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AND RELATED SUBJECTS OF BIOCHEMISTRY

Edited by F. F. NORD

FORDHAM UNIVERSITY, NEW YORK, N. Y.

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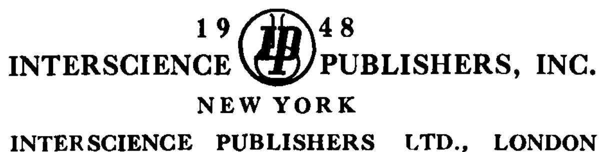

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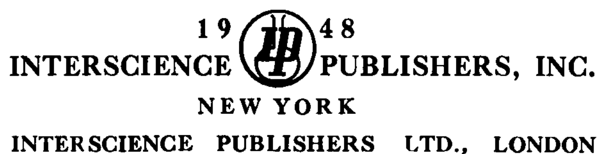
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FUNCTIONING OF THE CYTOPLASM

By LUDWIK MONNÉ, *Stockholm, Sweden*

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I. Introduction

In this article a critical and concise review of the recent literature dealing with the structure and the function of the protoplasm is presented. Moreover, an attempt is made to synthesize our knowledge and to give a consistent picture of the functioning of the cytoplasm. At the present moment this task does not appear to be too daring because of the great progress made during the last ten years in the field of chemistry and submicroscopic morphology of the protoplasm. A general survey of this field may be of some value for further investigations, even if the gaps in our knowledge must be filled by some hypotheses. At any rate, there will be a clear distinction between well-established facts and theories.

Structure and function of the protoplasm are intimately corre-

lated with each other. In biology, structure and function are two concepts similar to matter and energy in physics; they are merely two different aspects of the same thing. Any structure is the expression of the function of the protoplasm. Intimate collaboration between morphologists and physiologists is necessary in order to elucidate the life phenomena of the protoplasm. This collaboration has yielded the most splendid results in the study of heredity, but it is still very feeble in investigations dealing with the life phenomena of the cytoplasm, particularly with the mechanics of embryological differentiation. This article is therefore devoted to the regular, microscopic, and submicroscopic structural changes underlying any function of the cytoplasm.

II. Substrata of the Basic Life Phenomena

Anabolism, catabolism, and irritability are the three basic life phenomena. Growth, reproduction, and development are the expressions of the anabolic activity of the living substance. Irritability is the faculty of the purposeful regulation of both the anabolic and catabolic activities of the protoplasm.

Teleological thinking is required in order to describe life phenomena adequately. Causal thinking is necessary in order to find a scientific explanation of the indubitable purposefulness in nature (56). This purposefulness cannot be explained by assuming the existence of a mystic agent. It must be sought in the organization of the living substance. The power of reacting purposefully persists even when the organization of the protoplasm is disturbed to a minor degree. If, however, this organization is severely disturbed, nonpurposeful and detrimental processes appear. This is the case in many pathological conditions. Life phenomena are purposeful because they are always the result of a compromise among a great variety of antagonistic, stimulating, and inhibiting agents: *p*H buffering is the simplest inanimate system exhibiting a certain "purposefulness" (56).

The following units of life should be distinguished:

I. Ultimate units of life (viruses, genes, microsomes, etc.)
II. Cells.
   1. Prokaryote cells (bacteria, *Cyanophyceae*).
   2. Eukaryote cells (cells of all other organisms).
III. Multicellular organisms.
IV. Colonies of multicellular organisms.

Nucleic acid is concerned with the anabolic activity of the protoplasm, particularly with protein synthesis (see page 58). This is evidenced by the following facts: The amount of nucleic acid is increased in all kinds of cells when growth is initiated (13,19). Nucleic acid is not diffusely distributed; it is present within small self-perpetuating bodies (chromomeres, chromidia = microsomes, macromolecules or particulates), which are the most essential constituents of the protoplasm. Moreover, the simplest viruses are pure nucleoproteins (148) that are able to grow and to reproduce themselves within host cells. The catabolic activity of the protoplasm is due to the enzymes of hydrolysis, respiration, fermentation, phosphorylation, etc. Lipides* are concerned with the irritability of the protoplasm (page 42).

Since the simplest viruses are pure nucleoproteins, they exhibit anabolic activity only. They contain no enzymes or lipides and therefore do not exhibit catabolism and irritability. The host cells provide the energy and the material necessary for the anabolic activity of these viruses. There exist all intermediary forms between viruses and bacteria. The highest viruses, such as vaccinia virus, contain some enzymes and lipides (148) and therefore may be endowed not only with anabolic activity but also with a certain catabolism and irritability. At any rate, anabolism, catabolism, and most probably, irritability are displayed by the chromidia (microsomes) extracted from numerous cells.

The simplest protoplasm is represented by viruses, which are, as stated above, pure nucleoproteins. The protoplasm of vaccinia virus has a slightly higher organization, and that of prokaryote and eukaryote cells an even higher organization. It has long been supposed that cells are composed of still smaller units exhibiting all the essential features of life (Hermann, Pflüger, Verworn, Altmann *et al.*; see 58 and 61). This is confirmed by recent investigations (for literature, see 13 and 42). Viruses should be regarded, at least in a restricted sense, as ultimate units of life. But cells cannot be regarded as ultimate units of life. The protoplasm of prokaryote and eukaryote cells is an organized system of several types of self-perpetuating nucleic-acid-containing bodies comparable to viruses. These bodies, however, are not free, but concatenated with each other into self-

* Phosphatides and cholesterol are chiefly in question here.

perpetuating fibrils (cytoplasmic fibrils, chromosomes, etc.). Consequently the cells must be regarded as organized systems of several kinds of self-perpetuating fibrillar components. The higher the organization of the living substance, the more differentiated are the life phenomena. The simplest are exhibited by the primitive viruses, the most complicated by multicellular organisms.

Eukaryotc cells have a higher organization than prokaryote cells. The former have a well-developed nucleus that forms chromosomes during mitosis. The latter contain a primitive nucleus (for literature, see 37) very different from the nucleus of eukaryote cells.

III. Structure of Cytoplasm

A. CHROMIDIA AND CYTOPLASMIC FIBRILS

In the first decennium of this century much effort was spent in order to prove that the chromatin regularly migrates from the nucleus into the cytoplasm. Hertwig denominated as chromidia all the cytoplasmic substances supposed to originate directly from the nuclear chromatin. The chromidial hypothesis was developed chiefly by Goldschmidt. For details and literature concerning this subject the reader is referred to Cowdry's book (30). It has been assumed that both the chromatin and the chromidia contain nucleic acid. Moreover, it has been demonstrated by Van Herwerden (see Brachet, 13) that the chromidia in preparations previously exposed to the action of enzymes which break down nucleic acid can no longer be stained with pyronine. Not only minute granules scattered throughout the whole cytoplasm, but also large inclusions of various shapes have been described as chromidia. Unfortunately only a few investigators have been able to distinguish chromidia from mitochondria. The two components of the cytoplasm have been confused by most cytologists, who, as a result, have denied the real existence of the chromidia.

