cells. Clearly there is no structural unity at the cellular level. Yet it is known that at the subjacent macromolecular level of organization all cells are, generally speaking, similar. Their common functions presuppose similarly integrated multienzyme systems, and these in turn may be expected to form similarly structured organs upon their assembly. These contradictions to the widely accepted concept of the unitary organization of living matter could be explained by assuming that the various functions now supposed to be connected with definite cell organs are actually carried out by smaller elementary particles of which the ribosomes would be the first relatively well-defined example, and the "oxisomes"[69] (mitochondrial elementary particles) and "quantasomes" the next possible candidates.

The bacterial cell apparently represents the minimal but sufficient formula for the cellular level in the hierarchy of patterns of living matter. We can assume that its emergence was a crucial event in the history of life: it made replication possible, or greatly improved its efficiency; it established life and thereby triggered the evolution process. The next difficult step was probably the addition of one or two more levels of organization to the structural framework of the first simple cells leading to the emergence of the more complex animal and plant cells. The systems which succeeded in making this step apparently acquired more independ-

FIG. 15.—Bacterial cell (*Diplococcus pneumoniae*) in the process of division. The nuclear regions (*nr*) contain a network of fine fibrils, and are surrounded by masses of ribosomes (*r*). The protoplasm contains, in addition, three mesosomes (*ms*), two of which are in continuity with the cell membrane. The cell wall is marked *cw*. Courtesy of Drs. A. Tomasz and J. Jamieson, The Rockefeller Institute. ×250,000. Marker 1 gives the transverse diameter of this cell at a magnification equal to that of the alga in Fig. 13, and marker 2 at the same magnification as the pancreatic cell in Fig. 5.

ence from the external medium; the possibility of using other energy sources than residual organic compounds of pre- or postbiotic origin; the ability of handling matter in mass; and the possibility of accommodating a more and more elaborate genome and of controlling its activity in time. An elaborate and controllable genome was probably the prerequisite for cell differentiation, hence for the emergence of multicellular plants and animals. Examined at the cellular level, all that followed this step appears as a rather easy exercise: a long series of variations on a common, durable, and versatile theme. It is worthwhile noting that the simple and the complex cell type stand out as sharp discontinuities in the spectrum of still extant cellular forms.

Whenever possible, I followed a historical approach in this presentation, to indicate that recent findings and present concepts are only the last approximation in a long series of similar attempts which, of course, is not ended. Time will tell how far we are at present from our final goal: the full understanding of the organization of living matter.

[1] Mostly C, N, H, O, P, and S supplemented with a series of trace elements.

[2] In the morphological sense.

[3] The abbreviations used in this article are: DNA, deoxyribonucleic acid; RNA, ribonucleic acid; ATP, adenosinetriphosphate.

[4] Michaelis, L., *Arch. f. mikroskop. Anat. Entwicklungsmech.*, **55**, 558 (1900).

[5] Palade, G. E., *J. Histochem. Cytochem.*, **1**, 188 (1953); Sjöstrand, F. S., and J. Rhodin, *Exptl. Cell Res.*, **6**, 426 (1953).

[6] Fernández-Morán, H., *Circulation*, **26**, 1039 (1962).

[7] Claude, A., *J. Exptl. Med.*, **84**, 51 and 61 (1946); Hogeboom, G. H., W. C. Schneider, and G. E. Palade, *J. Biol. Chem.*, **172**, 619 (1948).

[8] Hogeboom, G. H., A. Claude, and R. D. Hotchkiss, *J. Biol. Chem.*, **165**, 615 (1946).

[9] Kennedy, E. P., and A. L. Lehninger, *J. Biol. Chem.*, **179**, 957 (1949).

[10] Schneider, W. C., *J. Biol. Chem.*, **176**, 259 (1948); Schneider, W. C., and V. R. Potter, *J. Biol. Chem.*, **177**, 893 (1949).

[11] In retrospect, it was realized that the characteristic staining of mitochondria with Janus green was due to the local concentration of oxidative enzymes, cf. Lazarrow, A., and S. J. Cooperstein, *J. Histochem. Cytochem.*, **1**, 234 (1953).

[12] Lehninger, A. L., *Harvey Lectures*, **49**, 176 (1954–55); Green, D. E., *Harvey Lectures*, **52**, 177 (1956–57); Chance, B., and G. R. Williams, *Advan. Enzymol.*, **17**, 65 (1956).

[13] Estabrook, R. W., and A. Holowinsky, *J. Biophys. Biochem. Cytol.*, **9**, 19 (1961).