There is no evidence that the chromidia orginate directly from the nuclear chromatin; therefore this opinion cannot be accepted by modern cytologists. The general idea, however, that certain cytoplasmic granules are similar to, although not identical with, chromatin is correct and is strongly supported by modern investigations. Moreover, the presence of nucleic acid within the chromidia should be regarded as definitely proved. Nevertheless, an important difference between the chromidia and the chromatin escaped the

previous workers in cytology, because no methods were available to distinguish ribo- from thymonucleic acid in microscopic preparations. These methods have been provided by the independent investigations of Brachet, Caspersson, and co-workers (for literature, see 13,42). It has been demonstrated by Caspersson and collaborators (Schultz, Hydén, Landström-Hydén, Aquilonius, *et al.*) that the cytoplasm of various rapidly growing cells is Feulgen negative and that it strongly absorbs ultraviolet light of the wave length 2600 Å; both properties are characteristic of pure ribonucleic acid. Moreover, it has been found that the chromosomes also strongly absorb the same ultraviolet light and that they are, in contrast to the cytoplasm, Feulgen positive; these properties are characteristic for pure thymonucleic acid. From these facts it has been concluded that ribonucleic acid is present within the cytoplasm and thymonucleic acid within the chromosomes. Another method has been invented by Brachet (13), who found that the cytoplasm of various cells is deeply stained with pyronine when the microscopic preparations are treated with Unnas dye mixture and that the staining fails to occur when the preparations are subjected to the action of the enzyme ribonuclease before treatment with the above-mentioned dye mixture. Thus, it is evident that staining with pyronine is due to ribonucleic acid and that it does not occur when this acid is removed under the influence of the above-mentioned enzyme. Ribonucleic acid is not diffusely distributed throughout the cytoplasm; it is present within minute granules only, long since described as chromidia. Only these granules absorb ultraviolet light, stain with pyronine, and are Feulgen negative.

Recent investigations definitely prove that the chromidia and the mitochondria are entirely different from each other. This is clearly evidenced by the fact that, in sea urchin eggs stratified by centrifuging, the chromidia and the mitochondria are found in two different layers. Only the chromidial layer stains distinctly with pyronine (Monné 98,100) and strongly absorbs ultraviolet light of 2600 Å wave length (57). In contrast to the chromidia, the mitochondrial layer does not stain with pyronine and does not absorb ultraviolet light of the mentioned wave length. It has also been found that the mitochondria of some other cells do not absorb ultraviolet light to any considerable degree (78). Thus, the mitochondria, in contrast to the chromidia, do not contain any considerable amount of ribo-

nucleic acid. Both mitochondria and chromidia are Feulgen negative.

The term chromidia should be restricted to the minute granules only, which exhibit the above-mentioned properties. Nevertheless, the same properties are also exhibited by several other, large structural entities which have also been described as chromidia. These large components of the cytoplasm have the form of thick cords, spirals, concentric lamellar bodies, etc., and do not correspond to the single chromidia. They are compact aggregates of numerous chromidia, present within strongly condensed regions of the fibrillar ground cytoplasm. The known term ergastoplasm should be reserved for all these condensations of the cytoplasmic texture. Like chromatin, the chromidia are purely morphologic entities; their chemical composition is variable. Both the amount of ribonucleic acid present within the chromidia and the amount of thymonucleic acid present within the chromatin granules are subject to great variations under different physiologic conditions. The ribonucleic acid content of the chromidia is high in rapidly growing cells and low in cells that have ceased to grow. In the former case, the chromidia have a more acid character and therefore they are preferentially stainable with basic dyes. In the latter case the chromidia have a more basic character and therefore they are preferentially stainable with acid dyes.

In preparations fixed in Bouin fluid and stained with iron hematoxylin or other basic dyes, the chromidia of sea urchin eggs appear as minute granules whose size is just on the limit of microscopic resolution. Thus, the diameter of the fixed and stained chromidia cannot exceed 0.2 μ. Under the influence of acetic acid contained in Bouin fixative the chromidia swell, similarly to the chromatin granules. The chromidia also increase in size under the influence of the dye, which is heavily adsorbed on their surfaces. Thus the chromidia must be much smaller in living cells, probably 0.1 μ or less. The chromidia are, as a rule, not densely packed within the cytoplasm and therefore they clearly appear as single bodies in fixed preparations.

The microsomes, macromolecules, plasmagenes, or particulates separated from various minced cells by Claude, Stern, Brachet, Jeener, *et al.* (for literature, see 13,42) contain ribonucleic acid and have a diameter of 0.3 to 0.05 μ. There is no doubt that these corpuscles are identical with the chromidia previously discovered in

fixed preparations. Therefore it is proposed in this article to call these corpuscles chromidia instead of microsomes, etc. The term chromidium is excellent because it means that the corpuscle in question is similar to but not identical with chromatin. Moreover, proper credit should be given to those investigators who first recognized that chromatin-like, nucleic-acid-containing bodies are present within the cytoplasm, and to the man who first coined a suitable term (R. Hertwig) to designate these bodies. The terms microsomes, macromolecules, plasmagenes, or particulates are either too vague or incorrect. Nevertheless, the separation of the chromidia from minced cells is a very important discovery because it renders possible the investigation of the above-mentioned bodies by exact chemical methods.

It is universally accepted that nucleic acid is concerned with protein synthesis (13,19), and that consequently self-perpetuating corpuscles must be nucleoproteins. Thus, it is very probable that the chromidia are self-perpetuating bodies similar to chromatin. The chromidia are an organized system of numerous chemical compounds. The chromidia contain proteins rich in sulfhydryl groups, ribonucleic acid, lipides (among them phosphatides), and respiratory and hydrolyzing enzymes (for details, see page 31; for literature, see 13,42). It is possible that among other proteins the chromidia also contain basic proteins such as histones. In microincinerated preparations heavy ash deposits occur wherever ribo- or thymonucleic acid is present (31,40). Calcium and magnesium are the chief components of this ash. During the development of nerve cells the amount of the ash-yielding substances is strongly decreased within the nuclei and increased within the cytoplasm (76). Simultaneously the amount of nucleic acid (thymonucleic acid) is also strongly decreased within the nuclei and increased (ribonucleic acid) within the cytoplasm. It is evidenced by this fact how intimately nucleic acids are associated with calcium and magnesium. The amount of calcium and magnesium is increased wherever the amount of nucleic acids is increased, and inversely. Moreover, it is known that calcium and magnesium are not free, but bound to the components of the protoplasm (143). Thus, it appears very probable that the phosphoric acid parts of phosphatides and nucleic acids are linked by means of the bivalent cations calcium and magnesium. At any rate, there is no doubt that calcium and magnesium are present

within the chromidia. The role of the lipides is to separate the nucleoproteins from the numerous enzymes present within the chromidia. The chromidia are not scattered in a disorderly manner within the cytoplasm, but concatenated with each other into long fibrils.

Many theories concerning the structure of the protoplasm have been advanced. It is not the right occasion to discuss all these theories here, inasmuch as this problem appears to be already solved. For literature concerning this matter the reader is referred to the books of Henneguy (60) and Seifriz (144). In the present review only some recent investigations definitely proving the fibrous structure of the protoplasm are reported. Polarization optics proved to be a very valuable method for investigating the submicroscopic structure of living and fixed cells. For literature concerning this subject the reader is referred to the books and papers of Schmidt (138,140).

The fibrillar structure of the ground cytoplasm of living and fixed cells can be safely demonstrated by means of the polarization microscope. Various birefringent inclusions of microscopic size scattered throughout the cytoplasm may conceal and simulate the birefringence of the ground cytoplasm. Therefore living uninjured eggs of sea urchins (98,108*) and frogs (115) were stratified by centrifuging, and investigated between crossed nicols in a polarization microscope. A distinct birefringence could be detected within the clear layer of ground cytoplasm deprived of all inclusions. In sea urchin eggs long streaks of variable thickness negatively birefringent in longitudinal direction have been observed within this layer (98). No doubt the fibrillar components of the ground cytoplasm were oriented parallel to each other by centrifuging. The eggs do not suffer any injury when exposed to this experimental treatment. This is evidenced by the well-known fact that centrifuged eggs cleave upon fertilization. In normal eggs the fibrils are oriented in any direction and therefore the ground cytoplasm appears isotropic. Under the influence of centrifuging the fibrils are oriented in a certain direction and therefore the ground cytoplasm becomes birefringent. This birefringence may be increased by weakly hypertonic sea water (98). The described facts prove that the ground cytoplasm of normal living cells has a fibrillar structure.

---

* Unfortunately it is very difficult for the reader to imagine what kind of optical phenomena have been observed by Moore and Miller (108). The very short note does not seem to be sufficiently clear.

Runnström (122,123) has demonstrated that under the influence of hypertonic sea water great, but completely reversible, structural alterations are produced within the cytoplasm of the sea urchin *Psammechinus miliaris*. Monné (100,103) investigated in detail these alterations by means of the polarization microscope and gave the correct interpretation of the phenomena observed. It was found that under the influence of hypertonic sea water the fibrillar texture of the ground cytoplasm is condensed in the central region of the eggs. Simultaneously a liquid (enchylema) is released and all inclusions of microscopic size (yolk, mitochondria) are forced out from the interstices between the cytoplasmic fibrils. These inclusions form a layer beneath the cortex that is only 1 μ thick. The whole ground cytoplasm condensed in the central region of the egg presents a polarization cross which is positive in radial direction. In some cases the described alterations do not occur, but the whole egg becomes positively birefringent in radial direction (94,98).

Under the influence of undiluted glycerol either a mosaic of birefringent structures (122,123) appears, or the whole egg becomes positively birefringent in radial direction (126). The whole egg becomes negatively birefringent in radial direction when fixed in absolute alcohol (98,140). Evidence has been provided (98, 100) that this birefringence is due to the submicroscopic structure of the ground cytoplasm, and not to the birefringence and orientation of the microscopic inclusions of the egg.

Fixed eggs treated with absolute alcohol are negatively birefringent in radial direction because the polypeptide chains of the ground cytoplasm are preferably oriented in tangential direction. This birefringence cannot be due to ribonucleic acid associated with the proteins of the cytoplasm, because the hydrophilic nucleic acid molecules can only be oriented parallel (140) and not perpendicular to the polypeptide chains of the proteins, and because proteins and nucleic acids have an intrinsic birefringence of opposite sign. In eggs treated with hypertonic sea water the whole ground cytoplasm is positively birefringent in a radial direction. Nevertheless, even in this case the birefringence is not due to nucleic acid, but to the rod-shaped lipide molecules oriented in radial direction. This is plainly evidenced by the fact that this birefringence is reversed under the influence of lipide-dissolving, but protein- and nucleic-acid-preserving agents. The cytoplasm of pancreas and nerve cells is known to be particularly rich in ribonucleic acid (20,100). Nevertheless, even in this case the birefringence phenomena (100,120) displayed by the cytoplasm cannot be due to ribonucleic acid (100). Either the amount of ribonucleic acid present within the cytoplasmic fibrils is not sufficient to reverse the sign of birefringence of the proteins, or the ribonucleic acid molecules are arranged in a disorderly manner because

they adhere to the sections of cytoplasmic fibrils which are permanently folded. The latter assumption is strongly supported by the facts cited below (page 14). Thus, it is certain that the birefringence phenomena of the ground cytoplasm are due to proteins and to lipides only. The rod-shaped lipide molecules have one hydrophilic and one hydrophobic pole and therefore they must be oriented perpendicular to the hydrophilic polypeptide chains of the proteins (46,140). The described birefringence phenomena make it evident that the ground cytoplasm has a fibrillar structure. The birefringence phenomena, due to lipides and to proteins, are of opposite sign and therefore they strongly compensate each other. In living sea urchin eggs the birefringence, which is due to the lipide component of the cytoplasmic fibrils, appears distinctly when these fibrils are arrenged in a certain order under the influence of some experimental agent. The birefringence due to lipides is increased when the polypeptide chains are stretched under the influence of dehydrating agents. No doubt the ordered arrangement of the lipide molecules is increased when this occurs. This birefringence is reversed when the lipides are dissolved. The birefringence due to the proteins of the cytoplasmic fibrils appears and is still more increased when the polypeptide chains are stretched to the maximum upon complete dehydration.

Not only in the mentioned cells, but in practically any cell, birefringence of the ground cytoplasm may appear under the influence of various agents which cause orientation and stretching of the cytoplasmic fibrils, without serious injury (94,95,97). Detailed investigations have been performed in order to distinguish the birefringence of the ground cytoplasm from the birefringence of its inclusions (94, 97). Also under normal physiologic conditions orientation of the cytoplasmic fibrils and temporary birefringence of the ground cytoplasm may appear (94,140).

The chromidia are a component of the ground cytoplasm. This is evidenced by the fact that in eggs stratified by centrifugal treatment the chromidia are always strongly condensed in the layer which corresponds to the ground cytoplasm (57,98). Birefringence appears within this layer because the fibrillar components of the ground cytoplasm are also strongly condensed here under the influence of centrifugal treatment. The chromidia cannot be separated from the cytoplasmic fibrils by means of centrifugal treatment. The chromidia

are an essential component of these fibrils. It can be seen in fixed preparations that the chromidia are concatenated with each other by means of very thin threads which do not show any distinct affinity to any dye. The intermediate threads do not exhibit the characteristic properties of ribonucleic acid. Thus, it must be concluded that the cytoplasmic fibrils are constructed of the ribonucleic-acid-containing chromidia and the ribonucleic-acid-free interchromidia, regularly alternating with each other.

Ample evidence in support of this conclusion is provided by the following experiment (103,104): Mature unfertilized eggs of the sea urchin *Psammechinus miliaris* were exposed for several hours to the action of a 0.2 *N* sodium azide solution in sea water. Cytolysis does not occur but the structure of the cytoplasm is slowly and gradually altered. The extremely thin cytoplasmic fibrils are oriented parallel to each other. Bundles of cytoplasmic fibrils are formed. The bundles gradually become thicker because of the increasing number of cytoplasmic fibrils which join each other. All intermediate stages between single cytoplasmic fibrils and thick bundles are found. The bundles are cross striated (Fig. 1). The thickest bundles contain several hundred, perhaps even one thousand, cytoplasmic fibrils (see page 13). The fact should be stressed, however, that the chromidia conjugate only with the chromidia, and the interchromidia only with the interchromidia. Evidently, there exists a mutual attraction between the corresponding parts of the cytoplasmic fibrils.* The thick bundles of cytoplasmic fibrils consist of ribonucleic-acid-containing and ribonucleic-acid-free sections regularly alternating with each other. They exhibit a great similarity to the salivary chromosomes of *Drosophila*, which consist of alternating sections, with and without thymonucleic acid.