[14] Blair, P. V., T. Oda, D. E. Green, and H. Fernández-Morán, *Biochemistry*, **2**, 756 (1963).

[15] Chance, B., D. F. Parsons, and G. R. Williams, *Science*, **143**, 136 (1964).

[16] Brierley, G. P., E. Bachmann, and D. E. Green, these PROCEEDINGS, **48**, 1928 (1962); De Luca, H. F., G. W. Engstrom, and H. Rasmussen, these PROCEEDINGS, **48**, 1604 (1962); Vasington, F. D., and J. V. Murphy, *J. Biol. Chem.*, **237**, 2670 (1962).

[17] Parsons, D. F., *Science*, **140**, 985 (1963); Stoeckenius, W., *J. Cell Biol.*, **17**, 443 (1963).

[18] Lehninger, A. L., *J. Biol. Chem.*, **190**, 345 (1951); Van den Bergh, S. G., and E. C. Slater, *Biochem. J.*, **82**, 362 (1962).

[19] Lehninger, A. L., C. S. Rossi, and J. W. Greenawalt, *Biochem. Biophys. Res. Comm.*, **10**, 444 (1963).

[20] Peachey, L. D., *J. Cell Biol.*, **20**, 95 (1964).

[21] Garnier, Ch., *J. anat. et physiol.*, **36**, 22 (1900).

[22] Mathews, A., *J. Morphol.*, **15** (suppl.), 171 (1899).

[23] Porter, K. R., *J. Exptl. Med.*, **97**, 727 (1953), and "The ground substance; observations from electron microscopy," in *The Cell*, ed. J. Brachet and A. E. Mirsky (New York: Academic Press, 1961), vol. 2, p. 621; Palade, G. E., *J. Biophys. Biochem. Cytol.*, **2** (suppl.), 85 (1956).

[24] Palade, G. E., *J. Biophys. Biochem. Cytol.*, **1**, 59 (1955); Palade, G. E., and P. Siekevitz, *J. Biophys. Biochem. Cytol.*, **2**, 171 and 671 (1956).

[25] Dalton, A. J., and M. D. Felix, *Am. J. Anat.*, **94**, 171 (1954); Dalton, A. J., "Golgi apparatus and secretion granules," in *The Cell*, ed. J. Brachet and A. E. Mirsky (New York: Academic Press, 1961), vol. 2, p. 603.

[26] Watson, M. L., *J. Biophys. Biochem. Cytol.*, **1**, 257 (1955).

[27] Caro, L. G., and G. E. Palade, *J. Cell Biol.*, **20**, 473 (1964).

[28] de Duve, Ch., B. C. Pressman, R. Gianetto, R. Wattiaux, and F. Appelmans, *Biochem. J.*, **60**, 604 (1955); de Duve, Ch., "The lysosome concept," in *Ciba Foundation Symposium on Lysosomes*, ed. A. V. S. de Reuck and M. P. Cameron (London: J. & A. Churchill Ltd., 1963), p. 1; Novikoff, A. B., "Lysosomes in the physiology and pathology of cells; contributions of staining methods," in *Ciba Foundation Symposium on Lysosomes*, ed. A. V. S. de Reuck and M. P. Cameron (London: J. & A. Churchill Ltd., 1963), p. 36; and "Lysosomes and related particles," in *The Cell*, ed. J. Brachet and A. E. Mirsky (New York: Academic Press, 1961), vol. 2, p. 423.

[29] Palay, S. L., and G. E. Palade, *J. Biophys. Biochem. Cytol.*, **1**, 69 (1955).

[30] Warner, J. R., A. Rich, and C. E. Hall, *Science*, **138**, 1399 (1962); Wettstein, F. O., T. Staehelin, and H. Noll, *Nature*, **197**, 430 (1963).

[31] Roberts, B. R., ed., *Microsomal Particles and Protein Synthesis* (New York: Pergamon Press, 1958); Ts'o, P. O. P., *Ann. Rev. Plant Physiol.*, **13**, 45 (1962).

[32] Jacob, F., and J. Monod, *J. Mol. Biol.*, **3**, 318 (1961).

[33] Hoagland, M., *Biochim. Biophys. Acta*, **16**, 288 (1955); Hoagland, M., E. B. Keller, and P. C. Zamecnik, *J. Biol. Chem.*, **218**, 345 (1956).

[34] Holley, R. W., *J. Am. Chem. Soc.*, **79**, 658 (1957); Hoagland, M. B., M. L. Stephenson, J. F. Scott, L. I. Hecht, and P. C. Zamecnik, *J. Biol. Chem.*, **231**, 241 (1958); Zamecnik, P. C., *Harvey Lectures*, **54**, 256, 1958–59.

[35] Nathans, D., J. E. Allende, T. W. Conway, G. J. Spyrides, and F. Lipmann, in *Symposium on Informational Macromolecules* (New York: Academic Press, 1963), p. 349; E. B. Keller and M. F. Ferger, *Federation Proc.*, **23**, 164 (1964).

[36] Webster, G., and J. B. Lingrel, "Protein synthesis by isolated ribosomes," in *Protein Biosynthesis*, ed. R. J. C. Harris (London & New York: Academic Press, 1961), p. 301; Raake, J. D., *Biochim. Biophys. Acta*, **34**, 1 (1959).

[37] Le Page, G. A., and W. C. Schneider, *J. Biol. Chem.*, **176**, 1021 (1948); Johnson, M. R., *Biochem. J.*, **77**, 610 (1960).

[38] Newberg, R. W., V. H. Cheldelin, *J. Biol. Chem.*, **218**, 89 (1956).

[39] Porter, K. R., and C. Bruni, *Cancer Res.*, **19**, 997 (1959); *Anat. Rec.*, **136**, 260 (1960); Revel, J. P., L. Napolitano, and D. W. Fawcett, *J. Biophys. Biochem. Cytol.*, **8**, 575 (1960).

[40] Among subcellular components not covered by this discussion are: microbodies, multivesicular bodies, spindle fibers (tubules), centrioles, cilia, basal bodies, and microtubules, to name only the most frequently encountered.

[41] Miescher, F., *Hoppe-Seyler med. chem. Untersuch.*, **4**, 441 (1871).

[42] Roodyn, D. B., *Internat. Rev. Cytol.*, **8**, 279 (1959); Allfrey, V., "The isolation of subcellular components," in *The Cell*, ed. J. Brachet and A. E. Mirsky (New York: Academic Press, 1959), vol. 1, p. 193.

[43] Maggio, R., P. Siekevitz, and G. E. Palade, *J. Cell Biol.*, **18**, 267 and 293 (1963).

[44] Keir, H. M., R. M. S. Smellie, and G. Siebert, *Nature*, **196**, 752 (1962); Smith, M. J., and H. M. Keir, *Biochim. Biophys. Acta*, **68**, 578 (1963).

[45] Weiss, S. B., and L. Gladstone, *J. Am. Chem. Soc.*, **81**, 4118 (1959); Weiss, S. B., these PROCEEDINGS, **46**, 1020 (1960).

[46] Prescott, D. M., "RNA synthesis in the nucleus and RNA transfer to the cytoplasm in *Tetrahymena piriformis*," in *Biological Structure and Function*, ed. T. W. Goodwin and O. Lindberg (New York: Academic Press, 1961), vol. 2, p. 527; cf. also Yankofsky, S. A., and S. Spiegelman, these PROCEEDINGS, **49**, 538 (1963).

[47] Huang, R. C., and J. Bonner, these PROCEEDINGS, **48**, 1216 (1962); Allfrey, V. G., and A. E. Mirsky, these PROCEEDINGS, **48**, 1591 (1962); Frenster, J. H., V. G. Allfrey, and A. E. Mirsky, these PROCEEDINGS, **50**, 1026 (1963).

[48] Allfrey, V. G., and A. E. Mirsky, "Amino acid transport into the cell nucleus and reactions governing nuclear protein synthesis," in *Protein Biosynthesis*, ed. R. J. C. Harris (London & New York: Academic Press, 1961), p. 49.

[49] Stephenson, M. L., K. V. Thimann, and P. L. Zamecnik, *Arch. Biochem. Biophys.*, **65**, 194 (1956); App, A. A., and A. T. Jagendorf, *Biochim. Biophys. Acta*, **76**, 286 (1963); Eisenstadt, J., and G. Brawerman, *Biochim. Biophys. Acta*, **76**, 319 (1963).

[50] Roodyn, D. B., P. J. Reis, and T. S. Work, *Biochem. J.*, **80**, 9 (1961); Roodyn, D. B., J. W. Suttie, and T. S. Work, *Biochem. J.*, **83**, 29 (1962); Kroon, A. M., *Biochim. Biophys. Acta*, **72**, 391 (1963).