The parallel orientation of the cytoplasmic fibrils may be a phenomenon related to meiotic chromosome pairing. Identical, or at least a very similar, structure is the prerequisite for this pairing. It is well known that chromosomes of deviating structure do not pair. All cytoplasmic fibrils must have an identical, or at least very similar, structure because they are so easily attached parallel to each other with their corresponding regions. Thus, the cytoplasmic fibrils are, in contrast to the chromosomes, not differentiated along their length. It is evident that all chromidia of a certain cell are identical. The same holds true for the interchromidia.

Under the influence of sodium azide the cytoplasmic fibrils are stretched, a fact which undoubtedly favors their parallel orientation.

* The parallel conjugation of cytoplasmic fibrils may be brought about by a process similar to blood clotting. It has been recently demonstrated that the cross striated fibrin fibrils also adhere to each other with their corresponding regions (57a).

Only the interchromidia are strongly elongated and therefore the distances between the chromidia are increased. Evidently the polypeptide chains of only the interchromidia are unfolded by the action of sodium azide. The stretching of the polypeptide chains is due to dehydration. This is evidenced by the fact that a vacuole is expelled at the ends of the bundles of cytoplasmic fibrils. Under normal conditions the cytoplasmic fibrils are strongly hydrated and the poly-

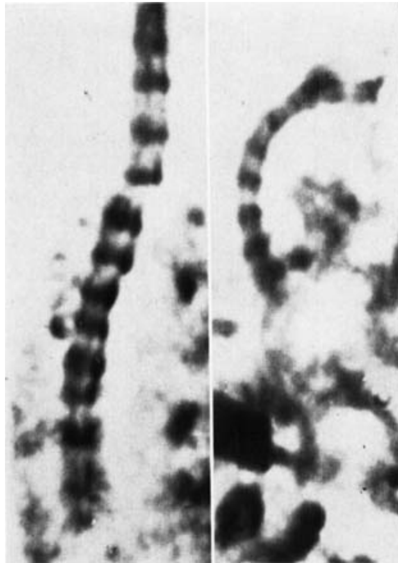

Fig. 1. Cytoplasmic fibrils arranged in cross-striated bundles under the influence of sodium azide.

peptide chains of their chromidia and interchromidia are folded. It seems that the polypeptide chains of the chromidia are permanently folded, in contrast to the polypeptide chains of the interchromidia, whose folding is subject to variations under different physiologic conditions.

Bundles of cytoplasmic fibrils are approximately as long as the egg diameter (about 90 μ). Thus, it is probable that the single cytoplasmic fibrils also have a similar length in eggs not subjected to any experimental treatment. No doubt the length of cytoplasmic fibrils is variable in various cells and depends upon the diameter of the

latter. It may be that the length of cytoplasmic fibrils is variable under different physiologic conditions and that this may be due to fragmentations and reunions. The cytoplasmic fibrils decrease strongly in length during cleavage because they are cut through in transverse direction simultaneously with any cell division. The length of the fibrillar constituents of the cytoplasm of slime molds has been estimated by Moore at about 10 μ (107).

The chromidia of the sea urchin eggs are about 100 mμ thick (see page 6). The interchromidia seem to be thinner. Thus, thickness of the single cytoplasmic fibrils may be estimated to 50–100 mμ. These thin fibrils are visible in the ordinary microscope only because of their length, which is above the limit of microscopic resolution. The cytoplasmic fibrils are comparable to the protofibrils constituting sperm tails. Ballowitz (2) showed, a long time ago, that sperm tails may be decomposed into several extremely thin fibrils, visible in the ordinary microscope. These protofibrils have recently been investigated by means of the electron microscope and it was found that they are about 50 mμ thick (57,134,137). Sperm tails are bundles of protofibrils whose number and thickness are strikingly constant. This fact indicates that fibrils which are about 50 mμ thick represent a very important submicroscopic structural unit. These protofibrils may be either constituents of thicker fibrils or may occur independently. Thus, the cytoplasmic fibrils seem to be independent protofibrils. Moore (107) found that the cytoplasm of slime molds may pass without injury through the pores of parchment, which are about 50 mμ in diameter. He inferred from this fact that the cytoplasm of slime molds must be composed of fibrils which are also about 50 mμ thick. Thus a structural unit corresponding to protofibrils may also be detected in this case. It would be of interest to know whether there exists yet another intermediary structural unit between the cytoplasmic fibrils, which correspond to the protofibrils, and the single polypeptide chains. At any rate, from muscles, fibrils as thin as 5–15 mμ have been separated (54). Thus, they are still thinner than the protofibrils of sperm tails. It is not known how many parallel polypeptide chains the cytoplasmic fibrils may contain. Nevertheless, it may be estimated that the cytoplasmic fibrils are bundles of about 2000 polypeptide chains (104), if it is assumed that the latter are as densely packed as in myofibrils (89). Thus, the ground cytoplasm cannot be regarded as a texture of single polypeptide chains. It has a much coarser structure.

Birefringent substances have been extracted from various tissues which were previously minced. Only in the case of muscles is it certain that these substances originate from the cytoplasm. The birefringent protein myosin is in question here. It has not been proved that the birefringent substances extracted from other cells derive from the cytoplasm. It seems that the birefringence of the materials hitherto separated from the cytoplasm of these cells is entirely due to contamination with thymonucleohistone, a substance

which occurs in the nuclei only (14). At any rate, the extracted chromidia (macromolecules, microsomes, particulates) appear globular in the electron microscope (151) and do not show any flow birefringence (14). This is in perfect harmony with the above-mentioned fact that the birefringence of the cytoplasm is not influenced by ribonucleic acid (see page 10). From this fact it has been inferred that the ribonucleic acid molecules are scattered in a disorderly manner because they intimately adhere to the permanently folded polypeptide chains of the chromidia (100,104).* The ground cytoplasm is a texture of fibrils, the latter consisting of the chromidia and interchromidia which regularly alternate with each other. It must be concluded from the above-mentioned facts that the birefringence phenomena displayed by the ground cytoplasm of living and fixed cells are due to the interchromidia. It is obvious that the birefringent interchromidia are dissolved by the methods employed to extract the isotropic chromidia. In this connection it is of interest to note that the cross-striated muscles may also be fragmented in transverse direction into anisotropic (Bowman discs) and isotropic discs. Agents are known some of which dissolve the former, and some the latter (for literature, see 58). Proteins which are birefringent or which may easily be made birefringent are present in the cytoplasm of any cell. This is clearly evidenced by the birefringence phenomena exhibited by the cytoplasm of living and fixed cells. With the exception of myosins in the case of muscles these proteins have not yet been extracted in pure state.

B. COMPARISON OF VARIOUS LIVING FIBRILS

A longitudinal structural periodicity seems to be the essential feature of all, both inanimate and living, protein fibrils. This periodicity is in some cases above, and in others below, the limits of resolution of the ordinary microscope. Structural periodicity of the cytoplasmic fibrils, the prophase chromosomes, and the cross-striated myofibrils can be investigated easily by means of the ordinary microscope. Structural periodicity of smooth myofibrils, sperm tails, fibrin, and connective tissue fibrils (54,57a,134) appears distinctly only in the electron microscope. All these fibrils exhibit a distinct cross striation. It is very probable that other protoplasmic fibrils, particularly neurofibrils, also have a similar periodic structure.

* Nevertheless the isotropy of the chromidia may also be due to compensation.

This is plainly evidenced by the specific mode of nerve conduction (see page 47). Differences in the folding of the polypeptide chains are probably the essential feature of this structural periodicity (134). In some cases, sections with stretched polypeptide chains may regularly alternate with sections with folded polypeptide chains; in other cases, the two alternating sections may differ from each other only in the degree of folding of their polypeptide chains and, in still other cases, the degree of folding of the polypeptide chains may be the same in both sections which differ from each other only in the relative ease with which their polypeptide chains are unfolded. It is probable that this kind of structure represents a buffering mechanism by which the resistance of protein fibrils against disruption is increased. It is certain that inanimate protein fibrils do not contain enzymes and nucleic acids. Within living fibrils nucleic acids together with the enzymes of the energy-yielding activities and of hydrolysis are present and periodically distributed (see page 33). Nucleic acid and enzymes are probably always associated only with the sections of fibrils whose polypeptide chains exhibit the tendency to remain folded.

The metaphase chromosomes are spirally twisted and strongly contracted; therefore their internal structure is not visible. Nevertheless, the prophase chromosomes are stretched and exhibit a distinct structural periodicity. This kind of structure is displayed most distinctly by the famous salivary chromosomes of dipterans. These chromosomes are bundles of several hundreds of chromonemata and in this regard they are very similar to the bundles of cytoplasmic fibrils described in the preceding section. The single chromonemata are probably as thick as the single cytoplasmic fibrils of sea urchin eggs (0.1 μ). This is probably the thickness of the univalent chromosomes of the resting nuclei. The chromosomes consist of thymonucleic-acid-containing and thymonucleic-acid-free sections regularly alternating with each other.

Pfeiffer (113,114) and Schmidt (139) demonstrated that the living salivary chromosomes display a very weak birefringence which is negative in longitudinal direction. This birefringence is entirely due to the thymonucleic acid present within the chromomeres. Frey-Wyssling (46) calculated that only a very small percentage (about 3%) of the thymonucleic acid molecules is regularly oriented within the chromosomes. Caspersson (18) arrived at the same conclusion earlier by employing his method of dichroism in ultraviolet light. Evidently the elon-

gated thymonucleic acid molecules are poorly oriented because they intimately adhere to the strongly folded polypeptide chains of the chromomeres.

Also in this respect the chromomeres and the chromidia are very similar to each other. The chromomeres remain unchanged when the salivary chromosomes are stretched. Nevertheless, the distances between the chromomeres are increased, because the interchromomeres are strongly elongated (16,113,114). No doubt, the polypeptide chains of both the chromomeres and the interchromomeres are strongly folded under normal conditions. The polypeptide chains of the interchromomeres only are unfolded when the chromosomes are stretched; the polypeptide chains of the chromomeres remain permanently folded. Thus, yet another similarity between the chromosomes and the cytoplasmic fibrils may be detected. Nevertheless, in contrast to the interchromidia, the interchromomeres remain isotropic, even when strongly stretched (113,114). Under the influence of stretching the polypeptide chains of the interchromomeres are certainly very well oriented, parallel to each other. The isotropy can only be due to compensation, probably brought about by lipide molecules which are oriented perpendicularly to the polypeptide chains. This is strongly supported by the fact that large amounts of lipides are present within the nuclei (152). Unfortunately it is not yet known whether the interchromomeres become positively birefringent in longitudinal direction, when stretched salivary chromosomes are treated with absolute alcohol and similar lipide solvents.

The cytoplasmic fibrils and the chromosomes are very similar to cross-striated myofibrils. Certain important differences, however, exist. The cross-striated myofibrils consist of the anisotropic (*A* bands) and the relatively isotropic bands (*I* bands), which regularly alternate with each other. In general it is held that the polypeptide chains of the *I* bands are permanently folded in contrast to the polypeptide chains of the *A* bands, which are stretched upon relaxation and folded upon contraction of the muscle. Stretched chromosomes and stretched cytoplasmic fibrils are similar to relaxed myofibrils, while cytoplasmic fibrils and chromosomes in normal condition are similar to contracted myofibrils. The *I* bands of muscles are analogous to the chromomeres and chromidia, where the polypeptide chains are permanently folded, while the *A* bands of muscles are analogous to the interchromomeres and the interchromidia, where the

folding of the polypeptide chains is variable. When chromosomes and cytoplasmic fibrils are subjected to strain the polypeptide chains of the interchromomeres and interchromidia only are unfolded. In relaxed muscles the polypeptide chains of the *A* bands are stretched to a maximum and therefore they cannot be extended any more. The polypeptide chains of the *I* bands are folded and therefore only these polypeptide chains are stretched when relaxed muscles are subjected to strain (54). The similarity between *I* bands, chromomeres, and chromidia is also supported by some other facts which are cited below. It has been demonstrated that in microincinerated preparations of resting muscles heavy ash deposits coincide only with *I* bands (41) where adenylnucleic acid is also present (23). This ash consists chiefly of calcium and magnesium. Also within the chromidia and the chromomeres nucleic acid is associated with these bivalent metals.

However, there exists an essential difference between the chromosomes on the one hand and the myofibrils and cytoplasmic fibrils on the other hand. The chromomeres (genes) of the same chromosome differ from each other, while the corresponding sections of cytoplasmic fibrils and myofibrils, respectively, are identical with each other. Probably the same holds true for the interchromomeres on the one hand and the interchromidia and *A* bands on the other hand.

All protein fibrils seem to have a periodic structure and therefore it is probable that not only the cross-striated myofibrils, but also all other fibrils, may be fragmented into their two different component parts. It would be of interest to subject to chemical analysis these two fractions of various fibrils.

C. CORTEX AND PLASMA MEMBRANE

The cortex, which represents the superficial layer of the living cytoplasm, is an essential component of both animal and plant cells. The cortex differs distinctly from the underlying cytoplasm. Moreover, the cortex is entirely different from the extraneous coats covering the cells. In contrast to the cortex, the above-mentioned coats may be removed without injuring the cells (25). The extraneous coats are merely inanimate cellular envelopes. They appear as well-defined membranes or as indistinct layers of various substances of protein or carbohydrate nature. The real plasma surface must be uncovered by means of experimental agents, which fact has been particularly emphasized by Chambers (25).

The ground cytoplasm beneath the cortex is a relatively coarse texture of fibrils oriented in any direction. In many cells this cytoplasmic texture is strongly condensed in the peripheral region and loosened in the central region. Therefore the peripheral part of these cells is a pronounced jelly in contrast to the fluid interior. Chambers refers to this peripheral cytoplasmic region when using the term "cortex." This peripheral cytoplasm is also called ectoplasm, particularly if it is deprived of all inclusions. There is, however, no essential difference between the ectoplasm and the entoplasm. The only difference is the degree of condensation of the cytoplasmic texture. In this article the term cortex is not used in the same sense as Chambers uses it. The real cortex is sharply delimited and it is very different from the jelly-like cytoplasm underlying the cortex. The term cortex is used here to designate a peripheral, very thin, birefringent, jelly-like layer of the cytoplasm. The thickness of this layer is about 1 μ. The cortex maintains the integrity of the whole cytoplasm. The substance of the cortex is much more condensed than the substance of the cytoplasm underlying the cortex.