[51] Chun, E. H. L., M. H. Vaughan, Jr., and A. Rich, *J. Mol. Biol.*, **7**, 130 (1963); Sager, R., and M. R. Ishida, these PROCEEDINGS, **50**, 725 (1963); Kirk, J. T. O., *Biophys. Biochem. Acta*, **76**, 417 (1963); Gibor, A., and M. Izawa, these PROCEEDINGS, **50**, 1164 (1963); Nass, M., and S. Nass, *J. Cell. Biol.*, **19**, 593 and 613 (1963).

[52] Ris, H., and E. Plaut, *J. Cell Biol.*, **13**, 383 (1962).

[53] Robertson, J. D., *Prog. Biophys. Biophys. Chem.*, **10**, 343 (1960).

[54] Wilbrandt, W., *Int. Rev. Cytol.*, **13**, 203 (1962); Christensen, H. N., *Biological Transport* (New York: W. A. Benjamin, Inc., 1962).

[55] Sjöstrand, F. S., *J. Ultrastruct. Res.*, **8**, 517 (1963); Farquhar, M. G., and G. E. Palade, *J. Cell Biol.*, **17**, 375 (1963).

[56] Essner, E., A. B. Novikoff, and B. Masek, *J. Biophys. Biochem. Cytol.*, **4**, 711 (1958); Farquhar, M. G., and G. E. Palade, these PROCEEDINGS, **51**, 569 (1964).

[57] Robertson, J. D., *Biochem. Soc. Symp.*, **16**, 3 (1959); Yamamoto, T., *J. Cell Biol.*, **17**, 413 (1963).

[58] Ernster, L., P. Siekevitz, and G. E. Palade, *J. Cell Biol.*, **15**, 541 (1962); Novikoff, A. B., E. Essner, S. Goldfischer, and M. Heus, "Nucleosidephosphatase activities in cytomembranes," in *The Interpretation of Ultrastructure*, ed. R. J. C. Harris (New York & London: Academic Press, 1962), p. 149.

[59] Tedeschi, H., and D. L. Harris, *Biochim. Biophys. Acta*, **28**, 392 (1958); Tedeschi, H., J. James, and W. Anthony, *J. Cell Biol.*, **18**, 503 (1963).

[60] Sager, R., and G. E. Palade, *J. Biophys. Biochem. Cytol.*, **3**, 463 (1957).

[61] Granick, S., "The chloroplast: inheritance, structure and function," in *The Cell*, ed. J. Brachet and A. E. Mirsky (New York: Academic Press, 1961), vol. 2, p. 489.

[62] Luck, D. J. L., *J. Cell Biol.*, **16**, 483 (1963); Zalokar, M., *J. Biophys. Biochem. Cytol.*, **9**, 731 (1961); Blondel, B., and G. Turian, *J. Biophys. Biochem. Cytol.*, **7**, 207 (1960).

[63] Robinow, C. F., "Outline of the visible organization of bacteria," in *The Cell*, ed. J. Brachet and A. E. Mirsky (New York: Academic Press, 1960), vol. 4, p. 45; Ryter, A., and E. Kellenberger, *Z. Naturforsch.*, **13b**, 597 (1958).

[64] A similar structure in a cell unit of even smaller dimensions ($d \sim 0.2\ \mu$) has been reported in Mycoplasmataceae (microorganisms of pleuropneumonia group) which at present are assumed to be the smallest free living cells (van Iterson, W., *J. Ultrastruct. Res.*, **3**, 282 (1960)).

[65] Glauert, A. M., and D. A. Hopwood, *J. Biophys. Biochem. Cytol.*, **7**, 479 (1960); van Iterson, W., *J. Biophys. Biochem. Cytol.*, **9**, 183 (1961); Fitz-James, P. C., *J. Biophys. Biochem. Cytol.*, **8**, 507 (1960).

[66] Vander Winkel, E., and R. G. E. Murray, *J. Ultrastruct. Res.*, **7**, 185 (1962); van Iterson, W., *J. Cell Biol.*, **20**, 361 (1964).

[67] Weibull, C., *J. Bact.*, **66**, 696 (1953); Marr, A. G., "Localization of enzymes in bacteria," in *The Bacteria*, ed. J. C. Gunsalus and R. Stanier (New York: Academic Press, 1960), vol. 1, p. 443.

[68] Park, R. B., and N. G. Pon, *J. Mol. Biol.*, **3**, 1 (1961).

[69] Chance, B., R. W. Estabrook, and C. P. Lee, *Science*, **140**, 379 (1963).