Birefringence on the surface of various cells has been demonstrated by several authors (Schmitt, Bear, Ponder, Chinn, Schmidt, *et al.*; for literature, see 140). Of particular interest are the investigations of Schmitt, Bear, and Ponder dealing with the induced birefringence of the stromata of hemolyzed erythrocytes of mammals. It was found that this induced birefringence is due, in some cases, to the lipide and in other cases to the protein component of the stromata. Nevertheless, further detailed investigations were necessary in order to elucidate the two questions: (*1*) Whether the birefringence demonstrated on the cellular surface is always due to the real surface layer of the cytoplasm or whether this birefringence is sometimes simulated and concealed by the birefringence of some extraneous coat; and (*2*) whether a distinct birefringence may be exhibited by the real surface layer of the living cytoplasm of typical cells, such as sea urchin eggs which are not injured and not subjected to any experimental treatment. Investigations of these questions have been performed by Runnström, Monné, and Broman (129), Runnström and Monné (127), Monroy and Monroy-Oddo (106), and Monné (102–104).

The surface of living uninjured sea urchin eggs not subjected to any experimental treatment exhibits a distinct birefringence which is

positive in radial direction (106,127,129). This birefringence remains unchanged when all extraneous coats of the sea urchin eggs are removed by means of trypsin digestion (129). It is known that proteolytic enzymes do not attack and do not injure living protoplasm. It must be added that cleavage takes place upon insemination of eggs treated with trypsin. From these facts indisputable evidence is provided to prove that the birefringence on the surface of sea urchin eggs is due to the cortex only, and not to some extraneous coat intimately adhering to this cortex. The cortical birefringence disappears when the eggs are subjected to the action of lecithinases (bee venom) and various lipide-dispersing agents (detergents, etc.). Thus, it is certain that this birefringence is due to lipides. The sign of birefringence of the cortex is reversed when the proteins are precipitated subsequent to the dissolution of the lipides. From these facts it is concluded that the rod-shaped lipide molecules are oriented perpendicular to the surface of the cortex and the polypeptide chains of the proteins tangential, in any direction within the plane of the cortex. The cortex consists of protein foils and lipide lamellae regularly alternating with each other.

Not in sea urchin eggs only, but also in several other cells it has been found that the cortex is positively birefringent in radial direction (101,102,104). This also holds true for the living uninjured erythrocytes of the polychaete *Glycera rouxi* (99). This birefringence is reversed upon hemolysis. No doubt the cortex has a similar structure in all kinds of cells.

It is possible that the cortex is constructed of fibrils which are similar to the fibrils of the underlying cytoplasm (see page 11). The cortex is much denser than the underlying cytoplasm and therefore it is probable that the cortical fibrils are much thinner than the cytoplasmic fibrils. The structure of both kinds of fibrils may, however, be similar. This statement can be supported by some facts. It has been found that in microincinerated preparations of various cells heavy ash deposits are always present on the site of the cell surface (142). This ash contains chiefly calcium and magnesium. It is known that these ash deposits occur wherever large amounts of nucleic acid are present. Thus, it is possible that some kind of nucleic acid is also present within the cortex. At any rate, adenylnucleic acid was found within the surface layer of the cytoplasm of plant cells (85). Therefore it is possible that the cortical fibrils also consist of nucleic-acid-containing and nucleic-acid-free sections regularly

alternating with each other. However, the rod-shaped lipide molecules must be oriented perpendicular to the polypeptide chains of the cortical fibrils. The polypeptide chains of the cortical fibrils which are oriented in tangential direction constitute the protein foils, and the lipide molecules oriented in radial direction represent the lipide lamellae of the cortex. It is probable that also in the case of the cortex the phosphoric acid parts of the phosphatides and the nucleic acids are kept together by means of the bivalent cations, calcium and magnesium. The enzymes, particularly the enzymes of the energy-yielding activities, must be associated with the nucleic-acid-containing sections, as in the case of the cytoplasmic fibrils (see page 33). The meshes between the cortical fibrils represent the pores which are important for the permeability phenomena.

The structure of the cortex is very similar to the postulated structure of the hypothetical plasma membrane. Nevertheless the cortex was calculated to be 1000 mμ thick in contrast to the plasma membrane which was estimated to be only 10 mμ thick (see 66). The last-mentioned estimation, which is based on various experiments, is, however, not very reliable. It is not certain whether the cortex is identical with the plasma membrane. It may be that the plasma membrane only corresponds to the superficial layer of the cortex. At present it is impossible to decide whether the selective permeability is due to the whole cortex or to its superficial layer only.

D. MITOCHONDRIA, GOLGI BODIES, AND CHLOROPLASTS

Bensley and Hoerr (7,67) were the first to succeed in separating mitochondria from minced tissues. Since then numerous investigations on the chemical composition of the extracted mitochondria have been performed (for literature, see 42). Moreover, numerous cytochemical reactions have been tested. The mitochondria contain proteins, phosphatides, cholesterol, glycerides, respiratory enzymes (7,27,34, and others) amylase (69), and vitamins A and C (for literature, see 11). The amount of cholesterol present within the mitochondria is higher than in any other cellular component (34). In fixed preparations it was found that the mitochondria do not absorb ultraviolet light (2600 Å) to any considerable degree (57,78). This means that no considerable amount of nucleic acid can be present within the mitochondria. This result is more convincing than the result of certain chemical analyses performed on extracted mitochondria, where considerable amounts of ribonucleic acid could be demonstrated (27). Evidently these extracts were still strongly con-

taminated with the chromidial substance which contains large amounts of this acid.

Short rod-shaped and long filamentous mitochondria are positively birefringent in longitudinal direction (51,97,138). This birefringence is due to the protein component of the mitochondria.

Up to the present nobody has succeeded in extracting Golgi bodies from minced tissues. Investigations on this cytoplasmic component have been performed on living and fixed tissues only. For literature concerning this subject the reader is referred to the books of Cowdry (30) and Hirsch (64).

The Golgi apparatus is a universal component of the cytoplasm. It exhibits a certain morphologic, physical, and chemical variability, just as any other cellular component of widespread occurrence. In particular, there exist some important although not essential differences between the Golgi bodies of germ and tissue cells, and between the Golgi bodies of vertebrates and invertebrates. In the author's opinion all these differences are explained if it is assumed that the amount of proteins present within the Golgi bodies is variable and that cholesterol is in some cases present and in others absent; the phosphatides are the essential and most important chemical constituent of this cytoplasmic component.

In living germ cells (spermatocytes, oocytes) of various invertebrates the Golgi bodies may be distinctly seen by means of the ordinary microscope. The phase contrast microscope is a splendid method for detecting Golgi bodies in these cells (15,102). The Golgi bodies appear black on a dark ground. They are distinctly optically differentiated from the surrounding cytoplasm probably because they contain some amount of cholesterol which is a highly refringent substance and which, moreover, has the property of strongly condensing phosphatide films. These Golgi bodies also appear to be relatively rich in proteins. The Golgi bodies in the above-mentioned cells (*e.g.*, male germ cells of pulmonates and chilopodans) are birefringent (91,94). Their birefringence may be greatly increased under the influence of hypertonic salt solutions and vital staining with chrysoidine (94). Moreover, in fixed preparations these Golgi bodies are easily stained with Heidenhain iron alum hematoxylin, particularly at the temperature of 37°C. This staining may be due to the presence of cholesterol within the Golgi bodies. It is known that iron alum, which combines with hematoxylin, is easily reduced by choles-

terol at this temperature. The mitochondria which do not contain any considerable amount of nucleic acid, but large amounts of cholesterol, are deeply colored when this method is applied.

In contrast to germ cells, the Golgi bodies are, with few exceptions, invisible in living tissue cells and they cannot be demonstrated either by means of the phase contrast or the polarization microscope. Moreover, in fixed preparations the Golgi bodies of these cells cannot be stained with iron hematoxylin. They may be detected only by impregnation with osmium and silver; these methods are also successfully applied to demonstrate Golgi bodies in germ cells. The Golgi bodies of tissue cells probably do not contain any cholesterol and that may be the reason they are not optically differentiated from the surrounding cytoplasm. Moreover, they are very poor in proteins. Under the influence of various experimental agents the ground cytoplasm and the Golgi bodies become simultaneously birefringent and therefore they cannot be distinguished from each other. In spite of these differences the Golgi bodies may have the same structure in both germ and tissue cells. When living tissue cells are exposed to high-speed centrifuging, the Golgi bodies are displaced, which may be demonstrated in fixed preparations (5). This is indisputable evidence that the Golgi bodies are real components of the cytoplasm even in the cells, where they are invisible in living condition.

The Golgi bodies have the shape of platelets, lenses, globules, hemispheres, and invaginated gastrula-like bodies. Net-like Golgi apparatus has never been observed in living cells. It is very probable that net-like Golgi apparatus is a fixation artifact due to fusions and alterations of the single Golgi bodies. This is strongly supported by the model experiments of Holtfreter (71). The Golgi bodies always consist of two components: the externum and the internum. The former is membranous while the latter is globular or lens shaped. The interna are always compact and therefore they cannot be regarded as vacuoles.

It is universally accepted that large amounts of phosphatides are present within the Golgi bodies. Osmium tetroxide is strongly reduced only by the externa of the Golgi bodies. Moreover, under the influence of various agents, only the externa are transformed into myelin figures at room temperature (92). Both facts indicate that phosphatides with unsaturated fatty acids in their molecules prevail within the externa of the Golgi bodies, and phosphatides with saturated fatty acids within the interna. It is supposed that cholesterol may or may not be present within the Golgi bodies. Moreover, small

but variable amounts of proteins are present within the Golgi bodies. The latter do not absorb ultraviolet light to any considerable degree and therefore they cannot contain any considerable amount of nucleic acid (63). In microincinerated preparations no distinct ash deposits are found in the region occupied by the Golgi apparatus (142). Thus, it is very poor in minerals, which is also evidence that it does not contain nucleic acid (see page 7).

Neutral red is a vital stain which affects the Golgi bodies. Vacuoles deeply stained with this dye appear, as a rule, in the region of the cytoplasm which is occupied by the Golgi bodies. Moreover, it may be demonstrated that neutral-red vacuoles are formed in association with the Golgi bodies, when the latter are visible in living cells. The externa of the Golgi bodies are only exceptionally stained with this dye. Neutral red is strongly accumulated within the interna of the Golgi bodies, where local cytolysis is produced. The lipides are separated from the proteins and a vacuole stained with neutral red is exuded. Thus, the Golgi apparatus is vacuolized under the influence of neutral red. A similar effect is produced by morphine (102). The mentioned neutral-red vacuoles are pure artifacts, which do not correspond to any real component of the cytoplasm. Also this conclusion is strongly supported by the model experiments of Holtfreter (71).

The lamellar externa of the Golgi bodies of living germ cells of invertebrates are or may easily be made birefringent (91,94). The birefringence is positive in the direction which is perpendicular to the surface of the lamellae. This means that the rod-shaped lipide molecules are also oriented perpendicular to the surface of the lamellar externa. The globular interna of the Golgi bodies of the spermatocytes and spermatides of *Lithobius* are positively birefringent in radial direction (94). Thus, the rod-shaped lipide molecules of the interna must also be oriented in this direction. The Golgi bodies should be regarded as systems of lipide lamellae and protein foils regularly alternating with each other. The above-mentioned foils must be very thin, because the protein content of the Golgi bodies appears to be very low.

The chloroplasts of plant cells are also systems of alternating protein foils and lipide lamellae. They show a similar, although stronger, birefringence than the Golgi bodies (for details and literature, see 45,87).

E. ORGANIZATION OF THE CELL

The relatively firm cortex covers the underlying ground cytoplasm. The latter consists of the cytoplasmic fibrils (see page 4) and the enchylema. The term enchylema has been employed by previous cytologists to denote the liquid which fills the interstices between the fibrillar components of the protoplasm. The ground cytoplasm is not a network of single polypeptide chains; it is a relatively coarse texture of cytoplasmic fibrils which are bundles of numerous polypeptide chains. The cytoplasmic fibrils are not interconnected by means of transverse threads. This would prevent the previously described (see page 11) regular parallel conjugation of the cytoplasmic fibrils. The ground cytoplasm is not a network, but rather a texture of fibrils (98,100,119). The cytoplasmic fibrils adhere weakly to each other by means of lipide molecules perpendicularly oriented to their length. The cytoplasmic fibrils adhere somewhat more strongly to each other by means of their protein constituents. These lateral linkages between the protein molecules are brought about by a process similar to blood clotting (see page 28).

Within the enchylema various inclusions are suspended, such as the small, globular or rod-shaped mitochondria, yolk, free glycogen, and many others. These inclusions move when the enchylema is induced to flow. Thus, the small globular or rod-shaped mitochondria do not generally adhere to the cytoplasmic fibrils. Nevertheless, it is possible that under certain physiologic conditions the small mitochondria are reversibly attached to the cytoplasmic fibrils, so that long thread-like structures (chondrioconts) are produced.

The living cytoplasms and the living nuclei are practically neutral, their *p*H being fairly constant (for literature, see 59 and 144). Small deviations from the point of neutrality (*p*H 6.8 to 7.0 for the cytoplasm, *p*H 7.5 to 7.6 for the nucleus) may be due to the slight injury caused by microinjection of indicator dyes. No doubt only the *p*H of the buffered enchylema is in question here. Moreover, there is strong evidence that the electrical charges of the fibrillar components of the living protoplasm are neutralized by inorganic anions and cations, but chiefly by phosphatides in association with the bivalent calcium and magnesium ions (see p. 50). Temporary electrical charges seem to appear only upon stimulation. Permanent electrical charges can be demonstrated in fixed preparations when the cells are preserved in lipide-dissolving fixatives (99,100). Large amounts of

lipides are present both within the cytoplasm and the nucleus (152).

The architecture of the cytoplasm is variable because the distribution of cytoplasmic fibrils is different in different cells. This structure is, however, very plastic; it may easily be disturbed and later reconstituted. Reversible, experimentally produced alterations of the cytoplasmic architecture of the sea urchin eggs have been observed by Runnström (123,125), and thoroughly investigated by Monné (98,100,103). In large, globular, undifferentiated cells the cytoplasmic fibrils may be irregularly distributed in all directions. In some cases, however, tangential orientation of the cytoplasmic fibrils weakly prevails (94). There exists in these cells a certain tendency to form concentric cytoplasmic layers. Radial orientation of the cytoplasmic fibrils may prevail during mitosis. A spiral arrangement of these fibrils was also observed (101). In elongated cells the cytoplasmic fibrils tend to be oriented in the direction of the largest extension. In greatly flattened cells the cytoplasmic fibrils are oriented in any direction but always parallel to the largest surface.

The texture of cytoplasmic fibrils may be either uniform or condensed in certain regions and loosened in other regions of the cell. The condensed cytoplasmic texture may form a layer of variable thickness, contiguous with the cortex. This layer may be free from all inclusions when the cytoplasmic texture is strongly condensed. A similar layer may be formed on the surface of the nuclear membrane. Several concentric alternating layers of condensed and loosened cytoplasm may be present. The cytoplasmic texture is frequently condensed around the centrosomes. The polar and dorsoventral organization of various eggs is due to unequal condensation of the cytoplasmic texture (28,98,101,121). The ergastoplasm is a local condensation of the cytoplasmic texture. It may have the form of long streaks, spirals, and concentric lamellar bodies known as yolk nuclei.

Gram-positive and Gram-negative bacteria differ greatly from each other in their morphologic and physiologic properties. The protoplasm of the former is differentiated into an interior core and an exterior layer, the latter containing large amounts of ribonucleic acid (see 37). The fibrillar ribonucleic-acid-containing components of the cytoplasm appear to be strongly condensed within the exterior layer of the Gram-positive bacteria as in the case of certain eukaryote cells (see 100, Pl. 1, Fig. 12). Cytoplasm of Gram-negative bacteria does not exhibit this differentiation probably because the ribonucleic-

acid-containing fibrils are uniformly distributed throughout the whole cell.

The cell is an organized system of several kinds of self-perpetuating fibrils probably all consisting of nucleic-acid-free and nucleic-acid-containing sections regularly alternating with each other, the latter being associated with enzymes, particularly the enzymes of the energy-yielding activities (see page 33). In the case of undifferentiated cells the most important fibrillar systems are the chromosomes, the cytoplasmic fibrils, the cortical fibrils, and the caryoplasmic fibrils (the latter occupy the interstices between the chromosomes of the resting nuclei). The specific fibrillar systems of differentiated cells are the myofibrils, neurofibrils, epithelial fibrils, cilia, flagella, etc.

IV. Functioning of Cytoplasm

A. DYNAMIC STATE OF THE STRUCTURE OF PROTOPLASM

Living protoplasm is continuously in a dynamic state. Energy is required to maintain the structure of the protoplasm, to bring about the cyclic structural alterations underlying any specific function, to produce various structures during embryologic development, to reconstitute the structure during regeneration, and to modify, within certain limits, the structure of the protoplasm in adaptation to changing environmental conditions.

Protoplasm is a highly organized colloidal system. Colloids are known to change their properties spontaneously. Syneresis and hysteresis occur. This quality of the protoplasmic colloids is regarded to be the cause of aging (132). The colloidal structure is not permanent. It breaks down spontaneously. The structure of the protoplasm would also soon be impaired and would break down spontaneously with ensuing cytolysis if there were not a mechanism present tending to prevent it. This mechanism is the metabolism by which the living substance is continuously broken down and reconstituted. Thereby the protoplasmic colloids are continuously rejuvenated. For this reason the protoplasm is potentially immortal. Aging of the protoplasm of single cells is prevented and aging of multicellular organisms is delayed. This metabolism is not stimulated in inactive organs, which therefore atrophy. In these organs the living substance is not renewed to the same extent as it is impaired spontaneously as a consequence of the metastability of its colloids. This occurs for some reason in multicellular organisms and therefore

aging is caused. The continuous breakdown and reconstitution of the chemical constituents of the protoplasm must be regarded as an established phenomenon well supported by the experiments on cellular metabolism performed by the use of labeled (radioactive) elements (for literature, see 141). Runnström (124,125) and Monné (103) demonstrated that the structure of the cytoplasm of sea urchin eggs is altered when the respiration of the eggs is depressed by various agents, particularly potassium cyanide and sodium azide. Moreover, it has been emphasized by the former investigator that fermentation (glycolysis) is not sufficient for the maintenance of the normal structure of the cytoplasm of the sea urchin egg. Nevertheless, this does not mean that fermentation is without importance for the maintenance of this structure. Fermentation must suffice for the maintenance of the normal structure of protoplasm in the case of organisms which normally live under anaerobic conditions. It is known that nerve cells of warm-blooded animals are very sensitive to lack of oxygen. Evidently considerable work must be performed in order to maintain the normal structure of these cells. Not the inhibition of the respiration as such is the cause of cell death, but the irreversible structural alterations of the protoplasm which invariably occur when the energy-yielding cell activities are inhibited. These rapid structural alterations cannot be due to the metastability of the protoplasmic colloids only, but to the appearance of certain detrimental substances or to the disappearance of other substances necessary for the maintenance of the normal structure of the protoplasm. Evidently energy is required to produce or to maintain the former substances and to remove or inactivate, in some way, the latter. Probably the cytoplasmic fibrils perform continual active movements which cease when the energy-yielding activities are inhibited. The cessation of these movements and the permanent stretching of the polypeptide chains of the cytoplasmic fibrils may also contribute to the appearance of these structural alterations.

It is universally accepted that the protoplasm is a soft thixotropic gel (24,144) which continuously changes its state under physiologic and experimental conditions. Nevertheless the thixotropy of the protoplasm is not a passive physical thixotropy, but an active biological thixotropy controlled by several antagonistic substances, particularly enzymes. Some of these substances present within the cell tend to liquefy the protoplasm by breaking down fibrils, while other

substances in the cell tend to solidify the protoplasm by building up fibrils and by producing lateral linkages between these fibrils. Therefore the structure of the protoplasm cannot be seriously disturbed by microneedles. The fibrils are reconstituted almost at the same moment as they are cut through. The solidifying agents may be similar to enzymes and other substances concerned with blood clotting. The presence of this clotting system within the protoplasm has been particularly postulated by Heilbrunn (59). Meiotic chromosome pairing, the above-described conjugation of cytoplasmic fibrils, the reunion of chromosome fragments, and the formation of membranes and of cytoplasmic nodes may be processes similar to blood clotting. The viscosity of the cytoplasm is increased when the number of lateral points of attachment [*Haftpunkte* of Frey-Wyssling (45)] between the protein components of fibrils is increased (p. 24).

Runnström (124,125) and Monné (103) demonstrated that the structure of the cytoplasm of sea urchin eggs coarsens when respiration is inhibited. Obviously respiration is necessary in order to control the clotting system of the protoplasm. Death occurs when this clotting is strong. The spotted or striated appearance of neurons of warm-blooded animals is due to condensation of the cytoplasmic fibrils which consist of the ribonucleic-acid-containing chromidia (Nissl substance) and the ribonucleic-acid-free interchromidia alternating regularly with each other. It is evident that the clotting and coarsening of the structure of the cytoplasm of the neurons occur rapidly when respiration ceases at the moment of death of warm-blooded animals. It is of interest to note that this change does not occur in excessively stimulated neurons. In these cells the structure of the cytoplasm does not coarsen, the fibrils do not agglutinate with each other, and consequently, the chromidia remain diffusely distributed. A similar phenomenon has been observed by Runnström and Monné (128) in sea urchin eggs. Clotting and coarsening of the structure of the cytoplasm are brought about much more easily in mature unfertilized than in fertilized eggs. The latter are stimulated to development and therefore they are comparable with the excessively stimulated neurons. In cells whose activity is depressed, the structure of the cytoplasm is coarse or it may easily be made coarse by means of experimental agents.

Energy is required to produce the cyclic changes of the microscopic and submicroscopic structure underlying any specific function. The